Seroprevalence and Epidemiological Correlates of HTLV-I Infection in U.S. Blood Donors

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Screening for human T-lymphotropic virus type I (HTLV-I) antibodies was performed on sera from 39,898 blood donors at eight blood centers in geographically distinct areas of the United States. Ten donors (0.025 percent) showed evidence of HTLV-I seropositivity by enzyme immunoassays; this was confirmed by protein immunoblot and radioimmunoprecipitation. Seroprevalence rates ranged from 0 to 0.10 percent at the locations sampled, with HTLV-I antibodies found predominantly in donors from the southeastern and southwestern United States. Matched case-control interviews and laboratory studies were performed on five seropositive women and two seropositive men who participated in an identity-linked collection of sera from a subset of 33,893 donors at six of the eight blood centers. Four of the women and both men are black; one woman is Caucasian. Four of the seven seropositive individuals admitted to prior intravenous drug abuse or sexual contact with an intravenous drug user. Sexual contact with native inhabitants of an HTLV-I endemic area was the only identified risk factor for one male. The distribution of HTLV-I antibodies in this U.S. blood donor sample corroborates the previously reported epidemiology of this agent and suggests that additional donor screening measures, including the testing of donated blood for HTLV-I markers, may be necessary to prevent the spread of HTLV-I to transfusion recipients.

HUMAN T-LYMPHOTROPIC VIRUS type I (HTLV-I) has been identified as a cause of adult T-cell leukemia/lymphoma (ATL) on the basis of virus isolation from ATL patients, as well as extensive cross-sectional serological studies; these studies demonstrate an association of HTLV-I antibodies and ATL within distinct geographic clusters in southwestern Japan, Africa, and the Caribbean basin (*1*). In seroepidemiological studies, HTLV-I has been linked with neurological diseases designated as tropical spastic paraparesis (TSP) and HTLV-I–associated myelopathy (HAM) (*2*). Recently, reports have also proposed an association between HTLV-I *tat* gene products and the induction of chronic B-cell leukemia in man as well as a wide range of mesenchymal tumors in transgenic mice (*3*).

Several lines of evidence suggest that HTLV-I can be transmitted from infected blood donors to recipients of cellular blood products. Retrospective studies of donor-recipient pairs in Japan (*4*) demonstrated a 48 to 82% seroconversion rate in recipients of HTLV-I seropositive blood. Two cross-sectional studies of recipient populations have been conducted in New York City. The first showed a 3.0% prevalence of HTLV-I seropositivity in chronically transfused hematology patients, whereas the second showed a 2.8% prevalence of HTLV-I infection in leukemia patients who had multiple transfusions (*5*). Unlike human immunodeficiency virus type 1 (HIV-1), which is present in cell-free plasma, HTLV-I is highly cell-associated, and recipients of products made from plasma pools do not show an increase in HTLV-I infection beyond that which can be attributed to transfusion of cellular products. This observation is supported by reports that HTLV-I is most readily isolated in vitro by cocultivation with uninfected lymphocytes (*6*).

Although ATL, TSP, and HAM are rare outside of Japan, Africa, and the Caribbean basin, reports have indicated that HTLV-I infection is currently endemic in intravenous (IV) drug abusers in certain urban areas of the eastern United States (*7*). Because of an apparent reservoir of HTLV-I infection in U.S. IV drug abusers and the unknown prevalence of infection in the general population, there is increasing concern that asymptomatic HTLV-I carriers may be present at low levels in the U.S. blood donor pool. Although all volunteer blood donors are highly screened by general health history information, specific exclusion criteria for AIDS risk factors, and laboratory tests for HIV-1 antibodies, hepatitis B surface antigen, antibodies to the hepatitis B core antigen, alanine aminotransferase (ALT), and syphilis serology, the efficacy of these measures to exclude HTLV-I carriers is unknown. HTLV-I infection, if present in the donor pool, would contribute to the introduction of the agent into populations of the country where infection is not endemic, and it may present a future health risk to blood recipients and their sexual partners (*8*). The study

