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Enhanced Specificity of Truncated Transmembrane Protein for Serologic Confirmation of Human T-Cell Lymphotropic Virus Type 1 (HTLV-1) and HTLV-2 Infections by Western Blot (Immunoblot) Assay Containing Recombinant Envelope Glycoproteins

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Immunoassays based on the highly immunogenic transmembrane protein of human T-cell lymphotropic virus type 1 (HTLV-1) (protein 21e) are capable of detecting antibodies in all individuals infected with HTLV-1 and HTLV-2. However, because of antigenic mimicry with other cellular and viral proteins, such assays also have a large proportion of false-positive reactions. We have recently identified an immunodominant epitope, designated GD21-I located within amino acids 361 to 404 of the transmembrane protein, that appears to eliminate such false positivity. This recombinant GD21-I protein was used in conjunction with additional recombinant HTLV type-specific proteins and a whole virus lysate to develop a modified Western blot (immunoblot) assay (HTLV WB 2.4). The sensitivity and specificity of this assay were evaluated with 352 specimens whose infection status was determined by PCR assay for the presence or absence of HTLV-1/2 proviral sequences. All HTLV-1-positive ($n = 102$) and HTLV-2-positive ($n = 107$) specimens reacted with GD21-I in the HTLV WB 2.4 assay, yielding a test sensitivity of 100%. Furthermore, all specimens derived from individuals infected with different viral subtypes of HTLV-1 (Cosmopolitan, Japanese, and Melanesian) and HTLV-2 (IIa0, a3, a4, IIb1, b4, and b5) reacted with GD21-I in the HTLV WB 2.4 assay. More importantly, HTLV WB 2.4 analysis of 81 PCR-negative specimens, all of which reacted to recombinant protein 21e in the presence or absence of p24 and p19 reactivity in the standard WB assay, showed that only two specimens retained reactivity to GD21-I, yielding an improved test specificity for the transmembrane protein of 97.5%. None of 41 specimens with *gag* reactivity only or 21 HTLV-negative specimens demonstrated reactivity to GD21-I. In an analysis of additional specimens ($n = 169$) from different geographic areas for which PCR results were not available, a substantial increase in the specificity of GD21-I detection was demonstrated, with no effect on the sensitivity of GD21-I detection among specimens from seropositive donors. Thus, the highly sensitive, GD21-I-based HTLV WB 2.4 assay eliminates the majority of false-positive transmembrane results, thereby increasing the specificity for serologic confirmation of HTLV-1 and HTLV-2 infections.

Infections with human T-cell lymphotropic virus type 1 (HTLV-1) and HTLV-2 are widely distributed among recipients of multiple blood transfusions, intravenous drug users, female prostitutes, and patients attending sexually transmitted disease clinics (11). HTLV-1 is the etiologic agent of adult T-cell leukemia and HTLV-1-associated myelopathy/tropical spastic paraparesis. HTLV-2 has not yet been etiologically linked to any particular disease, although rare, isolated cases of hairy cell leukemia and a neurological syndrome resembling HTLV-1-associated myelopathy have recently been reported (10, 11). Infection with HTLV-1 is endemic in certain geographic areas, including Japan, Melanesia, the Caribbean Islands, and parts of Africa. HTLV-2 is now recognized as a highly prevalent infection among intravenous drug users and as an endemic retrovirus of several indigenous Amerindian populations (11).

Both HTLV-1 and HTLV-2 are transmitted in a cell-associated manner, with major routes of transmission being sexual (predominantly male to female), mother to child (breast-fed infants of seropositive mothers have an approximately 25% probability of becoming infected), and parenteral injection of contaminated blood (blood transfusion or intravenous drug use) (11). Because of the risk of blood-borne infections associated with HTLVs, screening of volunteer blood donors was implemented first in Japan in 1986 and then in the United States in 1988 (3, 11). Donor screening is also performed in Canada, Trinidad and Tobago, the French West Indies, France, and The Netherlands.

