Serological Confirmation of Human T-Lymphotropic Virus Type I Infection in Healthy Blood and Plasma Donors


We wished to develop criteria for serological confirmation of human T-lymphotropic virus type I (HTLV-I) infection in healthy donors. Selected serum or plasma samples reactive by HTLV-I enzyme immunoassorbent assay or gel-agglutination assays with at least one viral-specific band on Western immunoblot (WIB) were tested in six laboratories by four WIBs and four radioimmunoprecipitation assays (RIPAs) for antibodies to HTLV-I encoded proteins by gag (p19 and p24), env (gp46 and/or gp61), and tax (p40?) genes. One hundred forty-two donor sera were obtained from 38 Japanese, 69 American, and 35 Caribbean blood or plasma donors. Among these samples, WIB assays appeared more sensitive to p24 antibodies, whereas RIPAs were significantly more sensitive to gp61 antibodies. All sera (137) with gp61 antibodies had p24 antibodies. Of the 137 sera positive for p24 and gp61 antibodies, 19 antibodies were detected in 129 sera, and 40? antibodies were detected in 108. In sera with p19 antibodies and antibodies to env- or tax-encoded proteins, p24 antibodies were always present. Antibodies to p40? were not found in the absence of gp61 antibodies. Virological evidence of infection was found in seven American donors by lymphocyte coculture (one HTLV-I, one HTLV-II) or by polymerase chain reaction (three HTLV-I, two HTLV-II). Sera from all seven donors showed p24 and gp46 and/or gp61 antibodies. We suggest that seroreactivity to both p24 and gp46 and/or gp61 by WIB or RIPA or both are suitable criteria to confirm but not to distinguish HTLV-I and HTLV-II infections.

HUMAN T-CELL lym photropic virus type I (HTLV-I), the first known human retrovirus, is associated with an aggressive lymphoma, adult T-cell leukemia/lymphoma (ATL), and a degenerative demyelinating neurological disease called tropical spastic paraparesis (TSP) in the Caribbean and HTLV-I-associated myelopathy (HAM) in Japan. Development of ATL may require years to decades after infection with HTLV-I. Preliminary data from Japanese patients with HAM and a history of blood transfusion suggest a mean interval of 4 years between transfusion and development of neurologic symptoms (Osame M., unpublished observations, November, 1988). HTLV-I endemic areas have been identified in southern Japan, the Caribbean, and parts of Africa. HTLV-II is a closely related retrovirus which shares considerable genomic homology with HTLV-I and is believed to be indistinguishable from HTLV-I with current serologic techniques. Little is known about the epidemiology and clinical outcomes of HTLV-II infections except for their apparent high frequency among drug abusers and occasional isolation from patients with T-hairy cell leukemia and some B-cell lymphoid malignancies. In the absence of virus isolation or characterization of gene sequences by polymerase chain reaction (PCR), the virus type of seropositives in the United States is best classified as HTLV-I/II. Antibodies to HTLV-I/II were recently detected in 10 (0.025%) of 39,898 random blood donors in eight US cities, and HTLV-I/II seroconversion in multiply transfused US hospital patients has been documented.

On November 29, 1988, enzyme immunoassay (EIA) screening tests designed to detect antibodies to HTLV-I were licensed by the US Food and Drug Administration (FDA). Implementation of HTLV-I EIA screening of donated whole blood and cellular components is currently underway in many blood establishments nationwide. An estimated 13,600 US blood donors may test positive in the first year of HTLV-I EIA screening. (The number of blood donors testing positive [repeatably reactive] in the first year of HTLV-I EIA screening was estimated as the product of 8 x 10^6 blood donors times 0.17%, repeatably reactive rate). Although blood from these donors will not be used for transfusion, less than one of six is likely to be confirmed positive with additional, more specific serological tests. Thus, criteria used to confirm HTLV-I/II seropositivity will affect notification and counseling strategies used for thousands of healthy donors.