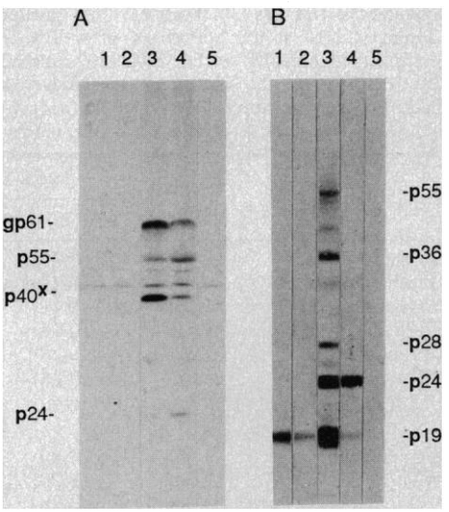

Fig. 1. Radioimmunoprecipitation (**A**) and protein immunoblot (**B**) assays for confirmation of HTLV-I antibody reactivity. Portions of samples that were repeatedly reactive by EIA were sent to four different laboratories simultaneously for further testing to confirm the presence of HTLV-I antibody. Research laboratories at Du Pont/Biotech and at Cellular Products tested the samples by protein immunoblot analysis with antigen preparations identical to those used in the HTLV-I EIA kits. RIP assays were performed at SUNY Upstate Medical Center and at UCLA School of Medicine with [^{35}S]cysteine and [^{35}S]methionine double-labeled cell lysate of HTLV-I–infected human T cells as the reacting antigens. Specific antigen-antibody complexes were precipitated by *Staphylococcus aureus* protein A after incubation. Finally, viral antigens precipitated by HTLV-I–specific antibodies contained in the serum were subjected to polyacrylamide gel electrophoresis and autoradiography. Lanes 1 and 2 of (A) and (B) are samples considered "indeterminate" as a result of protein immunoblot reactivity against only viral core proteins p19 or p28 or both, and no reactivity by RIP. Lanes 3 and 4 are samples considered to be positive for HTLV-I antibodies because of reactivity against p19, p24, and other viral specific bands by protein immunoblot analysis, and against gp61, p55, p40^x, and p24 by RIP. Lane 5 is a negative sample.

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reported here measures the prevalence of HTLV-I antibody in a geographically diverse sample of the U.S. donor population and assesses the epidemiological variables associated with HTLV-I seropositivity.

Blood (10 ml) was collected with identitylinkage from 33,893 sequential blood donors at six blood centers (Table 1). These donors provided written informed consent after reading a study information sheet explaining the nature and possible consequences of this investigation. Additional anonymous (identity unlinked) serum samples from 6005 sequential blood donors were collected in Miami and Los Angeles. All donors enrolled in this study were negative for antibodies to HIV-1 by enzyme immunoassay (EIA) (Abbott HIV EIA, Abbott Laboratories, Chicago). A total of 68 serum samples (0.17%) were found to be repeatedly reactive by HTLV-I EIA (Du Pont/Biotech HTLV-I ELISA, Wilmington, DE/Rockville, MD, or Cellular Products HTLV-I ELISA, Buffalo, NY). Additional testing of the sera that were repeatedly reactive by HTLV-I EIA included protein immunoblot analysis by two laboratories (Du Pont/Biotech and Cellular Products) and radioimmunoprecipitation (RIP) testing at two sites (UCLA School of Medicine, Los Angeles, and SUNY, Syracuse, NY). All confirmation testing of sera obtained from study subjects was completed before donor notification and interview.

Table 1. Results of screening for HTLV-I antibodies of blood donor sera. EIAs from Cellular Products and Du Pont/Biotech were selected as the initial screening tests after an evaluation of available commercial test kits (*9*). Both tests use disrupted semipurified HTLV-I as the solid-phase capture antigen coated at the bottom of 96-well microtiter plates. Viral antigen was obtained from the transformed human T-cell line HUT-102 for the Du Pont/Biotech assay whereas HUT-102B2 was used by Cellular Products. Samples giving initial reactive results (IR) were retested in duplicate with tests from both manufacturers. Those samples with absorbance greater than the manufacturer's cutoff level in retesting were considered EIA repeatedly reactive (RR) and subjected to further confirmation.

Region	Samples tested	Initial EIA		Repeat EIA		PIB/RIP
		IR (%)	GZ* (%)	Total (%)	RR (%)	Positive† (%)
Atlanta	5,946	23 (0.39)	8 (0.13)	31 (0.52)	12 (0.20)	2 (0.034)
Washington	4,949	21 (0.43)	9 (0.18)	30 (0.61)	12 (0.24)	3 (0.061)
St. Paul	6,060	7 (0.12)	6 (0.10)	13 (0.22)	3 (0.05)	0
Portland	5,563	8 (0.14)	5 (0.09)	13 (0.23)	4 (0.07)	0
Birmingham	5,773	16 (0.28)	7 (0.12)	23 (0.40)	8 (0.14)	2 (0.035)
Baltimore	5,602	19 (0.34)	6 (0.11)	25 (0.45)	6 (0.11)	0
Identity-linked total	33,893	94 (0.28)	41 (0.12)	135 (0.40)	45 (0.13)	7 (0.021)
Los Angeles	3,008	9 (0.30)	3 (0.10)	12 (0.40)	7 (0.23)	3 (0.100)
Miami	2,997	21 (0.70)	8 (0.27)	29 (0.97)	16 (0.53)	0
Identity-unlinked total	6,005	30 (0.50)	11 (0.18)	41 (0.68)	23 (0.38)	3 (0.050)
Total	39,898	124 (0.31)	52 (0.13)	176 (0.44)	68 (0.17)	10 (0.025)

*Gray zone (GZ) indicates test signals under, but within 15% of, the cutoff value. †Sera showing specific reactivity to two or more HTLV-I gene products by protein immunoblot (PIB) or RIP are considered to be confirmed.