The ability to make accurate and reliable diagnoses of HTLV-1 and HTLV-2 infections is essential both for ensuring the safety of blood and cellular blood products that are collected for transfusion and for supporting surveillance and prevention programs (3, 18). On the basis of recommendations by a U.S. Public Health Service Working Group and by the World

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Health Organization, the serologic confirmation of HTLV-1 and HTLV-2 infections requires the demonstration of antibodies to *gag* (p24) and *env* (gp46 and/or gp68) proteins (4, 14). Specimens reacting with any of the protein bands, but not satisfying the criteria given above, are designated "seroindeterminate" and in most cases do not represent infection with HTLVs (14, 16). The sensitivities of Western blot (WB; immunoblot) assays for the detection of antibodies to envelope proteins of HTLVs have been substantially enhanced by the addition of the recombinant transmembrane protein r21e, which can detect antibodies in all HTLV-1- and HTLV-2-infected individuals (2, 17). The added sensitivity of r21e-spiked WB assays, however, has resulted in an increased proportion of false-positive results (12, 13). Because of the lack of absolute specificity of this assay, it is recommended that the p21e WB assay not be used as a confirmatory test for the notification of blood donors (12). Therefore, until a more specific p21e antigen is available, it is necessary to confirm p21e reactivities by other serologic tests for *env*, such as the cumbersome radioimmunoprecipitation assays, or by PCR assays (4, 14).

We have recently identified an immunodominant epitope within the transmembrane envelope protein of HTLV-1. This epitope, designated GD21-I, is located within amino acids 361 to 404 of the envelope glycoprotein (GD21-I$^{361–404}$) and reacts specifically with HTLV-1- and HTLV-2-positive sera (9). In this report, we describe the development and performance of a modified WB that contains the truncated transmembrane protein GD21-I in addition to type-specific recombinant proteins from the external glycoproteins of HTLV-1 and HTLV-2. This assay has allowed the elimination of a large proportion of false-positive WB results, while maintaining the 100% sensitivity for detection of all HTLV-1- and HTLV-2-positive specimens from diverse geographic areas where various subtypes of HTLV are found.

MATERIALS AND METHODS

Construction and purification of GD21-I. The recombinant clone expressing GD21-I was synthesized as described previously (9). Briefly, the PCR-amplified HTLV-1 gene fragments from the transmembrane containing appropriate linkers were inserted into a modified version of the vector pGEX-2 (Pharmacia). The vector used in these studies encodes for a truncated version of *Schistosoma japonicum* glutathione *S*-transferase, and GD21-I is expressed as a fusion protein of 15 kDa. Induction of a modified GD21-I clone was achieved by isopropyl-β-D-thiogalcotoside, and GD21-I was extracted from crude cell lysates with 1% (wt/vol) Triton X-100 in phosphate-buffered saline and was solubilized in 6 M urea. The extract containing GD21-I was further purified with an S-Sepharose Fast Flow cation-exchange column (Pharmacia) and a Sephacryl S-100 size exclusion column, and appropriate fractions were pooled.

WB analysis. The modified WB strips containing r21e (representing a conserved epitope between HTLV-1 and HTLV-2), recombinant gp46^1 (rgp46^1; MTA-1$^{162–209}$, representing an HTLV-1-specific epitope), and rgp46^2 (K-55$^{162–205}$, representing an HTLV-2-specific epitope) (HTLV Blot 2.3; Genelabs Diagnostics, Singapore) were run as described previously (1, 20). We developed a similar assay format in which r21e was replaced by the recombinant GD21-I (amino acids 361 to 404; IVKNHKNLLKIAQYAAQNRRGLDLLFWEQGGLCKAL QEQCRFPN) fusion protein. GD21-I, like r21e, was electrophoresed with HTLV-1 viral lysate and was Western blotted onto nitrocellulose membranes. The type-specific envelope proteins rgp46^1 (MTA-1) and rgp46^2 (K-55) were subsequently slot blotted onto these membranes, similar to the HTLV Blot 2.3 format. These membranes incorporating GD21-I were designated modified HTLV WB 2.4, and assays with these proteins were run as described for the WB 2.3 assay.