As with screening tests for human immunodeficiency virus-1 (HIV-1), a distantly related but not serologically cross-reactive retrovirus, current approaches to confirmation of HTLV-I/II seropositivity rely on additional, more specific tests such as Western immunoblot (WIB) and radioimmunoprecipitation assay (RIPA). Both WIB and RIPA enable separate demonstration of antibodies to HTLV-I proteins encoded by viral genes. However, there are important differences between WIB and RIPA in terms of viral antigen...
preparation and the physicochemical milieu of antigen–
antibody interaction. Viral antigen commonly used in WIB
formats is derived from density-gradient purified virions
rather than extracts of infected cells. Viral proteins in WIB
formats therefore consist predominantly of virus particle-
associated structural proteins (gag-encoded proteins p19,
p24, and their precursor p55 and env-encoded proteins
gp14, gp46, and their precursor gp61/68). After boiling,
sodium dodecyl sulfate (SDS) treatment, reduction and
electrophoretic transfer, all of which may modify the anti-
gens, the antigen–antibody interaction occurs on a two-
dimensional nitrocellulose membrane. In contrast, viral anti-
gens commonly used in RIPA formats are extracts from
cronically-infected cells and include structural as well as
nonstructural (tax-encoded p40) regulatory proteins at vari-
stages of posttranslational modification. The antigen–
antibody interaction in a RIPA occurs in a solution (three-
dimensional) of whole cellular lysate under nondenaturing
conditions.

Investigators have used various confirmatory criteria and
differing combinations of WIB and RIPA to identify asym-
ptomatic HTLV-I/II seropositive persons. Indeed, valida-
tion of serological confirmatory criteria in this setting is
complicated by the inability of current assays to distinguish
antibodies to HTLV-I from antibodies to HTLV-II, a lack of
common positive reference sera, and incomplete knowl-
dge of variability in the pattern of HTLV-I–specific antibodies or
HTLV-II–specific antibodies among persons after infection.
An additional complication is insufficient data regarding the
comparative performance of WIB and RIPA in detecting
variations in the pattern of antibodies to specific HTLV-I/II
antigens, particularly when different cell lines are used for
antigen production.

Using a variety of assays in six different laboratories, we
undertook to characterize the pattern of HTLV-I/II–
specific antibodies in healthy blood and plasma donors. Our
investigation represents a consensus approach to confirm-
tion of HTLV-I/II seropositivity and serves as the basis for
current Public Health Service and blood bank guidelines for
confirming positive results of antibody screening. The results
obtained provide a rationale for use of antibodies to certain
gag and env gene products in confirmatory criteria as well as
for assessing the suitability of WIB and RIPA for use in
confirmatory strategies.