Protein immunoblot assays performed by all collaborating laboratories were capable of demonstrating antibodies to HTLV-I *gag*-encoded core proteins p19, p24, p28, and p55 but showed minimal or no ability to identify serological reactivity to *env*-encoded glycoproteins gp46 and gp61. The genomic origin of the protein band appearing at the p36 has not been established (Fig. 1). By means of HTLV-I–infected whole cell lysates as the antigen for RIP assays, additional serological reactivities were identifiable, including core and envelope proteins and $p40^x$, the *tat*-encoded transcriptional activator. HTLV-I seropositivity was considered to be confirmed if antibodies to the products of two or more different HTLV-I genes were detected by protein immunoblot, RIP, or both (*9*).

When tested by protein immunoblot, 47 of the 68 samples that were repeatedly reactive by EIA showed combinations of bands in positions compatible with the size of HTLV-I core proteins. Ten of these sera showed antibody to p24 in addition to other bands. These ten sera were the only samples that were reactive by RIP: seven with antibodies to p24, $p40^x$, p55, and gp61; one with antibodies to p24, $p40^x$, and gp61; and two with antibodies to gp61 only (Table 2). On the basis of the identity of these band patterns with those obtained from ATL patients and seropositive blood donors in Japan and Jamaica (*9*), these ten individuals were considered to be confirmably seropositive for HTLV-I. Although the antibodies detected in this study are reactive against HTLV-I viral proteins, the possibility of cross-reacting antibodies stimulated by HTLV-II, or another antigenically similar human retrovirus, could not be excluded.

The identification of HTLV-I seropositive donors in four southern U.S. cities corroborates the earlier observations of in-

Table 2. HTLV-I–confirmed positive donors. Epidemiologic data for cases 1 to 7 were obtained by interview. Cases 8 to 10 were identified by screening of anonymous blood donors. The hierarchical risk of HTLV-I exposure is the variable (obtained from the oral interview) that was judged most likely to be associated with HTLV-I infection for a given individual. Abbreviations: M, male; F, female; B, black; C, Caucasian; and NA, not available.

Case	City	Age (years)	Sex	Race	Protein immunoblot	RIP	Hierarchical risk
1	Atlanta	61	M	B	19, 24, 26, 28, 36, 53	61	Sexual contact in Orient (1947–49)
2	Atlanta	41	F	B	19, 24	24, 40, 61	None identified
3	Washington	37	F	B	24	24, 40, 55, 61	IV drug abuse and sexual contact with IV drug abuser
4	Washington	44	F	C	19, 24	24, 40, 55, 61	Sexual contact with IV drug abuser
5	Washington	29	F	B	19, 24, 28, 36, 53	24, 40, 55, 61	Sexual contact with IV drug abuser
6	Birmingham	32	M	B	19, 24, 26, 28, 32, 36, 40, 46	24, 40, 55, 61	IV drug abuse
7	Birmingham	28	F	B	19, 24, 28, 36, 53	24, 40, 55, 61	History of three prior STDs
8	Los Angeles	NA	NA	NA	19, 24, 28, 53	24, 40, 55, 61	NA
9	Los Angeles	NA	NA	NA	24	24, 40, 55, 61	NA
10	Los Angeles	NA	NA	NA	24	61	NA

creased HTLV-I prevalence in the southeastern United States (*10*). However, comparisons of HTLV-I seroprevalence among the different geographic locations studied are likely to be confounded by the limited sample size of this study as well as demographic differences that may be inherent to the donor populations of each blood center.

Demographic information from the blood donor record was reviewed for all donors whose sera were repeatedly reactive by HTLV-I EIA but which could not be confirmed by protein immunoblot or RIP. The age and sex distributions of these donors, as well as all other routinely collected medical and demographic information, paralleled the overall distribution within the Red Cross collection system. These individuals were not notified of their test results, and no further information was obtained from them.