Interpretation of modified WB results. All specimens reacting with rgp46^1, p24, and GD21-I were classified HTLV-1; specimens reacting with rgp46^2, p24, and GD21-I were classified HTLV-2; and those specimens reacting with p24 and GD21-I were considered HTLV-1/2 (HTLV positive but untypeable). Serum specimens with some virus-specific bands but not fulfilling these banding pattern criteria were considered HTLV indeterminate. Specimens with no virus-specific bands were considered HTLV negative.

Serum specimens. Serum specimens from individuals whose HTLV status was determined by PCR analysis (n = 352) were tested to determine the sensitivity and specificity of the modified HTLV WB 2.4 assay (see Table 1). These included U.S. blood donors (n = 264), as well as individuals from Brazil (n = 26), Mexico (n = 12), Panama (n = 6), Colombia (n = 1), Jamaica (n = 14), Ghana (n = 4), Italy (n = 7), and Melanesia (n = 18). Of these 352 specimens, 102 were HTLV-1 positive, 107 were HTLV-2 positive, 27 were HTLV-1/2 untypeable (seropositive but PCR negative), 95 were HTLV indeterminate (PCR negative), and 21 were HTLV negative. An additional 169 specimens from diverse geographic areas for which PCR results were not available were also included to further test the sensitivity and specificity of the test. Serum specimens (n = 8) from monkeys infected with simian T-lymphotropic virus type I were also included in the study.

PCR. PCR was performed on peripheral blood mononuclear cells. Two gene regions from each patient were amplified with *pol* and *tax*/*rex*-specific primers and probes (16). Viral genotype and subtype analysis was performed as described previously (19, 23–25).

RESULTS

Modified WB spiked with GD21-I is highly sensitive. To determine whether replacing r21e with a truncated transmembrane protein GD21-I would result in an altered sensitivity of HTLV transmembrane detection, well-characterized PCR-confirmed specimens were analyzed by a modified WB assay spiked with GD21-I (HTLV WB 2.4) (Table 1). Of 102 HTLV-1-positive and 107 HTLV-2-positive specimens, all reacted with GD21-I and p24^{gag}. Although GD21-I represents an immunodominant epitope of HTLV-1, this epitope is conserved in HTLV-2 strains, thus allowing the detection of cross-reacting antibodies in all HTLV-2-positive individuals. Thus, the sensitivity of detection of antibodies to GD21-I was 100% in HTLV-positive specimens. Analysis of serum specimens from monkeys infected with simian T-lymphotropic virus demonstrated that all eight specimens reacted with GD21-I (data not shown).

Since both HTLV-1 and HTLV-2 have been shown to exist as different viral subtypes, we next examined serum specimens from populations from diverse geographic areas for which restriction fragment length polymorphism analysis of peripheral blood mononuclear cells had determined the specific viral genotype and subtype (selected specimens in Table 1). Restriction fragment length polymorphism analysis within the long terminal repeat region of HTLV-1 from 39 specimens (16 from the United States, 21 from Brazil, 2 from Melanesia) demonstrated that 25 were of cosmopolitan subtype a (formerly known as subtype II), 4 were Cosmopolitan subtype c, 2 were cosmopolitan subtype e, 6 were the Japanese subtype, and 2 were the Melanesian subtype. Similarly, long terminal repeat-restriction fragment polymorphism analysis of 64 HTLV-2-positive specimens (57 from the United States, 7 from Panama) showed that 40 were subtype a (33 were strain a0, 6 were strain a3, 1 was strain a4) and 24 were subtype b (7 were strain b1, 8 were strain b4, and 9 were strain b5). All 39 HTLV-1- and 64 HTLV-2-positive serum specimens, representing diverse genetic subtypes, reacted with the GD21-I and p24^{gag}, further confirming a sensitivity of 100% for the specimens tested. Results for representative specimens from each category are presented in Fig. 1.

