Transfusion-transmitted human T-lymphotropic virus Type I infection in a United States military emergency whole blood transfusion recipient in Afghanistan, 2010

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BACKGROUND: The United States introduced human T-lymphotropic virus Type I (HTLV-I) screening of blood donors in 1988. The US military uses freshly collected blood products for life-threatening injuries when available stored blood components in theater have been exhausted or when these components are unsuccessful for resuscitation. These donors are screened after donation by the Department of Defense (DoD) retrospective testing program. All recipients of blood collected in combat are tested according to policy soon after and at 3, 6, and 12 months after transfusion.

CASE REPORT: A 31-year-old US Army soldier tested positive for HTLV-I 44 days after receipt of emergency blood transfusions for severe improvised explosive device blast injuries. One donor's unit tested HTLV-I positive on the DoD-mandated retrospective testing. Both the donor and the recipient tested reactive with enzyme immunoassay and supplemental confirmation by HTLV-I Western blot. The donor and recipient reported no major risk factors for HTLV-I. Phylogenetic analysis of HTLV-I sequences indicated Cosmopolitan subtype, Subgroup B infections. Comparison of long terminal repeat and *env* sequences revealed molecular genetic linkage of the viruses from the donor and recipient.

CONCLUSION: This case is the first report of transfusion transmission of HTLV-I in the US military during combat operations. The emergency fresh whole blood policy enabled both the donor and the recipient to be notified of their HTLV-I infection. While difficult in combat, predonation screening of potential emergency blood donors with Food and Drug Administration–mandated infectious disease testing as stated by the DoD Health Affairs policy should be the goal of every facility engaged with emergency blood collection in theater.

Human T-lymphotropic virus Type I (HTLV-I) is an intracellular human RNA retrovirus that is associated primarily with adult T-cell leukemia in 2% to 5% and HTLV-I–associated myelopathy or tropical spastic paraparesis in 1% to 2% of infected carriers.[1-3] Six subtypes have been identified: a (Cosmopolitan)—Japan; b to f—Central Africa; c—Melanesia; and e—South and Central Africa.[4-9] Isolates from West African countries are almost identical to those from the French West Indies, Haiti, French Guyana, and Peru.[8] While the genetic variability of the HTLV-I proviral sequence is relatively low when compared to other viruses such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV), epidemiologically linked infections

ABBREVIATIONS: ASBPO = Armed Services Blood Program Office; DoD = Department of Defense; FOB = forward operating base; IED = improvised explosive device; LTR = long terminal repeat; MTF(s) = military treatment facility(-ies).

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TABLE 1. Countermeasures employed to reduce the risk of transfusion-transmitted viral infection from freshly collected blood products in the combat theater of operations

Countermeasure	HIV	HCV	HBV	HTLV-I/II
Periodic screening of the force	Every 2 years*			
Theater entrance screening	Within 120 days†			
Vaccination	NA*	NA*	Required‡	NA
Volunteer donor screening questionnaire	Yes	Yes	Yes	No§
Volunteer donor pools‖	Yes	Yes	Yes	Yes
Rapid test	Yes	Yes	Yes	NA

* Force screen policy 2001—current by service and component for active component; every 5 years for reserve component.
† Central Command (CENTCOM) theater entrance requirement for predeployment HIV testing within 120 days of deployment, as of December 2011 (MOD 11).
‡ Universal vaccination during initial entry training since 2001. Required to initiate vaccination prior to entry in to the CENTCOM combat theater of operations.
§ Not currently utilized; recommendation has been made to consider modification of the DoD donor screening questionnaire (DD572) and include questions pertaining to risk for HTLV-I/II infection.
‖ Volunteers at facilities with blood donation capacity are screened for HIV, HBV, HCV, HTLV-I/II, West Nile virus, and syphilis. Donors are admitted to the donor pool upon receipt of negative test results and rescreened every 90 days after readmission. At the time of donation, donated units are tested for HIV, HBV, and HCV with rapid diagnostic tests.

have been identified.[10-12] In HTLV-I–endemic areas such as southwestern Japan, the Caribbean, sub-Saharan Africa, South America, and parts of Iran, seroprevalence rates range from less than 5% to 10%.[13]Infection is lifelong with transmission of the virus primarily through breast milk, sexual contact, blood transfusion, or from sharing needles in intravenous drug use.