Of the ten confirmed HTLV-I seropositive donors, the seven individuals participating in the identity-linked portion of the cross-sectional study were notified of the results. Each seropositive case was randomly matched by sex and age (±2 years) with five control donors from the same blood collection center. Standardized questionnaires were administered in person to both case and control donors by trained interviewers. Questionnaires were designed to gather a broad range of donor characteristics, including general demography, travel, direct and indirect intravenous drug exposure, animal exposures, characteristics of sexual partners, donor medical history and current health status, and familial history of neurologic disease and cancer. All interviewed controls were confirmed to be seronegative by HTLV-I EIA.

The HTLV-I protein immunoblot and RIP reactivities of the five women and two men identified as HTLV-I seropositive were verified in the follow-up blood samples collected at the time of interview (Table 2). At the same time, peripheral blood was collected from five of the HTLV-I seropositive donors for viral culture studies.

Several questionnaire variables reported during the medical history interview distinguished the group of seropositive donors from their matched controls, including: a history of prior IV drug abuse (one donor), sexual contact with an IV drug user (two donors), or both (one donor); black race; and possibly a higher level of heterosexual activity with different partners, indicated indirectly by a tendency to be unmarried and to have more reported episodes of sexually transmitted diseases (STDs) (Table 3). The only identifiable risk for one 61-year-old male was a history of sexual contact with Japanese women when he was stationed with the U.S. occupation forces in Japan from 1947 to 1949. This individual had been married twice with the marriages lasting most of the 36-year period between 1949 and 1985; no other apparent risk factors for infection could be determined. A similar history was also reported by two of this donor's seronegative matched controls. Although the extent to which HTLV-I was endemic in Japan 40 years ago is unknown, it has been estimated that HTLV-I has been indigenous in Japan for at least several centuries (*11*). No risk for HTLV-I exposure could be identified for one seropositive donor. No evidence was found in this study for an association of HTLV-I infection with short periods of travel to HTLV-I endemic areas.

All interviewed donors considered themselves to be in good or excellent health. HTLV-I seropositivity could not be specifically associated with a history of cancer or neuromuscular disease in either the donors or their immediate family members. No donors reported a history of homosexual or bisexual activities.

Cocultivation of peripheral blood lymphocytes from two HTLV-I seropositive donors with lymphocytes obtained from random donors resulted in the isolation of HTLV-I from one donor (case 1), and HTLV-II from a second donor (case 3) on the basis of RIP of cellular lysates and DNA blot analysis. Details of viral isolations are reported elsewhere (*12*).

This study demonstrates that 0.025% of random blood donors in eight geographically diverse areas of the United States had serological evidence of HTLV-I infection by current EIA, protein immunoblot, and RIP criteria. If we assume that HTLV-I seropositivity can be equated with HTLV-I infection and that the efficiency of HTLV-I transmission by homologous blood transfusion is approximately 60% (*4*), we can predict, by a rough extension of these prevalence data, the infection of approximately 2800 blood recipients annually in the United States (*13*). Although as many as two-thirds of recipients may die within 1 year of transfusion from their underlying disease, we would anticipate some secondary spread

Table 3. HTLV-I seropositive cases were each matched with five seronegative controls by blood collection center, age (±2 years), and sex. After notification of test results, a standardized questionnaire was administered by oral interview. Odds ratios and confidence intervals (without correction for multiple intervals) were calculated by the Mantel-Haenszel procedure for matched case-control studies (*15*). Lower confidence limits for intravenous drug and STD exposure were determined by maximum likelihood estimate (*16*). The male to female ratio of cases compared with the known ratio of 51:49 for American Red Cross blood collection centers was not significant ($P > 0.1$, Fisher's exact test). Antibody to hepatitis A virus (HAV) was measured by HAVAB (Abbott); antibodies to herpes simplex virus (HSV) and cytomegalovirus (CMV) were measured by FIAX solid-phase immunosorbent assay (M.A. Bioproducts, Walkersville, MD).