Analysis of the immunoreactivity to type-specific epitopes derived from the external glycoprotein demonstrated that 98 of 102 HTLV-1-positive and 102 of 107 HTLV-2-positive specimens reacted with rgp46^1 and rgp46^2, respectively, in the modified HTLV WB 2.4 assay. Four specimens each of HTLV-1 and HTLV-2 missed by the modified HTLV WB 2.4 assay were the identical specimens that were missed in the previous HTLV WB 2.3 assay and reflect low antibody titers to the rgp46 epitope (data not shown). Thus, the overall ability to simultaneously confirm and type the infection with HTLV-1 and HTLV-2 was unchanged by using the modified recombinant HTLV WB 2.4 assay.

Modified WB spiked with GD21-I results in enhanced specificity. We and others have previously demonstrated that the

TABLE 1. Sensitivity and specificity of modified immunoblots spiked with recombinant envelope glycoproteins of HTLV-1 and HTLV-2 in PCR-confirmed specimens

Specimen[a]	No. of specimens tested	No. of specimens with PCR result of:		No. of specimens reactive by recombinant HTLV WB 2.3 to:			No. of specimens reactive by modified recombinant HTLV WB 2.4 to:		
		Positive	Negative	$r46^1$	$r46^2$	r21e	$r46^1$	$r46^2$	GD21-I
HTLV-1 positive									
Blood donors	50	50	0	49	0	50	49	0	50
Others[b]	52	52	0	49	0	52	49	0	52
HTLV-2 positive									
Blood donors	84	84	0	0	82	84	0	82	84
Others[c]	23	23	0	0	21	23	0	20	23
HTLV-1/2 positive ($r21e^+$, $p24^+$)									
Blood donors	21	0	21	0	0	21	0	0	1
Others[d]	6	0	6	0	0	6	0	0	0
HTLV indeterminate									
$r21e^+$, $p19^+$	14	0	14	0	0	14	0	0	0
$r21e^+$ only	40[e]	0	40	0	0	40	0	0	1
$p24^+$ only	22	0	22	0	0	0	0	0	0
$p19^+$ only	19	0	19	0	0	0	0	0	0
HTLV negative, Blood donors	21	0	0	0	0	0	0	0	0

[a] Superscript pluses indicate positivity.
[b] The specimens included 21 from Brazil, 14 from Jamaica, 13 from Melanesia, and 4 from Ghana. Of the Brazilian specimens, 8 were from patients with HTLV-1-associated myelopathy, and of the Jamaican specimens 5 were from patients with HTLV-1-associated myelopathy and 4 were from patients with adult T-cell leukemia.
[c] The specimens included four from Mexico, two from Brazil, three from Seminole Indians, 6 from Guaymi Indians, 1 from a Wayuu Indian, and seven from Italy.
[d] The specimens include five from Melanesia and one from Brazil.
[e] The specimens included 30 from the United States, 8 from Mexico, and 2 from Brazil.

use of r21e in WB assays results in false-positive results (12, 13), and therefore, an assay based on r21e detection alone could not be used as a sole confirmatory test in blood donor settings (4). The specificity of the modified HTLV WB 2.4 assay was next determined in specimens which were considered HTLV-1/2 positive (p24 and r21e positive) or HTLV indeterminate (any reactivity not fulfilling the criteria of seropositivity) by the standard WB assay. Of the 27 specimens that were considered HTLV-1/2-positive on the basis of the standard algorithm but that were confirmed to be negative for HTLV proviral sequences by PCR, only 1 specimen reacted with GD21-I. The remaining 26 specimens did not demonstrate any reactivity to GD21-I. Similarly, of the 54 HTLV-indeterminate specimens (14 with r21e-positive and p19-positive reactivities and 40 with r21e-positive reactivities only) that were shown to be PCR negative, only 1 retained GD21-I reactivity (Table 1). Thus, of the 81 specimens that had false-positive reactivity to r21e, only 2 were reactive to GD21-I, resulting in an improved test specificity of 97.5% for HTLV envelope detection.