Reports of transfusion-transmitted HTLV-I in the United States have been infrequent with the last such report in 1989.[14] The United States initiated HTLV-I screening of blood donors in 1988[15] to prevent transmission of the virus from transfusion of blood products.[14,16] As a lifesaving measure when stored blood products have failed and when existing US Food and Drug Administration (FDA)-approved blood products have been exhausted or are unavailable, the US military uses freshly collected blood products during conflicts for combat casualty resuscitation.[17,18] Health Affairs policy guidelines for non–FDA-compliant emergency blood collection include, in order of preference, 1) blood donors screened for FDA-mandated transfusion-transmitted pathogens within 90 days by a Clinical Laboratory Improvement Amendments–certified laboratory; military treatment facilities (MTFs) and US Naval vessels conducting predonation screening are required to maintain up-to-date rosters of eligible blood donors; 2) donors who self-report to have been nondeferred repeat donors; and 3) donors who neither have been screened for FDA-mandated transfusion transmitted pathogens nor have a history of donation.[19] The US military utilizes several countermeasures to reduce the risk of transfusion-transmitted viral infections from battlefield transfusion of emergency blood products collected in the deployed setting. Any MTF in the combat theater of operations with blood donation capability may initiate a walking donor program, which includes screening volunteers for HIV, hepatitis B virus (HBV), HCV, HTLV, West Nile virus, and syphilis. MTFs send samples collected from volunteers to a commercial laboratory in the continental United States for testing. After being tested, volunteers who screened negative are eligible to donate for a period of 90 days and are retested at 90-day intervals and at each donation event. Other countermeasures include universal HBV immunization and, in accordance with Health Affairs policy, screening of blood products for HIV, HBV, and HCV with rapid diagnostic test devices at the time of collection from donors in emergency situations who have not been screened in the combat theater of operations (Table 1). We report here the results of an investigation conducted as a result of the Department of Defense (DoD) retrospective testing program of non–FDA-compliant fresh whole blood.

CASE REPORT

The recipient, a 31-year-old US Army soldier, received 13 units of fresh whole blood in Jalalabad, Afghanistan, on the day of his injury after an improvised explosive device (IED) blast (Table 2). The soldier had sustained multiple injuries to the head, chest, midsection, groin, and lower extremities requiring chest tubes, fasciotomies, and washouts. Before arrival at the forward operating base (FOB) he had received basic care in the field on the day of the blast (Day 0, Table 2). He was transferred to Bagram Airfield from the FOB for a laparoscopic splenectomy and follow-up care, which included more washouts, external fixation placements, and splinting for his groin and fracture injuries. He received 4 units of platelets (PLTs). He was evacuated subsequently to a military hospital in Germany on Day 3 where he remained for 2 days for stabilization due to his traumatic brain injury, which required bolts for subarachnoid injuries.

On Day 5 he was transferred by a Critical Care Air Transport Team to a tertiary care military hospital in Texas where he remained for approximately 3 months before

TABLE 2. Timeline of events and testing for a lookback investigation of a donated unit which tested positive for HTLV-I, Afghanistan, 2010

Donor	Day	Recipient
1 unit donated in theater	0	IED blast; field medical care; 13 units fresh whole blood transfused, FOB Fenty, Jalalabad, Afghanistan
	2	4 units PLTs, BAF, Bagram, Afghanistan
	3	Medical evacuation to Germany
	4	Germany
	5	Transfer to a military hospital in United States for definitive care
Donated unit tested HTLV+	12	
	15	Lookback Test 1 = HTLV-I indeterminate
Notified in theater of HTLV diagnosis	31	
	44	Lookback Test 2 = HTLV-I ; notification and counseling
	180	Lookback Test 3 = HTLV-I+
	234	Reposed sample collected 142 days before transfusion = HTLV–
Reposed sample collected 265 days before donation = HTLV+	279	
	293	Epidemiologic interview; sample collection for HTLV sequencing
Epidemiologic interview; sample collection for HTLV sequencing	309	

BAF = Bagram Airfield.