MATERIALS AND METHODS

Positive and negative reference sera. The Center for Biologics
Evaluation and Research (CBER), US FDA distributed to each of
six collaborating laboratories and one in-house laboratory (Table 1)
eight uncoded sera (positive reference sera) from US patients
clinically diagnosed as having ATL (three cases) or TSP (five cases)
(B.J. Poiesz, unpublished observations, December, 1987). All cases
had evidence of HTLV-I–specific sequences in peripheral lympho-
cyte DNA extracts amplified by PCR, including six patients with
HTLV-I–specific sequences found by Southern blotting of unampli-
fied lymphocyte DNA extracts. Each laboratory chose to study
antibodies to one or several of the gag-encoded core proteins p19,
p24, and precursor p55, the env-encoded glycoproteins gp46 or gp61,
gp63, gp65, or gp68 (hereafter called gp61) or the tax-encoded
transcriptional activator p40 (Table 1). These particular viral
proteins were selected for study because, with the exception of gp68,
each represents the product of only one HTLV-I gene. Uncoded
sera from 20 normal US blood donors (negative reference sera) were
also provided. Normal donors included 15 males, mean age 24 ± 4
years (SD) and two females (aged 20 and 34 years) who donated
blood in Maryland and were found nonreactive for HIV-1 antibodies
(HIV-I EIA, Abbott Laboratories, N. Chicago, IL), hepatitis B core
antibodies (Corzyme, Abbott) and hepatitis B surface antigen
(Auszyme, Abbott). For all nine positive reference sera, each
laboratory found strong seroreactivity to all viral proteins listed for
its assays in Table 1. No detectable seroreactivity was found by any
laboratory for any of the 20 negative reference sera. To investigate
nonspecific interference, 37 sera representing a variety of serological
conditions that might interfere with detection of HTLV-I/II–
specific antibodies were distributed under code. These sera were
obtained from Boston Biomedica, Medford, MA, and included two
era from multiply transfused hospital patients, one grossly lipemic
serum, one serum with anti-HLA DR antibodies, two sera with
antibodies to HIV, three sera reactive for rheumatoid factor, and
sera with immunoreactivity to each of the following infectious agents
(three sera per agent): Toxoplasma gondii, cytomegalovirus
(CMV), rubella, Herpes simplex, and Epstein-Barr virus (EBV)
early antigen. In addition, three sera were positive for hepatitis B
surface antigen, one serum was positive for hepatitis C core antibod-
ies, three sera were reactive by the heterophile test, three sera were
reactive with antinuclear antibodies, and three sera were reactive by
the rapid plasma reagin test. No seroreactivity was found by any
laboratory (laboratory 3 performed WIB but not RIPA on these
samples) in any of the 37 samples. Thus, the performance of each
assay was similar among seven laboratories, and common positive
and negative reference sera were available as standards for assessing
reactivities of unknown test sera.

Donor samples. Serum or plasma samples from 153 HTLV-I
seroreactive blood and plasma donors from Japan, the United States,
and the Caribbean basin were obtained by CBER from blood
establishments and manufacturers (Table 2). Samples were
accepted for study if they met the following criteria: (a) volume ≥5
mL, (b) repeatably reactive by HTLV-I EIA or gel-agglutination
screening tests, (c) seroreactive to at least one HTLV-I viral protein
on WIB or reactive on HTLV-I indirect immunofluorescent assay
(IFA), and (d) remained frozen at less than –20°C since blood
drawing. Of 153 samples received in an 8-month period, 142 (donor
samples) met the above criteria for study and represented similarly
significant numbers of Japanese (38), American (69) and Caribbean
(35) donors. Of 142 donor samples, 37 were serum and the remain-
der were plasma. Identical aliquots of each donor sample were
simultaneously distributed under code to seven collaborating labora-
tories for investigation by serological assay methods as listed in
Table 1. All donor samples were found nonreactive by HIV-I EIA
(Originon-Technika, Durham, NC) except two plasma samples from
Martinique, both of which showed no HIV-specific bands by HIV-I
WIB (Biotech/DuPont HIV Western Blot Kit, DuPont, Wilming-
ton, DE).

Statistical methods. Associations between discrete variables
were evaluated for significance by Fisher’s exact test (corrected for a
two-tailed distribution). Differences in the performance of WIB and
RIPA for detection of p24 and gp61 antibodies were evaluated by
McNemar’s paired chi-square test.

RESULTS

Comparison of WIB and RIPA for detection of p24
antibodies. Seroreactivity to the viral core p24 antigen was
assessed by four WIB and four RIPAs performed in different
laboratories (Table 3). If two or more WIB assays were
positive, the sample was designated consensus positive (ie
Table 1. Serologic Methods of Collaborating Laboratories