We next examined whether the addition of GD21-I would result in any false-positive results in seroindeterminate or seronegative specimens that had previously not shown r21e reactivity. None of the specimens with p24-positive reactivity ($n = 22$) or p19-positive reactivity ($n = 19$) or seronegative specimens ($n = 21$), all of which were confirmed to be negative by PCR, demonstrated any reactivity to GD21-I (Table 1). Thus, replacement of GD21-I did not result in the addition of any false-positive results.

Test performance with serum specimens from diverse populations. We next examined the performance of the modified HTLV 2.4 WB with serum specimens from populations from diverse geographic areas (Table 2). Among 38 HTLV-1-positive specimens, as confirmed and typed on the basis of HTLV WB 2.3 results ($r46^1$ positive, p24 positive, and r21 positive), all retained reactivity to $r46^1$, p24, and GD21-I. Similarly, of the 53 HTLV-2-positive specimens ($r46^2$ positive, p24 positive, and r21e positive), 50 reacted with $r46^2$ and all but 1 specimen reacted with GD21-I. The one specimen negative for GD21-I but positive for $rgp46^2$ and p24 was from an individual from Ghana and, so far, has been the only specimen missed by GD21-I. Thus, the sensitivity of GD21 was 98.88% in non-PCR-confirmed HTLV-positive specimens.

Analysis of 44 HTLV-1/2-positive untypeable specimens (p24 positive and r21 positive by the modified HTLV WB 2.4 assay revealed GD21-I reactivity in 9 specimens, 7 of which also had p24 reactivity. In accordance with our previous studies (2, 12, 13), specimens with p24 and GD21-I reactivities may represent true HTLV infection, although we do not have PCR data to confirm such a finding. Of the 10 specimens positive for r21e and p19 reactivity, 8 were negative for GD21-I reactivity. Similarly, of the 24 specimens positive only for r21e reactivity, 22 specimens were negative for GD21-I reactivity. Thus, replacement of r21e with GD21-I resulted in a markedly enhanced specificity for the detection of HTLV *env* reactivity.

Comparison of GD21-I sequence. The sequence of GD21-I was next examined by using published data from GenBank. The alignments of the GD21-I sequences of representative isolates from diverse geographic areas and with diverse genotypes are given in Fig. 2. All strains examined demonstrated 2 amino acid substitutions of an R for a C residue at position 401 ($R^{401}\rightarrow C$) and $P^{403}\rightarrow L$, and these substitutions represent sequence errors in the original isolate (21). Among the HTLV-1 isolates, the remaining sequences were conserved except for $Y^{374}\rightarrow R$ in a Solomon Island strain. Similarly, all simian T-lymphotropic virus type I sequences were also conserved in the GD21-I region, although some strains had premature stop