being released to the Veterans Administration health system for follow-up care. While in care at the military hospital in 2012, recurring fever and increasing white blood cell counts in the soldier 29 days into his admission led to an infectious disease work-up for malaria, brucellosis, cytomegalovirus, Epstein Barr virus, *Clostridium difficile,* and Q fever. These tests, along with routine evaluation for nosocomial and other trauma-related wound infections, were unrevealing. In the midst of his infectious disease work-up, his providers received notification that the soldier's posttransfusion surveillance sample, drawn 19 days after the IED blast as part of the DoD retrospective testing program, had tested HTLV-I indeterminate: rg46-1 and -2 reactive, p19 and GD21 nonreactive (Day 19, Table 3). The sample had tested negative for HIV, HBV, HCV, and other pathogens. Retrospective testing performed 44 days after transfusion (Quest Laboratories, Irving, TX) indicated that the recipient had seroconverted and was HTLV-I infected: rgp 46-1, p19, GD21 reactive (Day 44, Table 3).

The Armed Services Blood Program Office (ASBPO) initiated a lookback investigation for the donors of the recipient. Mandatory testing of donation aliquots shipped to the United States after fresh whole blood combat theater donations had revealed a blood unit, donated 12 days prior in Afghanistan (Day 0, Table 2), that was positive for HTLV but negative for HIV, HBV, HCV, and syphilis.[19,20] The other 12 of 13 fresh whole blood units the recipient had received at the FOB had tested negative for HTLV and other blood-borne pathogens.

The ASBPO initiated another investigation to determine the HTLV-I infection status of the recipient and donor before transfusion and donation, respectively. The method has been previously described.[21] Briefly, the recipient's pretransfusion and donor's predonation reposed sera, residual sample from mandatory HIV force

TABLE 3. Results for donor and recipient samples tested in the lookback investigation of an HTLV-I–positive donated unit in Afghanistan, 2010

	Days in relation to donation/transfusion					
	Donor		Recipient			
Assay	–265*	128	–142*	19	44	180
HTLV-I/II EIA	R	R	NR			R
Western blot†						
Band interpretation				Ind	P	
P19				NR	R	
P24				NR	R	
GP46				NR	NR	
P26				NR	R	
GD21				NR	R	
P28				NR	R	
P32				NR	R	
RG46-1				R	R	
RG46-2				R	NR	
P53				NR	NR	
P36				NR	R	
Line immunoassay						
Interpretation	P	P				P
Streptavidin	NR	NR				NR
P19 I/II	R	R				R
P24	R	R				R
GP46	R	R				R
GP21	R	R				R
P19	R	R				R
GP46 I	R	R				R
GP46 II	NR	NR				NR

* Predonation and pretransfusion testing for donor and recipient, respectively.

† Positive for HTLV-I if P19, GD21, and RG46-I are reactive; positive for HTLV-II if P24, GD21, and RG46-II are reactive; indeterminate if criteria for positivity are not met; negative if HTLV bands are not present.

Ind = indeterminate; NR = nonreactive; P = positive; R = reactive.

testing, were retrieved from the DoD Serum Repository and sent to Quest Laboratories for HTLV testing (Days –142 and –265, respectively; Table 3).[22] For the donor, both a predonation sample, drawn 265 days before donation, and a postdonation sample, drawn 128 days after donation, were HTLV enzyme immunoassay (EIA) reactive and HTLV line immunoassay positive. The recipient's pretransfusion sample, drawn 142 days previously, was HTLV EIA nonreactive (Table 3). Since all evidence pointed to a case of transfusion-transmitted HTLV-I infection, an epidemiologic investigation was launched.