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Assay Type*</th>
<th>Antigen Source</th>
<th>Method (Reference No.)</th>
<th>Seroreactivities Evaluated†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Retrovirus Diseases Branch</td>
<td>WIB</td>
<td>MT-2 cells</td>
<td>12</td>
<td>gag-p19, p24, p55 env-gp61/68</td>
</tr>
<tr>
<td>Centers for Disease Control</td>
<td>RIPA</td>
<td>MT-2 cells</td>
<td></td>
<td>gag-p24, p55 env-gp61/68</td>
</tr>
<tr>
<td>Atlanta, GA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Laboratory of Tumor Cell Biology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>National Cancer Institute</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bethesda, MD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competitive EIA</td>
<td></td>
<td></td>
<td>13, 14</td>
<td>Positive/indeterminate/negative</td>
</tr>
<tr>
<td>WIB</td>
<td></td>
<td>HUT 102 cells</td>
<td>15</td>
<td>gag-p19, p24, p55 env-gp46</td>
</tr>
<tr>
<td>HUT 102 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Department of Cancer Biology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvard School of Public Health</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boston, MA</td>
<td></td>
<td>MT-2 cells</td>
<td>16</td>
<td>gag-p19, p24, p55 env-gp63/65</td>
</tr>
<tr>
<td>WIB</td>
<td></td>
<td></td>
<td></td>
<td>env-gp61/68</td>
</tr>
<tr>
<td>MJ cells</td>
<td>RIPA</td>
<td></td>
<td>17</td>
<td>env-gp61/68</td>
</tr>
<tr>
<td>Rockville, MD</td>
<td></td>
<td>HUT 102 cells</td>
<td></td>
<td>gag-p24</td>
</tr>
<tr>
<td>5. Division of Hematology/Oncology</td>
<td></td>
<td></td>
<td>19, 20</td>
<td>gag-p24</td>
</tr>
<tr>
<td>UCLA School of Medicine</td>
<td></td>
<td>SLB-1 cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Los Angeles, CA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Department of Pediatrics/Immunology</td>
<td></td>
<td></td>
<td>21</td>
<td>Positive, indeterminate, negative</td>
</tr>
<tr>
<td>University of Miami</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miami, FL</td>
<td></td>
<td>HUT 102 cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Laboratory of Retrovirology</td>
<td></td>
<td>MT-2 cells</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>CBER, FDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bethesda, MD</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*Laboratories 4 and 6 performed this assay by measuring counts from immunoprecipitable 125I-p24 in a γ-counter, whereas laboratories 1, 2, and 5 subjected precipitable proteins to gel electrophoresis and autoradiography.
†Each laboratory determined which seroreactivities to report based on previous experience or validation experiments with monoclonal antibodies or both.

Of 142 donor samples tested, 139 were consensus positive for p24 antibodies by RIPA. Of 142 donor samples tested, 139 were consensus positive for p24 antibodies by WIB and included all 134 that were consensus positive by RIPA. In terms of its performance as a group of assays, WIB appeared to be more sensitive for p24 antibodies than RIPA, but this difference did not reach statistical significance (P = 0.074). We considered all 139 donor samples consensus positive by WIB to have antibodies to p24.

Table 2. Donor Sera Characteristics

<table>
<thead>
<tr>
<th>Nationality</th>
<th>Donation Site</th>
<th>Donor Type</th>
<th>N</th>
<th>n/Sex/Mean Age (yr ± SD)</th>
<th>Initial Screening Test (Manufacturer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese</td>
<td>Japan</td>
<td>B</td>
<td>29*</td>
<td>21/M/38 ± 11</td>
<td>HTLV-I gel-agglutination (Fujirebio)†</td>
</tr>
<tr>
<td>Japanese</td>
<td>Okinawa</td>
<td>B</td>
<td>9</td>
<td>5/M/48 ± 11</td>
<td>HTLV-I EIA (DuPont)</td>
</tr>
<tr>
<td>Japanese-American</td>
<td>Hawaii</td>
<td>B</td>
<td>34</td>
<td>28/M/49 ± 11</td>
<td>HTLV-I EIA (DuPont)</td>
</tr>
<tr>
<td>American</td>
<td>United States</td>
<td>B</td>
<td>22</td>
<td>14/M/40 ± 15</td>
<td>HTLV-I EIA (Abbott, 4; DuPont, 15; CPI, 3)</td>
</tr>
<tr>
<td>American</td>
<td>United States</td>
<td>P</td>
<td>13</td>
<td>9/M/39 ± 10</td>
<td>HTLV-I EIA (Abbott, 13)</td>
</tr>
<tr>
<td>Jamaican</td>
<td>Jamaica</td>
<td>B</td>
<td>11</td>
<td>8/M/31 ± 4</td>
<td>HTLV-I EIA (DuPont)</td>
</tr>
<tr>
<td>Martinique</td>
<td>Martinique</td>
<td>B</td>
<td>24</td>
<td>12/M/49 ± 8</td>
<td>HTLV-I EIA (Abbott)</td>
</tr>
</tbody>
</table>