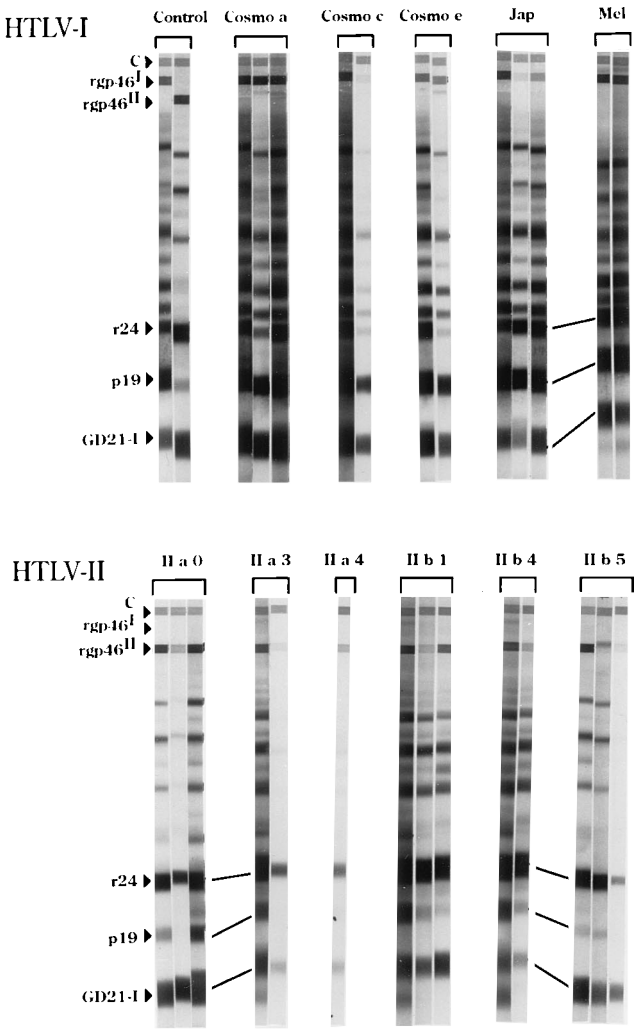

FIG. 1. Modified WB spiked with recombinant external glycoproteins representing immunodominant epitopes of HTLV-1 (rgp46^{1}) and HTLV-2 (rgp46^{2}) and truncated transmembrane protein GD21-I. An anti-immunoglobulin G control band at the top of the WB strip simply detects the presence of serum immunoglobulin in the well. Representative HTLV-1- and HTLV-2-positive specimens are shown where the viral genotype was determined by restriction fragment length analysis of peripheral blood lymphocytes. Of 39 HTLV-1-positive specimens, 25 were of the cosmopolitan a subtype (formerly known as subtype II), 4 were of the cosmopolitan c subtype, 2 were of the cosmopolitan e subtype, 6 were of the Japanese (Jap) subtype (formerly known as subtype III), and 2 were of the Melanesian (Mel) subtype. Of 64 HTLV-2-positive specimens, 40 were of subtype a (33 were a0, 6 were a3, and 1 was a4) and 24 were of subtype b (7 were b1, 8 were b4, and 9 were b5).

codons. In contrast, all HTLV-2 sequences had five additional substitutions ($K^{366}\rightarrow Q$, $L^{368}\rightarrow I$, $K^{370}\rightarrow R$, $I^{371}\rightarrow V$, and $L^{396}\rightarrow I$) that were conserved among all HTLV-2 isolates tested. None of these amino acid positions appear to represent crucial amino acids required for antibody binding, since all serum specimens from individuals infected with HTLV-1 or HTLV-2 reacted with the GD21-I protein derived from the prototypic HTLV-1 ATK strain.

DISCUSSION

Despite the highly immunogenic nature of the gp46 and gp21 envelope glycoproteins of HTLV-1 and HTLV-2 (14, 17), most serologic assays containing the purified viral antigens preferentially detect *gag* proteins. Incorporation of selected highly immunoreactive epitopes from the envelope glycoproteins has resulted in increased sensitivities for envelope detection (1, 12, 14, 17). While such modified WB assays containing the recombinant proteins derived from the external glycoprotein, representing type-specific epitopes of HTLV-1 (rgp46^{1}) and HTLV-2 (rgp46^{2}), and the transmembrane protein, representing a type-common epitope (r21e), have allowed simultaneous confirmation and typing of HTLV-1 and HTLV-2 infections (1, 20), the issue of specificity still remains. We have recently mapped a linear immunodominant epitope in the transmembrane protein of HTLV-1 which appears to be highly specific for HTLV infection (9). A recombinant protein, termed GD21-I$^{361–404}$, was synthesized and used in place of r21e for the detection of antibodies to the transmembrane proteins. However, it was important to determine that such a truncated protein would not compromise the sensitivity of envelope detection. The use of the modified WB assay allowed detection of antibodies in all (209 of 209; 100%) HTLV-1- and HTLV-2-positive specimens, maintaining the sensitivity of envelope detection with the GD21-I transmembrane epitope. The region represented by GD21-I not only appears to be highly immunogenic but also contains functionally important regions. Recent computer modeling analysis has shown that this immunodominant region can form a "knob"-like protrusion that can accommodate a "pocket"-like structure from the

