Evaluation of a Recombinant Human T-Cell Lymphotropic Virus Type I (HTLV-I) p21E Antibody Detection Enzyme Immunoassay as a Supplementary Test in HTLV-I/II Antibody Testing Algorithms

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To evaluate the usefulness of a human T-cell lymphotropic virus type I (HTLV-I) recombinant p21E immunoassay as a supplementary test in HTLV-I/II serologic testing algorithms, we used this assay to test 378 serum samples previously categorized as positive, indeterminate, or negative for HTLV-I/II antibody, as defined by U.S. Public Health Service guidelines. We found this test to be highly sensitive for detecting antibody to HTLV-I/II env (99.4%) but slightly less specific (96.0%), particularly among samples from intravenous drug users. Our data suggest that this test is most appropriately used to confirm the absence of env antibody in samples which are repeatedly reactive in an HTLV-I/II screening assay and gag reactive only by immunoblotting. Because of the high sensitivity of this recombinant p21E test, a negative result in this context could preclude radioimmunoprecipitation testing. However, pending further evaluation of the specificity of this assay, specimens testing positive for p21 env antibody may require confirmation by radioimmunoprecipitation, particularly in situations in which the results will be used for diagnostic purposes or blood donor counseling.

MATERIALS AND METHODS

Selection of serum samples. Serum samples previously categorized, by Public Health Service guidelines, as positive (n = 177), indeterminate (n = 70), or negative (n = 131) for HTLV-I/II were selected from earlier seroepidemiologic studies of HTLV in U.S. blood donors (n = 129), seropositive asymptomatic Japanese (n = 15), intravenous drug users (IVDUs; n = 190), clients of sexually transmitted disease clinics (n = 34), and patients with HTLV-I-associated myelopathy/tropical spastic paraparesis or adult T-cell leukemia/lymphoma (n = 10) (Table 1). Negative samples were nonreactive in a whole-virus lysate-based screening assay. Samples were stored at -20°C until thawed for this study.

HTLV-I/II antibody testing. Serum samples were tested in our laboratory by methods previously described (4). Briefly, samples were screened for HTLV with a commercially manufactured EIA kit (Dupont de Nemours, Wilmington, Del.) according to the manufacturer’s instructions. Samples repeatedly reactive in this assay were further tested by IB with an affinity-purified antigen derived from HTLV-I-infected MT-2 cells (Hillcrest Biologicals, Cypress, Calif.). Samples demonstrating reactivity to both gag p24 and env gp46 or gp68 were considered seropositive for HTLV-I/II. Samples demonstrating reactivity to at least one viral gene product but not meeting the seropositivity criteria after IB analysis were also tested by RIPA. Specimens demonstrating reactivity to both p24 and env proteins by IB and/or RIPA were considered seropositive for HTLV-I/II; samples demonstrating one or more virus-specific bands but not satisfying the criteria for HTLV seropositivity were considered indeterminate. Samples repeatedly reactive in the screening test with no virus-specific reactivity by IB were not tested by RIPA and were considered negative. None of these EIA-reactive, IB-negative specimens were included in this study.

Recombinant HTLV-I p21 env assay. The recombinant HTLV-I p21 env EIA (Cambridge Bioscience Corp.,

Before enzyme immunoassays (EIA) for human T-cell lymphotropic virus types I and II (HTLV-I/II) antibody detection were licensed in November 1988, a U.S. Public Health Service Working Group recommended an algorithm of supplementary tests to confirm the results of these screening assays. The recommendation stated that a serum specimen exhibiting reactivity to gag p24 and env gp46 or gp61/68 be considered positive for HTLV-I/II and that a combination of immunoblotting (IB) and radioimmunoprecipitation assays (RIPA) be used as supplementary tests (9). The Public Health Service Working Group and others have reported that IB is most useful for detection of antibodies to gag proteins, whereas RIPA is often required for detection of antibodies to env proteins (1, 3). In one study, RIPA detected antibodies to env proteins in nearly half of the serum specimens that demonstrated gag reactivity but failed to show env reactivity by IB (4).