An infectious disease clinician interviewed both the donor and the recipient for HTLV risk factors using a standardized case report form for transfusion-transmitted viral infections. The interview indicated that the recipient was white and US-born and had served 13 years in the US military at the time of his transfusion. At the time of the interview, he had no signs or symptoms of HTLV-I/II: skin lesions; numbness, stiffness, or weakness of the legs; difficulty walking; acute bronchitis; asthma; pneumonia; leukemia; arthritis; abscess; lymphadenopathy; bladder or kidney infection; and *Staphylococcus* or *Strongyloides* infections. There was no evidence of HTLV infection–related neurologic disease on physical examination. The only risk factors reported were blood transfusions received in theater reported here, ear piercings, and corrective surgeries as described above for injuries sustained in combat. The recipient revealed no history of: sexually transmitted diseases, sex with a commercial sex worker, or injection drug user; incarceration; residence in a group or halfway home; tattoos; rape; needlestick; blood splash to mucous membranes; organ, tissue, or marrow transplant; and sex or household contact with a person with hepatitis, HIV, or HTLV-I/II or a person who had received clotting factors. He recounted that he had traveled to Australia, France, and Germany.

The donor was a US-born 32-year-old white male from the Pacific Northwest region whose parents were white and not of mixed race. He was an Army Reservist who had been in service for 3 years at the time of his donation. He reported having no awareness of HTLV infection until he was informed of his donated unit's screening results (Table 2). At the time of his interview, he reported no signs or symptoms of his HTLV-I infection and revealed no risk factors other than acquiring a couple of tattoos at reputable facilities and having had surgery as a child. He indicated no history of blood transfusions and denied having sex or household contact with a person known to have HTLV-I/II infection and did not report having sex partners from HTLV-endemic regions of the world. His travel history included trips to Germany, Prague, Poland, Spain, and Amsterdam.

Molecular characterization of HTLV-I from both the donor and the recipient was initiated to determine whether HTLV-I infection in the recipient was transfusion

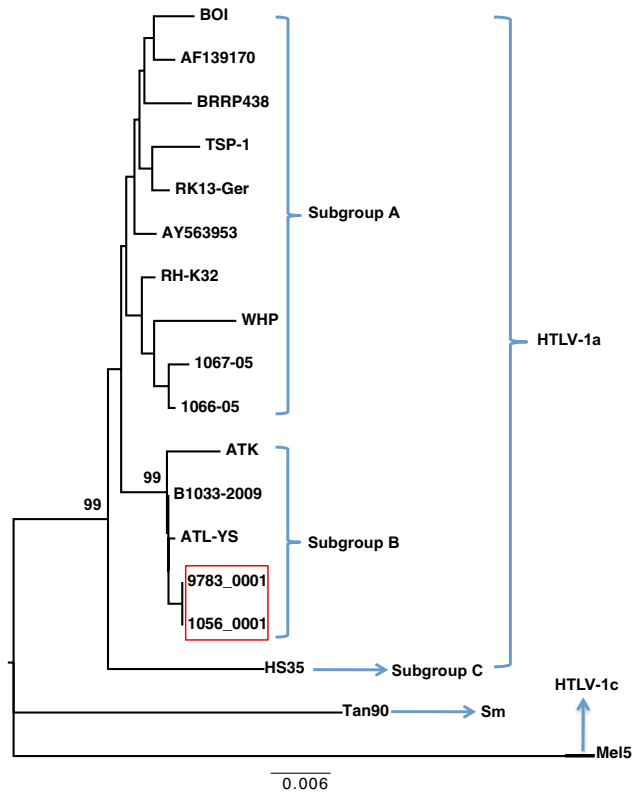

Fig. 1. A phylogenetic tree of HTLV-1 sequences from the donor (9783) and recipient (1056), as highlighted by the red box, and 16 HTLV-I subtype reference sequences was constructed by the maximum likelihood method using MEGA 5.05 software. The sequences were concatenated from 1353 nucleotides in envelope gene corresponding to ATK1 numbering Positions 5217 to 6569 and 433 nucleotides in LTR region corresponding to ATK1 numbering positions 8269 to 8700. The scale bar indicates the number of nucleotide substitutions per site estimated by general time reversible model with number of bootstrap replications at 1000.

transmitted. Blood samples provided at the time of interviews were sent to the US Military HIV Research Program for sequencing. DNA extracted from peripheral blood mononuclear cells was used for partial genome sequencing: 433 nucleotides of the long terminal repeat (LTR) region (ATK1 reference positions, 8269 to 8700) and 1353 nucleotides from the envelope (*env*) region (ATK1, 5217-6569). Phylogenetic analysis was performed using computer software (MEGA 5.05, http://www.megasoftware.net/). Due to the very low evolutionary rate of HTLV-1, a maximum likelihood approach with the number of nucleotide substitutions per site estimated by general time reversible model and number of bootstrap replications at 1000 was chosen for the analysis.