Variable	Cases	Controls	Odds ratio	95% Confidence interval
History of IV drug use or sex with IV drug user	4/7	0/35	20.0	(12.7–∞)
Black race	6/7	4/35	14.0	(3.2–62.0)
History of more than two STDs	1/7	0/35	14.0	(1.7–∞)
HAV seropositive	5/7	3/35	8.9	(3.5–17.1)
Unmarried	6/7	17/35	7.5	(1.7–33.4)
No education past grade 12	4/7	5/35	6.0	(1.3–8.8)
History of transfusion	3/7	2/35	5.6	(0.9–7.3)
History of skin rash	2/7	3/35	3.0	(0.7–3.4)
One or more STDs	2/7	3/35	3.0	(0.6–3.1)
Sexual contact in Orient	1/7	2/35	2.3	(0.7–4.4)
HSV seropositive	6/7	26/35	2.0	(0.6–5.2)
Travel to HTLV-I endemic areas	1/7	4/35	1.9	(0.6–3.1)
Family history of cancer	2/7	9/35	1.2	(0.7–2.4)
Exposure to swine	1/7	5/35	1.0	(0.5–2.7)
Breastfed as infant*	4/5	22/27	0.9	(0.7–1.8)
CMV seropositive	3/7	18/35	0.7	(0.6–1.7)
Birth outside of United States	0/7	0/35	0.0	0.0
Gay, lesbian, or bisexual	0/7	0/35	0.0	0.0
Exposure to cattle	0/7	2/35	0.0	0.0
Numbness or weakness, difficulty walking, or poor health	0/7	0/35	0.0	0.0
Neurologic disease in family	0/7	0/35	0.0	0.0

*Two seropositive cases and eight controls did not know whether they were breastfed as infants.

of infection by blood recipients who remain sexually active and by the small proportion of recipients who are women of child-bearing age.

No cases of transfusion-associated ATL have been reported to date. The absence of such reports, however, may not be an accurate indication of the impact of transfusion-transmitted HTLV-I infection in nonendemic areas of the world for the following reasons: first, a latency period of 10 to 30 years appears to exist between infection and clinical disease (*1*); and second, HTLV-I may play a role in indirectly mediating the development and growth of neoplasms other than ATL by mechanisms that are not fully understood (*3*).

Information obtained from this study and from continuing HIV-1 surveillance efforts indicates that the complete exclusion of potentially infective blood donors is not attainable solely by health history and self-deferral procedures (*14*). Refinements of donor selection methods are needed, as are continued efforts to develop blood sterilization technologies. In the meantime, the use of licensed assays to detect and exclude blood donors who have confirmed laboratory evidence of HTLV-I infection would appear to be the most prudent course of action.

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Dextran Sulfate Suppression of Viruses in the HIV Family: Inhibition of Virion Binding to CD4$^+$ Cells

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The first step in the infection of human T lymphocytes by human immunodeficiency virus type 1 (HIV-1) is attachment to the target cell receptor, the CD4 antigen. This step may be vulnerable to attack by antibodies, chemicals, or small peptides. Dextran sulfate (molecular weight approximately 8000), which has been given to patients as an anticoagulant or antilipemic agent for more than two decades, was found to block the binding of virions to various target T lymphocytes, inhibit syncytia formation, and exert a potent inhibitory effect against HIV-1 in vitro at concentrations that may be clinically attainable in human beings. This drug also suppressed the replication of HIV-2 in vitro. These observations could have theoretical and clinical implications in the strategy to develop drugs against HIV types 1 and 2.

DEXTRAN SULFATE, A LONG-CHAIN polymer of glucose that has a molecular weight of approximately 8000 and contains 17 to 20% sulfur, has been given orally to human beings for more than two decades as an anticoagulant or antilipemic agent (*1*). This agent was recently shown to be a potent agent against human immunodeficiency virus type 1 (HIV-1) in vitro (*2*). We have now investigated the possible mechanism of action of dextran sulfate against HIV-1 and related viruses.

When susceptible interleukin 2 (IL-2)–dependent helper T cells [strain ATH8 (*3*, *4*)] were exposed in vitro to HIV-1 in the form of cell-free virions, essentially all the cells were destroyed by the cytopathic effect of HIV-1 by day 7 (Fig. 1). However, when ATH8 cells were cultured in the presence of HIV-1 virions and more than 1.25 μM dextran sulfate, no cytopathic effects were observed and growth of the cells was comparable to that of the virus-free control ATH8 population.

Dextran sulfate may have two sulfate groups per glucose unit, and these sulfate groups appear to be associated with its antiviral activity (*2*). Indeed, nonsulfated dextran [molecular weight (MW) of 9400] showed no protective effect on ATH8 cells against the virus even at 125 μM.

Dextran sulfate also protected normal (tetanus toxoid–specific) helper/inducer clonal T cells (TM11 cells) against the cytopathic effect of HIV-1 (Fig. 1). At concentrations of 0.625 μM to 12.5 μM, this compound gave complete protection and did not suppress the growth of the T cells. At similar concentrations, dextran sulfate also inhibited the infectivity and cytopathic effects of human immunodeficiency virus type 2 (HIV-2), which may also cause an AIDS-like disease (*5*) (Fig. 1).

As an additional index of the antiretroviral effect of dextran sulfate, we looked for the presence of proviral DNA in susceptible ATH8 cells continuously exposed to HIV-1

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