However, as IB antigen preparations and preblotted strips are improved for detection of HTLV env proteins and new products become available, the role of RIPA in HTLV testing algorithms should be reevaluated. RIPA requires metabolic labeling of HTLV-infected cells with radioisotope-tagged amino acids. This process is time-consuming, expensive, and difficult to standardize and is not widely available. However, laboratories that report HTLV results based only on IB assays may fail to detect some samples with weak env reactivity, therefore regarding them as "indeterminates" reactive only to gag. This report describes our experience with a commercially manufactured assay available for research purposes only that detects seroreactivity to a recombinant HTLV-I p21 env protein (transmembrane glycoprotein) by using a simple EIA format.

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TABLE 1. Samples tested by Cambridge env EIA

<table>
<thead>
<tr>
<th>Source of sample*</th>
<th>No. of HTLV-I/II results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive b</td>
</tr>
<tr>
<td>U.S. blood donors</td>
<td>29</td>
</tr>
<tr>
<td>Japanese</td>
<td>15</td>
</tr>
<tr>
<td>HAM/TSP or ATL</td>
<td>10</td>
</tr>
<tr>
<td>IVDUs</td>
<td>103</td>
</tr>
<tr>
<td>STD</td>
<td>20</td>
</tr>
<tr>
<td>Total (n = 378)</td>
<td>177</td>
</tr>
</tbody>
</table>

* The Japanese were seropositive and asymptomatic. HAM/TSP or ATL, patients with HTLV-I-associated myelopathy/tropical spastic paraparesis or adult T-cell leukemia/lymphoma; STD, clients of sexually transmitted disease clinics.

b Seroreactivity to p24 and gp46/68.

c Seroreactivity to either gag or env protein.

RESULTS

The recombinant p21E EIA detected antibody to env protein in 175 of 177 positive samples, including 19 of 20 for which RIPA was required to detect env reactivity, resulting in a sensitivity of 98.9% (Table 2). The two discrepant samples were retested by HTLV-I RIPA; one failed to show env reactivity. This specimen, from a volunteer blood donor, was also tested by the American Red Cross National Reference Laboratory, which did not detect env reactivity by IB or RIPA. Peripheral blood lymphocytes obtained from this donor were negative for HTLV-I and HTLV-II sequences by the polymerase chain reaction. Removing this specimen from the calculations yields a sensitivity of 99.4% (175 of 176 positive samples).

Of the 70 indeterminate samples tested, the recombinant p21E EIA was negative for 66, resulting in a specificity of 94.3% (Table 2). Three discrepant samples were from IVDUs and demonstrated p24 reactivity by IB. All were retested by HTLV-I RIPA, and one sample demonstrated env reactivity. Because HTLV-II has been shown to be endemic among IVDUs (6, 8), these samples were also tested by HTLV-II RIPA; none of the three had reactivity to HTLV-II env. An additional discrepant sample, found to have reactivity to gp68 only, was from a volunteer blood donor; this specimen was retested by HTLV-I RIPA and again demonstrated env reactivity. Removing the two samples demonstrating env reactivity from the calculations resulted in a 97.1% specificity among indeterminate samples.

The recombinant p21E EIA was negative for 125 of 131 samples that previously tested negative by a whole-virus lysate HTLV-I/II screening assay and indeterminate by IB testing. Currently, the Public Health Service Working Group recommendations for confirmatory criteria for HTLV-I/II mention only immunoreactivity to gp46 and gp61/68 as fulfilling the criteria for the presence of antibody to env proteins; p21E was not included in this confirmatory scheme, primarily because of the difficulty of identifying reactivity to the native protein by IB and RIPA (7). Technical advances and the availability of new assays now make the detection of antibody to recombinant p21E possible.

