Transfusion-Transmitted HTLV-I/II Infection in Patients Undergoing Open-Heart Surgery


Sera from 5,244 blood donations collected between 1979 and 1987 were screened for antibody to HTLV-I with an enzyme immunoassay (EIA) whose result was confirmed with a two-step procedure including Western blot (WB) and radio immunoprecipitation. Of 20 repeatedly reactive samples, two were confirmed positive for HTLV-I infection. These blood units were transfused to patients undergoing cardiac surgery. Both recipients of the confirmed anti-HTLV-I positive units were infected with HTLV-I as evidenced by antibody seroconversion. In contrast, six recipients of EIA positive, WB indeterminate blood and nine recipients WB negative blood were not infected with HTLV-I. These results confirm a low prevalence of HTLV-I infection in US blood donors, the capacity of infected units to transmit the virus to recipients, and the importance of an appropriate confirmatory assay.

This is a US government work. There are no restrictions on its use.

MATERIALS AND METHODS

Patients and donors. Sera from 5,244 blood donations collected between 1979 and 1987 during a prospective study of posttransfusion hepatitis studies conducted between 1979 and 1987. Our data confirm the low prevalence of HTLV-I antibody in American blood donors and the transmissibility of HTLV-I infection by transfusion of seropositive units.

RESULTS

Among 5,244 donor samples, 54 samples (1.03%) were initially reactive by EIA; only 20 (0.38%) remained reactive when retested in duplicate (Table 1). These 20 samples were also tested using the second unlicensed EIA for the detection of antibody to HTLV-I; only three samples were repeatedly reactive by both tests. All repeatedly reactive samples by either screening assay were tested by RIPA and/or WB for confirmation (Table 1). Two of 20 and 2 of 3 samples positive by each screening assay respectively were confirmed. These confirmed positive samples were identified by both screening EIAs. Both sam-
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and envelope gp46); RIPA, radio immunoprecipitation assay.

In one sample, and only gp61 in the other sample. As shown in Table 1, 6 and 20 samples positive by one screening assay gave an indeterminate result with WB. In 4 of these 6 samples, only a p24 band was visible; in one sample both p19 and p24 bands were found, and in the last sample, only a gp46 band was present. RIPA performed on the same samples was negative in five and with a trace of gp61 band in the last sample. No samples indeterminate on WB were confirmed with RIPA. To assess the transmissibility of HTLV-I by transfusion, recipients of donations repeatedly reactive with the screening assay were examined. Of 19 recipients (one received 2 U), 17 could be traced (results are shown in Table 2). Only the two recipients of the confirmed HTLV-I antibody positive units were found positive by both EIAs and confirmed by WB and RIPA. Recipients of blood with any other combination of assay results, including those samples indeterminate by WB, did not develop serologic evidence of HTLV-I infection. In the control group of 360 recipients of EIA negative donations, one was positive by screening EIA, but not confirmed by WB or RIPA.

Sequential samples from the recipients of the two HTLV-I infected donations were tested with screening and confirmatory assays. Recipient 1 was transfused with 8 U of packed red cells containing approximately 80 mL of plasma, one of which had high titer of antibody to HTLV-I (sample/cut-off ratio 18.5). This female recipient had evidence of passive transfer of anti-HTLV-I IgG since the screening assay was positive up to 3 weeks posttransfusion and the WB showed p19 and p24 bands of decreasing intensity until 6 weeks posttransfusion. A positive EIA and multiple bands on WB reappeared 31 weeks posttransfusion. Although the sample at 31 weeks was indeterminate on confirmatory assay, a confirmed immune response to HTLV-I infection was observed in the next available sample, which was not obtained until 7 years after transfusion.

The serologic course in the second recipient of confirmed anti-HTLV-I positive blood unit is shown in Fig 1. The patient received a total of 13 U packed red cells, and 2 U fresh frozen plasma. One of the packed red cell units was weakly positive for antibody to HTLV-I, with a sample to cut-off ratio of 5.5. Pretransfusion and samples through the fourth week posttransfusion were negative by EIA, WB, and RIPA. A clear p24 band was seen on WB 6 weeks posttransfusion. Subsequent samples showed a progressively increasing intensity of the p24 band on WB and the appearance of a p19 band 8 weeks posttransfusion. At that time, screening EIA became positive. The first confirmed positive sample was observed 10 weeks posttransfusion when a gp61 band was observed on RIPA. Both screening EIA and confirmation assays were still positive 38 weeks posttransfusion.

The prevalence of antibody to HTLV-I in blood donors has been found to be exceedingly high in the southern Japanese island of Kiushiu (12%), moderately high in islands of the Caribbean basin (31%), and low in the United States (0.025%). This study confirms the low prevalence of antibody to HTLV-I (0.04%) and suggests that the prevalence did not substantially change over the 8 years in which the samples were collected (data not shown).