TABLE 2. Analysis of modified immunoblots spiked with recombinant envelope glycoproteins of HTLV-1 and HTLV-2 in specimens from blood donors and diverse geographic areas

Specimen and geographic location (no. of specimens)	HTLV WB 2.3 results[a]	No. of specimens with reactivity to the following by modified recombinant HTLV WB 2.4:		
		r46^{1}	r46^{2}	GD21-I
HTLV-1 positive	r46^{1+}, p24^{+}, r21e^{+}			
United States (12)		12	0	12
Peru (10)		10	0	10
Ghana (8)		8	0	8
Japan (8)		8	0	8
HTLV-2 positive	r46^{2+}, p24^{+}, r21e^{+}			
United States (20)		0	18	20
Brazil (Kayapo) (10)		0	10	10
Panama (Guaymi) (15)		1	15	15
Tanzania (4)		0	4	4
Ghana (4)		0	3	3
HTLV-1/2	p24^{+}, r21e^{+}			
United States (14)		0	0	2[b]
Brazil (Kayapo) (2)		0	0	1[b]
Tanzania (12)		0	0	1
Ghana (16)		0	0	5[c]
HTLV indeterminate	r21e^{+}, p19^{+}			
United States (9)		0	0	2
Tanzania (1)		0	0	0
HTLV indeterminate	r21e^{+} only			
United States (10)		0	0	1
Brazil (Kayapo) (4)		0	0	0
Tanzania (5)		0	0	1
Ghana (5)		0	0	0

[a] Superscript pluses indicate positivity.
[b] In addition to GD21-I reactivity, reactivity to p24 was positive as well.
[c] Four of the five specimens had both GD21-I and p24 reactivities.

Strains	Location	Sequence				
HTLV-I:						
ATK	Japan	IVKNHKNLLK	IAQYAAQNRR	GLDLLFWEQG	GLCKALQEQC	RFPN
H15	US	**********	**********	**********	**********	C*L*
H5 (HAM)	US	**********	**********	**********	**********	C*L*
SP (ATL)	US	**********	**********	**********	**********	C*L*
HS 35	Carribean	**********	**********	**********	**********	C*L*
EL	Africa	**********	**********	**********	**********	C*L*
Z17	Africa	**********	**********	**********	**********	C*L*
Mel 5	Solomon Island	**********	***R******	**********	**********	C*L*
Bel	Papua new Guinea	**********	**********	**********	**********	C*L*
MSHR-1	Australia	**********	**********	**********	**********	C*L*
STLV-I:	Consensus	**********	**********	**********	**********	C*L*
HTLV-II:						
MoT	US	*****Q*I*R	V*********	**********	*****I****	C*L*
NRA	US	*****Q*I*R	V*********	**********	*****I****	C*L*
G12	Panama	*****Q*I*R	V*********	**********	*****I****	C*L*
IIa	US	*****Q*I*R	V*********	**********	*****I****	C*L*
IIb	US	*****Q*I*R	V*********	**********	*****I****	C*L*
FLW	US	*****Q*I*R	V*********	**********	*****I**H*	C*L*

FIG. 2. Comparison of the GD21-I sequence of HTLV-1 ATK with the GD21-I sequences of other known strains of HTLV-1, simian T-lymphotropic virus type I (STLV-I), and HTLV-2.

external glycoprotein and that such linkage plays an important role in virus-cell membrane fusion (22).