DISCUSSION

We undertook this study of the Cambridge p21E EIA to evaluate its utility as a supplementary test for detecting env reactivity in serum samples repeatedly reactive in an HTLV screening assay and indeterminate by IB testing. Currently, the Public Health Service Working Group recommends for confirmatory criteria for HTLV-I/II mention only immunoreactivity to gp46 and gp61/68 as fulfilling the criteria for the presence of antibody to env proteins; p21E was not included in this confirmatory scheme, primarily because of the difficulty of identifying reactivity to the native protein by IB and RIPA (7). Technical advances and the availability of new assays now make the detection of antibody to recombinant p21E possible.

In our study, the recombinant p21E EIA was highly sensitive in detecting HTLV-I/II env antibody; it was reactive in 175 of 176 specimens (excluding the specimen that was retested and found to lack env reactivity by RIPA) demonstrating p24 gag and env reactivity by IB or RIPA. Other investigators have found the recombinant p21E EIA to be 100% sensitive for the detection of HTLV-I/II env antibody (10).
The specificity of the recombinant assay was excellent in samples classified as indeterminate by IB, except in the IVDU group. Importantly, if this test is used as a supplement to IB, these samples classified as gag-reactive indeterminates after IB and RIPA testing (i.e., lacking env reactivity in these tests) should be negative by the p21E EIA. Of 68 such specimens studied, the p21E EIA was nonreactive in 66, yielding a specificity of 97.1%. The two discrepant samples were from IVDUs, in whom specificity was 80% (eight of ten samples). Since most of the HTLV-I/II reactivity among IVDUs has been shown to be due to HTLV-II, the discrepant samples were tested by HTLV-II RIPA in addition to being retested by HTLV-I RIPA; neither demonstrated env reactivity on HTLV-II RIPA. Peripheral blood lymphocytes were not available from either of these individuals for testing by the polymerase chain reaction, and it is possible that one or both were infected with HTLV-II. In the absence of such information, however, we must assume that the p21E reactivity in these two persons who failed to demonstrate env reactivity on IB or RIPA represent false-positive test results.

Similarly, the specificity of the p21E EIA was high in testing samples classified as negative for HTLV-I/II after screening with an EIA using whole-virus lysate as the antigen, except, again, in IVDUs. All six discrepant samples in this group were tested by HTLV-I IB, HTLV-I RIPA, and HTLV-II RIPA, and none demonstrated env reactivity in any of these tests. Peripheral blood lymphocytes from these persons were not available for testing by the polymerase chain reaction.

Our findings are consistent with other studies demonstrating false-positive p21E EIA results in HTLV-I/II-indeterminate and -negative specimens (2, 10). However, the specificity among HTLV screening test-negative samples, which we evaluated in this study, is irrelevant to our intended role for this test. We believe that this recombinant env test is most useful within an HTLV-I/II antibody detection algorithm as a supplementary test performed on samples repeatedly reactive by the screening assay and reactive only to gag by IB. For the purposes of seroepidemiologic studies, in which serologic results are not reported to the individuals tested, the results of the p21E assay may be sufficient. However, when test results will be used for diagnostic purposes or blood donor counseling, we believe that specimens reactive in the p21E EIA should also be tested by RIPA to confirm the env reactivity, until further information is available.

In summary, this test is a sensitive supplementary assay for detecting antibody to env proteins in samples indeterminate after IB testing. On the basis of our results and those of others (2, 10), we believe that an IB-indeterminate specimen that fails to demonstrate reactivity in the Cambridge p21E EIA can be reported as indeterminate and need not be tested by RIPA.

However, the specificity of this test may be suboptimal, particularly in specimens from IVDUs. Since HTLV-I/II seropositivity in various populations is frequently associated with drug use (5, 11), we feel it is prudent to confirm p21E reactivity by RIPA, especially in nonblinded studies when the individual will be informed of the test results. Additional studies of samples that are reactive only to gag after immunoblotting, coupled with polymerase chain reaction analysis of peripheral blood lymphocytes, when available, will be required to further the specificity of the Cambridge p21E EIA and its role in HTLV-I/II antibody-testing algorithms.

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REFERENCES


7. Lairmore, M. D. Personal communication.