Maximum likelihood trees generated from LTR and *env* concatenated sequences (Fig. 1) showed that the virus sequences from the pair, 1056_001 (recipient) and

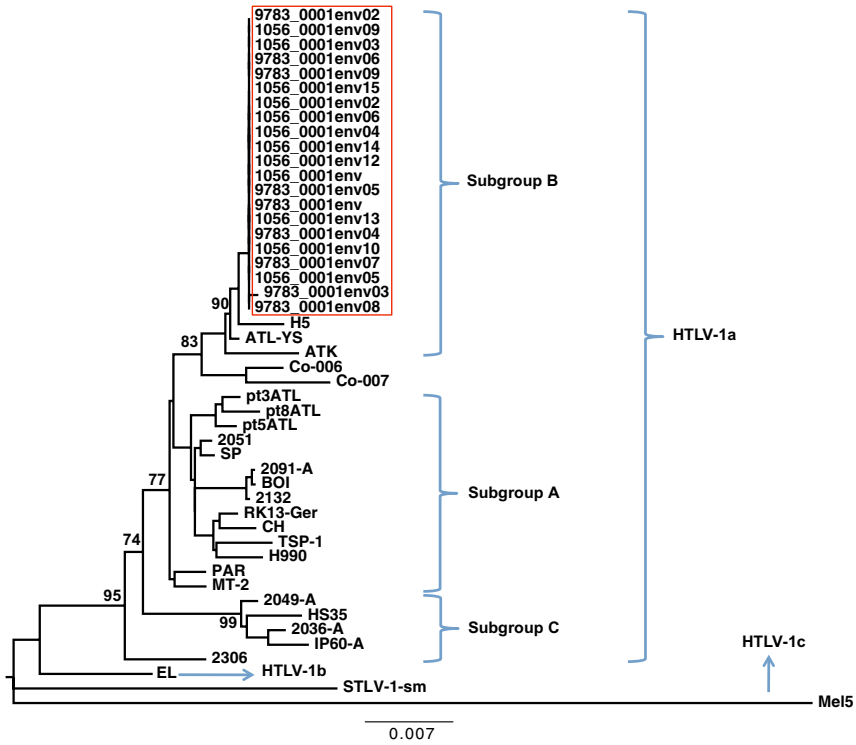

Fig. 2. A phylogenetic tree of HTLV-1 envelope genes corresponding to ATK Positions 5217 to 6569 was constructed by the maximum likelihood method using MEGA 5.05 software. There are 27 HTLV-1 subtype references and 21 envelope sequences from the donor (9783) and recipient (1056), as highlighted by the red box. The scale bar indicates the number of nucleotide substitutions per site estimated by general time reversible model with number of bootstrap replications at 1000.

9783_0001 (donor) clustered together with HTLV-I reference strains: Cosmopolitan subtype or Subtype a, Subgroup B (Japanese). To further confirm molecular genetic linkages between HTLV-I viruses from the donor and recipient, an independent polymerase chain reaction amplification reaction strategy was utilized. A DNA template at near endpoint dilution was used to generate an additional eight and 11 *env* gene sequences from the donor (9783) and recipient (1056), respectively. Comparison of these *env* sequences revealed nearly 100% sequence identity, except for a single-base pair (9783-0001*env*03) G-to-A transition. A maximum likelihood tree of the *env* sequences provided further evidence of a molecular genetic linkage between the viruses from these two individuals (Fig. 2).