The genomic diversity leading to antigenic variation within the immunodominant epitope could affect the binding of serum antibodies and, hence, could adversely affect the sensitivity of the assay (2). We therefore examined serum specimens representing various recently identified viral subtypes of HTLV-1 and HTLV-2 (24, 25). The reactivities of all specimens with different viral subtypes strongly suggest a lack of antigenic variation in the epitope identified by GD21-I. Indeed, a comparison of previously published HTLV-1/2 and simian T-lymphotropic virus type I sequences has also demonstrated a relative conservation of this epitope among all of the strains studied to date. The sequence variation observed in the HTLV-2 sequences appeared to have a minimal effect on antibody binding, since all HTLV-2-positive sera (with the exception of one serum specimen from an individual in Ghana for which no PCR results were available) have shown reactivity to GD21-I. Furthermore, our ability to detect antibody in serum specimens from individuals infected with known viral strains suggests that this modified WB assay would be applicable for seroepidemiologic studies in any part of the world.

Immunoreactivity to the type-specific epitopes $rgp46^1$ and $rgp46^2$ was observed in 98 of 102 HTLV-1-positive specimens and 102 of 107 HTLV-2-positive specimens. These results are in general agreement with those of previous studies, which have demonstrated that the sensitivity of the test can vary depending on the study population (1, 20). Such specimens with reactivity to *gag* p24 and rgp21, but no reactivity to either $rgp46^1$ or $rgp46^2$, have been referred to as HTLV positive but untypeable in the previous WB assay format. The fact that such untypeable specimens could represent true HTLV-1 or HTLV-2 infection has been demonstrated by amplification of proviral sequences from peripheral blood mononuclear cells (6, 26). Our group has further demonstrated that the lack of reactivity to $rgp46^2$ in specimens from Seminole Indians was not due to sequence variation but, rather, reflected lower titers of HTLV-2 antibodies (15). A larger study with HTLV enzyme immunoassay-negative specimens (PCR positive for HTLV-2) has further revealed that specimens containing low titers of antibodies to HTLV-1/2 also can be missed by this test (5). We therefore strongly recommend that all diagnostic specimens with reactivity to p24 and GD21-I continue to be referred to as HTLV-positive untypeable, requiring further testing by more sensitive assays.

Of greater importance is the finding that the use of GD21-I resulted in a substantially increased specificity for envelope detection. Seventy-nine of 81 PCR-negative specimens that had shown r21e-positive reactivities, with and without *gag* reactivities, did not react with the GD21-I protein, resulting in an improved test specificity of 97.5%. This finding has important implications for blood donor screening assays, in which the use of conventional r21e results in a greater number of false-positive results. Such false-positive results not only advertently increase the costs of the blood donor screening but also affect donor counseling (4). The replacement of the conventional r21e protein with GD21-I in screening immunoassays will help to further improve the specificities of these tests. The specificity of the assay was further illustrated by similar analyses of seroindeterminate specimens and seronegative specimens that had no false-positive results among all samples tested.

The extremely high sensitivity and specificity of the modified HTLV WB 2.4 assay for serologic confirmation and differentiation of HTLV-1/2 led us to evaluate its performance in specimens from diverse geographic locations. Comparison of the results obtained by the modified HTLV WB 2.4 assay with those of the HTLV WB 2.3 assay demonstrated that 90 of 91 specimens reacted with GD21-I, and all 38 HTLV-1- and 50 of 53 HTLV-2-positive specimens were accurately typed. Of the 44 HTLV-1/2-positive specimens, 7 retained their reactivities to GD21-I and may represent true HTLV infection. Alternatively, these specimens may represent infection with a recently identified divergent primate T-lymphotropic virus (7, 8). Indeed, serum specimens from primates infected with primate T-lymphotropic virus did show immunoreactivity to GD21-I (7a). Of the remaining indeterminate specimens, only 4 of 34 specimens reacted with GD21-I. Thus, the use of GD21-I resulted in significantly enhanced diagnostic specificity.

In conclusion, we have demonstrated that the modified HTLV WB 2.4 assay containing the recombinant rgp46 proteins from external glycoproteins of HTLV-1/2 and a conserved epitope within the transmembrane protein of HTLVs

results in an assay which provides serologic confirmation and differentiation of HTLV-1 and HTLV-2 infection in one step and eliminates the majority of false-positive test results observed with WB assays containing the entire p21e protein.

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