Abbreviations: B, blood donor; P, plasma donor.
*Age and sex data could not be obtained for seven Japanese blood donors.
†Fujirebio is located in Tokyo, Japan.
Comparison of WIB and RIPA for detection of env-encoded proteins gp46 and/or gp61. Seroreactivity to env-encoded proteins was assessed in different laboratories by three WIB assays for envelope gp46 and/or precursor gp61 and by three RIPAs for gp61 (Table 4). If two or more WIB assays were positive (i.e., greater than 50% concordance), the sample was designated consensus positive by WIB. Likewise, if two or more RIPAs showed evidence of gp61 antibodies (i.e., greater than 50% concordance), the sample was consensus positive by RIPA. Of 142 donor samples tested, 137 were consensus positive for gp61 antibodies by RIPA and included all 125 samples that were consensus positive by WIB for either gp46 or gp61. As a group, RIPAs detected antibodies to env-encoded proteins in significantly more donor samples than did WIB (P = .0015). We considered all 137 donor samples consensus positive by RIPA to have antibodies to gp61.

Correlation of p24 and gp61 antibodies. It is important that the 137 donor samples consensus positive by RIPA for gp61 antibodies were all consensus positive by WIB for p24 antibodies. These 137 samples were also repeatably reactive when blindly tested by each of the FDA-licensed HTLV-I EIA tests (Abbott, Dupont, and Cellular Products, Buffalo, NY). When tested by HTLV-I IFA (Table 1), 131 of the 137 samples were positive and six were indeterminate owing to weak reactivity.

Five samples, all from US donors, were not seroreactive by consensus to gp61 (Table 5) and showed predominantly p19 seroreactivity. Antibodies to p19 and p24 were compared on four WIB in different laboratories (Table 6). One hundred thirty-one donor samples were seroreactive by consensus (i.e., two or more WIB assays positive for each p19 and p24) to both p19 and p24; 129 of these were also seroreactive by consensus to gp61. Among the remaining 11 donor samples, eight were seroreactive by consensus to p24 and not to p19; there was a significant association (P = .007) between the presence of antibodies to p24 and antibodies to gp61 among these 11 samples. In contrast, none of three donor samples with p19 antibodies in the absence of p24 antibodies was seroreactive to env-encoded proteins. Antibodies to the tax-encoded protein p40* were detected by RIPA in 108 donor samples, of which 108 were seroreactive to both p24 and gp61.

Virological studies of peripheral blood lymphocytes. Follow-up studies of peripheral blood lymphocytes (PBLs) were performed for seven US blood donors who were positive for HTLV-I/II antibodies by EIA screening tests and who were available for further studies (Table 7). Antibodies to gag and env were identified by consensus in all seven cases. Lymphocyte cocultures in two donors were positive by endonuclease restriction mapping for HTLV-I in one case and human T-lymphotropic virus type II (HTLV-II) in one case. Lymphocyte DNA extracts from five other donors were positive for HTLV-I-specific sequences but negative for HTLV-II-specific sequences by PCR in three cases and positive for HTLV-II-specific sequences but negative for HTLV-I-specific sequences by PCR in two cases. The HTLV-I/II-specific antibody pattern of the four HTLV-I-infected donors was not distinguishable from that of the three HTLV-II-infected donors by WIB or RIPA.