DISCUSSION

We present a case report wherein evidence indicates that a service member was infected with HTLV-I after transfusion of non–FDA-licensed fresh whole blood. This transfusion-transmitted HTLV case is the first such report in the US military and a rarely reported occurrence in the United States; the last documented transmission in the United States occurred more than a decade ago but not since universal donor screening.[14,23] Transfusion-transmitted HTLV is strongly suggested for several reasons: 1) The recipient's pretransfusion reposed sample indicated no evidence of HTLV-I, whereas the donor's predonation reposed sample demonstrated HTLV-I infection; 2) the timing of the recipient's anti-HTLV status was consistent with a new infection: EIA positivity and indeterminate Western blot profile on Day 19 after transfusion, but full complement of HTLV-I bands by Day 44; 3) both the donor and the recipient were infected with HTLV-I Subtype a, Subgroup B; 4) viral sequences from the donor and recipient were nearly 100% homologous, indicating molecular genetic linkage; 5) the recipient had no major risk factors for HTLV-I; 6) clinical presentation of the recipient 29 days after transfusion fits the 30- to 90-day incubation period reported for HTLV before seroconversion.[24] The lookback investigation identified the donor who was unaware of his HTLV-I infection until notification of his test results in theater.

Prevention of transfusion transmission of HTLV in combat settings is challenging. While a voluntary HTLV screened donor pool is available near combat support hospitals and MTFs, in mass casualty scenarios, where prepositioned FDA-approved blood component supplies have been exhausted, or at smaller outposts, limited screening of emergency blood donors is possible. In the 2010 incident described herein, although 13 screened blood donors of

groups O and A were standing by at the FOB, the IED blast injuries necessitated additional donors since 54 fresh whole blood units were required for the ensuing mass casualties. Emergency donors are called from among volunteers who might be members of the receiving in-theater MTF, the recipient's military unit, or other civilian workers on the base and are referred to as the "walking blood bank." Theater infrastructure precludes donor screening with a FDA-approved screening assay and a Western blot investigational assay. Furthermore, a HTLV rapid kit has not been licensed for point-of-care use and questionnaires used in theater to screen emergency blood donors do not inquire about HTLV-I/II infection.

Since the issuance of the FDA guidance in November 1988 to screen blood donors for HTLV antibodies, transmission of HTLV-I/II has decreased in the United States.[25] A prevalence of 0.11 per 10,000 donations was found among first-time and repeat male US donors at the American Red Cross in 2009.[25] HTLV-I seroprevalence among US blood donors has been associated with older age, female sex, black race, birthplace outside the United States, and positive HCV serology.[26] US military blood donation centers in the continental United States, Hawaii, Germany, and Japan have routinely screened donors for HTLV-I/II since universal donor screening began in the United States (ASBPO). In 2011, of 91,656 donated units, 81 were repeat reactive by EIA of which 1 unit was confirmed positive by Western blot for a seroprevalence rate of 0.001% (0.11 per 10,000 units), which is consistent with that seen in US first-time donors (ASBPO). Donor screening and deferral in the US military for HTLV are based on FDA's 1997 guidance.[27] Donors repeatedly reactive for licensed HTLV screening tests are deferred from donation and placed under surveillance; in the combat theater, any donor testing HLTV positive on an initial screen is deferred indefinitely from theater donations. These donors are indefinitely deferred if repeatedly reactive a second time using screening assays. Although military health care providers may at their discretion request supplemental Western blot confirmatory testing for repeat-reactive donors, this information is not relayed systematically to the deployed environment. Screened donor pools at FOBs would be the best course of action to prevent future cases of transfusion-transmitted HTLV.

While the HTLV seroprevalence among blood donors in the US military and the general US population is low, and HTLV survival in stored red blood cells is limited,[16] the threat of transfusion transmission of HTLV-I/II among fresh whole blood recipients in the combat theater remains. Additionally, the seroprevalence of HTLV-I among US military personnel is unknown. Whereas modification of the predonation screening questionnaire administered to emergency blood donors to include questions regarding HTLV-I/II infection may be helpful, this would not have deferred donation in this instance. However, the utility of modifying the DoD donor screening questionnaire (DD572) to include questions pertaining to risk for HTLV-I/II infection should be considered. Since no licensed confirmatory assay is currently available for blood establishments, the use of HTLV Western blot assays should be employed to confirm any EIA HTLV reactive or repeat-reactive donor samples. Rapid detection of HTLV-I/II for emergency blood donations would be beneficial to prevent transfusion transmissions.

SEQUENCE DATA

Sequences described here were submitted to GenBank and are available under Accession Numbers JX984801-JX984802 and JX885208-JX885228.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

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