The results of the screening assay and the two-step confirmatory procedure illustrate the difficulties inherent in a screening assay based on semi-purified viral antigens. Most repeatedly reactive samples were not reproducible in a second EIA procedure or confirmed by WB or RIPA. WB frequently showed an indeterminate pattern, primarily an isolated p24 band. This apparently false reaction does not appear with RIPA and reinforces the utility of a two-step confirmatory process. Similar false reactions have been described with viral antigen based HIV screening assays. This study directly addresses the issue of false positive reactions for HTLV-I by assessing the infectivity of samples that could or could not be confirmed by a complete WB pattern and/or a positive RIPA. Only donors who were both WB and RIPA-positive transmitted HTLV-I infection to

Table 1. HTLV-I/II Antibody in Blood Donors

<table>
<thead>
<tr>
<th>Donors Tested</th>
<th>EIA Repeat Reactive (% of total)</th>
<th>EIA 2nd Manufacturer</th>
<th>Confirmation Testing</th>
<th>WB</th>
<th>RIPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neg*</td>
<td>Ind†</td>
<td>Conf‡</td>
</tr>
<tr>
<td>5,244</td>
<td>20 (0.38%)</td>
<td>3</td>
<td>12</td>
<td>6</td>
<td>2§</td>
</tr>
</tbody>
</table>

* Neg, negative; no viral specific bands. † Ind, indeterminate; viral specific band to one gene product only. ‡ Conf, confirmed antibody to at least two gene products by WB alone or a combination of WB and RIPA. § WB confirmed and RIPA confirmed correspond to the same donor sample.

Table 2. HTLV-I/II Seroclonversion in Recipients as a Function of Donor Anti-HTLV-I/II Status

<table>
<thead>
<tr>
<th>Donors Anti-HTLV-I/II Status</th>
<th>Recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conf, confirmed</td>
<td>No.</td>
</tr>
<tr>
<td>EIA + /WB - RIPA -</td>
<td>9</td>
</tr>
<tr>
<td>EIA + /WB/RIPA -</td>
<td>6</td>
</tr>
<tr>
<td>EIA + /WB/RIPA +</td>
<td>1</td>
</tr>
<tr>
<td>EIA - /Conf ND</td>
<td>360</td>
</tr>
</tbody>
</table>

* Of 12 donors in this category, three recipients were not followed. EIA + or - (positive or negative); WB, western blot, negative (no marker band), I indeterminant (one marker band or two core protein bands: p19 and/or p24) or + (positive bands of two gene products: core p19 or p24 and envelope gp46); RIPA, radio immunoprecipitation assay. † Pretransfusion serum was also EIA positive, confirmation for both pre- and posttransfusion samples were negative. ‡ WB and RIPA not performed on 359 recipients of EIA negative blood.
Fig. 1. Serologic profile of recipient 2 who seroconverted. The left panel shows results obtained with WB using purified HTLV-I antigens. gp46, p33, p24, p19, and p15 indicate the molecular weight of HTLV-I structural proteins in kilodaltons. NC, negative control; PC, positive control. Numbers on top of each strip indicates the number of weeks pre-1- or posttransfusion (O). The right panel shows results obtained with the same samples tested with RIPA. gp61, p40x, and p24 indicate the molecular weight of env, tax/rex, and gag gene products, respectively. WB shows a p24 core band in sample 6 and both core protein bands (p24 and p19) in subsequent samples. RIPA shows a clear gp61 (envelope precursor) in sample 10. HTLV-III seropositivity is confirmed in sample 10 and subsequent samples.

recipients of their blood. Donors whose sera were EIA-positive, but G-negative or indeterminate, were RIPA-negative and did not transmit HTLV-I to their blood recipients (Table 2). In addition to helping to discriminate between true and false reaction at the p24 band level, RIPA allows recognition of antibody to the envelope antigen of HTLV-I for which WB is rather insensitive and fulfills the two-gene product band criteria recommended by the Food and Drug Administration for a confirmed positive HTLV-I antibody result.12 Our study further illustrates the transmission of HTLV-I infection by transfusion. A transmission rate of approximately 66% by cell-containing blood products has been observed in Japan.4 In our study, each recipient of confirmed HTLV-I antibody positive blood was infected with this agent. Recipient 1 initially had evidence of passive transmission of antibody to HTLV-I, followed by a prolonged seronegative period and then, several months after transfusion, the development of serologic markers typical of HTLV-I/II infection was observed. A similar pattern was observed with recipient 2 (Fig 1). The HTLV-I seroconversion pattern observed differs somewhat from that which has been observed after transfusion-related transmission of HIV-I. In HIV infection, the window period between exposure and seropositivity is shorter (4 to 8 weeks) and the completion of the typical banding pattern observed by WB generally occurs in a matter of days or a few weeks. However, the early detection of a solitary p24 core protein band by WB as an initial marker of seroconversion is noted in both HTLV-I and HIV infections. In both infections also, an early confirmed diagnosis can be obtained by the detection of antibody to the external membrane glycoprotein by RIPA combined with the core protein band observed with WB.12,13

It has been shown that screening assays using HTLV-I viral antigens for the detection of specific antibodies also recognize antibodies to HTLV-II.14 This high level of serologic cross-reactivity is consistent with the 75% overall homology of amino acid sequences between these two viruses.15 The current procedure of confirmation combining results of WB and RIPA does not permit differentiation between antibodies to these viruses. Differential diagnosis has been achieved by hybridization of DNA probes complementary to specific regions of each virus genome amplified by polymerase chain reaction.16 Because this study was done retrospectively, no cellular DNA from the infected donors and recipients was available for such identification. It is therefore possible that one or both of the seropositive donors in this study was infected with HTLV-II and transmitted that infection.

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REFERENCES

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