**DISCUSSION**

Serological diagnosis of asymptomatic HTLV-I/II infection among persons living in the United States presents several challenges. The epidemiology of HTLV-I and HTLV-II in the general US population has not been evaluated systematically. Retroviral infection involves proviral
CONFIRMATION OF HTLV-I/II SEROPOSITIVITY

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Table 7. HTLV-I/II-Specific Antibodies Detected by WIB and RIPA in Blood Donor Samples

<table>
<thead>
<tr>
<th>Viral proteins</th>
<th>Antibodies Detected by WIB</th>
<th>Antibodies Detected by RIPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p19</td>
<td>p24</td>
</tr>
<tr>
<td>Total number of assays used</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Virus identified (technique used)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTLV-I (Lympocyte coculture†)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>HTLV-I (PCR†)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>HTLV-I (PCR‡)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>HTLV-I (PCR‡)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>HTLV-II (Lympocyte coculture†)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>HTLV-II (PCR#)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>HTLV-II (PCR#)</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

*Number of assays positive.
†Cocultivated lymphocytes were extracted, and whole DNA was digested with the following restriction endonucleases: XbaI, EcoRI, and BamHI.
Southern blots of digested DNA were probed with an AccI to HindIII fragment from the 3' end of the HTLV-I genome (bases 7346-8534) and a BamHI to BamHI fragment from the 5' end of the HTLV-II genome (bases 361-5090) (Slamon D, unpublished observations, February, 1989).
‡Whole DNA extracts from PBLs were subjected to PCR with the following primer pairs: SK43, 44, a tax gene sequence common to HTLV-I and HTLV-II; SK54, 55, a pol gene sequence specific for HTLV-I; and SK58, 59, a pol gene sequence specific for HTLV-II.10
#Foung S, personal communication, January, 1989.

integration; therefore, diagnostic virological methods such as lymphocyte coculture or PCR should allow direct identification of viral sequences in cellular DNA of a person infected many years before. However, these methods are not yet available for widespread routine use. Thus, serological tests will be used both to screen for and confirm most cases of HTLV-1/II infection in the immediate future. To examine serological confirmatory strategies we studied a cross-section of serum and plasma samples from two foreign HTLV-I endemic areas and the United States that were repeatedly reactive on HTLV-I screening tests and had evidence of viral-specific antibodies on WIB or IFA. The presence of antibodies to specific viral proteins was defined by a consensus of seroreactivities in both WIB and RIPA formats.

Our data confirm the observations of other investigators9 that the presence of antibodies to p24 and env-encoded proteins are highly correlated in sera from healthy donors. Antibodies to p19 usually accompanied p24 and gp61 antibodies. Antibodies to gp46 and/or gp61 were detected in 9% fewer donor samples by WIB assays than by RIPAs (P = .0015). The capability of RIPAs to detect antibodies to env-encoded proteins not apparent on WIB has been confirmed by other investigators9 for small numbers of samples; the physicochemical basis for this remains unclear. Optimal antibody binding to major epitopes of gp61 may require the conformational presentation of antigens as in the milieu of solubilized cellular lysate used in a RIPA. Alternatively, ultracentrifugal purification and other preparative processes commonly used in WIB assays may adversely affect gp61 epitope presentation. In contrast, WIB appears to be the more sensitive format for p24 antibody detection, although this trend did not reach statistical significance (P = .074).

Several serological confirmatory criteria for HTLV-I were proposed recently. Agius et al10 required antibodies to both p19 and p24 by WIB whereas Fang et al9 required antibodies to two or more gene products by a combination of WIB and RIPA. We showed that seroreactivity to gp61 could be detected by RIPA in 129 of 131 (98.5%) of donor samples with p19 and p24 antibodies by WIB. In this regard, the two sets of confirmatory criteria are similar. However, as shown in Table 6, two donor samples without gp61 antibodies showed p19 and p24 antibodies by consensus and in three other samples p24 antibodies were detected by one of four WIB assays. Confirmatory criteria that require antibodies to at least two independent gene products would not be satisfied for these five samples and are thus more conservative than a requirement of antibodies only to p19 and p24.

We have assumed that HTLV-I/II-specific antibodies to two independent gene products are prudent confirmatory criteria. On the basis of the present data, we suggest that antibodies to p24 and to the env-encoded proteins gp46 and/or gp61 be demonstrated by one or a combination of serologic tests. This strategy would maintain a requirement for antibodies to two independent gene products while excluding the presence of antibodies to p19 or p40* as criteria to be used in confirmation; ie, we found no samples with p19 antibodies but without p24 antibodies that contained antibodies to gp61 at routine sample dilutions. Furthermore, antibodies to p40* were present only in a subset (79%) of samples with p24 and gp61 antibodies. Conversely, we have no evidence that persons lacking p24 and gp46 and/or gp61 antibodies are infected with HTLV-I or HTLV-II. All seven samples for which the donor’s lymphocytes yielded virologic evidence of HTLV-I or HTLV-II infection (Table 7) showed seroreactivity to p24 and gp61. It is of interest that p19 antibodies were detected by two or more WIBs in only three of these seven samples whereas antibodies to p40* were found in six of seven samples. In the absence of more virologic data, samples which show antibodies to at least one suspected HTLV-I gene product (such as p19 only, p19 and p28, p40* only) should be regarded as indeterminate to leave open the question of infection in these cases.

We recommend examination of samples by WIB for p24 and gp46 and/or gp61 antibodies. If antibodies to p24 are present but antibodies to gp46 and/or gp61 are lacking, a
RIPA for gp61 antibodies is recommended. Application of this algorithm to our samples with p24 antibodies by WIB would result in the need for a RIPA to evaluate further 7 of 32 (22%) US blood donors and 6 of 34 (18%) Japanese-American (Hawaii) donor samples for gp61 antibodies. In contrast, only 1 of 29 (3%) Japanese, 0 of 11 Jamaican, and 0 of 24 Martinique donor samples with p24 antibodies by WIB would have required further evaluation by RIPA. These differences among the populations may relate to higher measurable antibody titers among persons living in areas of greater prevalence of HTLV-I, as in the general populations of Japan and Caribbean countries. The lower concordance rate in US samples may reflect differences in virus type, HTLV-I versus HTLV-II, or differences in expression of virus in vivo resulting in titer differences to various antigens, or both. Further studies carefully characterizing the nature of the virus by PCR and culture as well as epitope mapping of various reactivities should help clarify these alternatives.

The confirmatory criteria we developed have obvious limitations. First, as demonstrated by the HTLV-I/II–specific antibody patterns from US blood donors (Table 7), infection with HTLV-I can not be distinguished from HTLV-II infection. Second, virological evidence of infection was sought for only 7 of the 142 donor samples studied. Our lack of knowledge of the demographic distribution of virologically proven HTLV-I and HTLV-II infection in the United States remains a significant obstacle to correct interpretation of antibody patterns of all serological tests (eg, seroreactivity to p19 and other gag-encoded proteins might be found to reflect cross-reacting antibodies to other, yet uncharacterized human retroviruses). Moreover, cross-reaction of p19 antibodies with thymic epithelial antigens might account for some false-positive EIA and WIB results. Comparison of seroreactivity patterns in donors with virologically defined infections, both longitudinally after seroconversion and cross-sectionally in larger numbers of donors will be necessary to assess any set of serological confirmatory criteria thoroughly. Options for use of antibodies to p19, p40, or other env-encoded proteins such as gp21 may well be justified by such studies. The uncertain seroreactivity of pol-encoded proteins also deserves greater study. In the interim, given the low estimates of HTLV-I/II infection among US blood donors (0.025%), the low attack rates of associated diseases and the possibility of serological follow-up of persons with some but not all required antibodies, a conservative approach to serological confirmation of HTLV-I/II infection seems warranted. Sera from thousands of US blood donors are expected to be found repeatedly reactive by HTLV-I screening assays over the next few years. An understanding of serological confirmatory criteria and their limitations will be crucial for the health care professionals who counsel these persons.

REFERENCES


Serological confirmation of human T-lymphotropic virus type I infection in healthy blood and plasma donors

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