Transfusion-Transmitted Human T-Cell Lymphotropic Virus Type I Infection in Taiwan: A True Risk and Occasional Coinfection With Hepatitis C Virus Shown in a Prospective Study

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To study the incidence of human T-cell lymphotropic virus (HTLV) after blood transfusion in Taiwan, serum samples from 699 patients in a prospective study were examined for seroreactivity of anti-HTLV. By an enzyme immunoassay, 9 of the 699 recipients were repeatedly positive. Serial serum samples of these 9 patients were then confirmed with a Western blot analysis and with a polymerase chain reaction (PCR) assay for HTLV-I genome. Four were already positive for anti-HTLV before transfusion, 1 carried antibodies to HTLV-I transiently after transfusion, and only 4 cases had de novo seroconversions. These patients and their family members were called back and tested for HTLV-I genome in the peripheral blood mononuclear cell (PBMC) and plasma. All the serologically positive patients, except the "transient one," were positive for HTLV sequences in the PBMCs. Viral sequences could also be detected in several serum or plasma samples. In the family members, only the spouse of a pretransfusion-positive patient was infected. These results suggested that approximately 0.6% of the blood recipients were infected by HTLV-I through transfusion in Taiwan, and that the frequency of intrafamilial HTLV-I transmission is low. We also observed the unusual coinfection by both HTLV-I and hepatitis C virus in 2 patients, and superinfection of hepatitis C virus after blood transfusion in 1 HTLV-I carrier. Cases of coinfection suggest a prevalence of both viruses in blood donors and warrant further screening.

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Blood TRANSFUSION is a vital therapeutic intervention, but it always carries the risk of blood-transmitted viral infections. Posttransfusion hepatitis is one of the major problem associated with the use of blood or blood products. With the introduction of sensitive tests to screen blood donors for the hepatitis B surface antigen (HBsAg), the exclusion of paid donors, and the recently developed antibody assay to hepatitis C virus (HCV), the risk of posttransfusion hepatitis has declined greatly. However, there has been considerable concern about the transmission by transfusion of human retroviruses such as human immunodeficiency virus (HIV) and human T-cell lymphotropic virus type I (HTLV-I). In Taiwan, screening of donated blood for antibody to HIV (anti-HIV) began in 1985, although the seroprevalence rate was extremely low. In contrast, screening of HTLV-I infection, which is more frequent than HIV in Taiwan, has not yet been initiated.

The seroprevalence of anti-HTLV in Taiwan was calculated to be around 0.5% in a large-scale, community-based study. This rate is lower than the neighboring endemic area, but higher than most of the Western countries. Because the major risk of HTLV-I associated with blood transfusion is the development of a tropical spastic paraparesis/HTLV-I–associated myelopathy (TSP/HAM) in some of the recipients, whether such a prevalence rate carries an significant risk of posttransfusion HTLV infection and neurologic complication needs investigation. We have conducted a prospective study of posttransfusion hepatitis in Taiwan since 1987. Therefore, the serum samples in these prospectively followed recipients can be used to serve this purpose. The HTLV-infected subjects and their family members were called back to receive a check up and to study the frequency of intrafamilial transmission of this virus.

PATIENTS AND METHODS

Patients. From June 1987, we conducted a prospective study of posttransfusion hepatitis in Taiwan. Interim results for hepatitis viruses infection have been reported before. Patients who received blood transfusion and met the following criteria were recruited: normal liver function tests before transfusion; no transfusions received for 1 year; no previous history of liver diseases; and no history of alcoholism, drug addiction, or exposure to hepatotoxic drugs. After transfusion, the recipients were observed every 2 to 4 weeks for 6 months. Blood samples were obtained during each visit and kept frozen at −80°C until testing. As of May 1993, a total of 699 patients completed the 6-month follow-up. Among them, 649 received a transfusion before July 1992, when antibody to HCV was screened in volunteer blood donors. The recipients were predominantly patients with open heart surgery (Table 1) and had a mean donor number of 18.6 ± 14.6 (range, 1 to 97).

Donors. The blood or blood components were donated by volunteers negative for HBsAg, Venereal Disease Research Laboratory test (VDRL), and anti-HIV who also had serum alanine aminotransferase activities (ALT) less than 45 IU/L (normal, < 31 IU/L). Antibody to HCV was added to the screening list in July 1992.

Families. Patients who were positive for anti-HTLV and their family members (including parents, siblings, spouse, and children of the index patient) were called back. After oral consent, blood samples were obtained to test anti–HTLV-I in the plasma as well as HTLV-I sequences in the peripheral blood mononuclear cells (PBMCs).

Sero logic tests. Anti-HTLV assay was performed in the first posttransfusion serum samples and in the samples taken 6 months after transfusion with an enzyme immunoassay (ELA) kit (Organon Teknika Corp, Durham, NC). Reactive samples were repeated in duplicate, and were considered positive only if at least two of the three test determinations were reactive. The cut-off value of optical density (OD) was defined as the mean of negative samples plus 0.36

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TRANSFUSION-TRANSMITTED HTLV-I INFECTION
confirmed by a commercial immunoblot analysis according to the recipient before and after transfusion were tested if posttransfusion products was electrophoresed in a 2% agarose gel and stained with samples registered as positive. A viral-specific band that was present was interpreted as negative. A viral-specific band that was present but whose pattern did not meet the criteria for a positive result was interpreted as indeterminate.

Polymerase chain reaction (PCR). Serial serum samples of the anti-HTLV-IV-positive patients were tested for viral RNA and proviral DNA by a nested PCR using two independent primer pairs. The first primer pair was from the 5' long terminal repeat (LTR) region. The outer primers were LTR1R (nucleotide 691-672, 5' ATG CGC CGG TAC TTG GCC GTT, antisense) and LTR1 (nucleotide 401-425, 5' ATC CAC GGT TGA GTC GCC TTC T, sense). The inner primers were LTR2R (nucleotide 645-625, 5' TGA AAG GGA AAG GGG TGG AAC, antisense) and LTR2 (nucleotide 439-460, 5' TGT GGT GCC TCC TGA ACT GGG T, sense). The expected product was 206 bp. The second primer pair was from the splicing junction of tax1/ex1 gene. The outer primers were RPX2 (nucleotide 7066-7047, 5' GTA GCC GGG CGC AAC ATA GT, antisense) and RPX1 (nucleotide 4721-4740 AAG CGG TAG TTC TGC CCA GT, sense). The inner primers were RPX4 (nucleotide 7004-6985, 5' AAC ACG TAC ACT GGG TAT CC, antisense) and RPX3 (nucleotide 4743-4762, 5' ATC CGG TGG AGA CTC CTC A, antisense).

The expected products amplified from viral RNA and proviral DNA were 2,262 bp in length, but the spliced mRNA was of 145 bp. Nucleic acid was extracted from 100 μL of serum or PBMCs were extracted from 10 mL of EDTA blood and reverse transcribed (RT), as described previously.16 Nucleic acids from 20 μL of serum were then subjected to a nested PCR in a 50-μL mixture containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 0.01% gelatin, 2.5 U recombinant Taq DNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT), 200 μmol/L of dNTP, 0.6 μmol/L of each outer primer, and 5 μL of the cDNA mixture. Forty cycles of 96°C for 30 seconds, 56°C for 15 seconds, and 74°C for 30 seconds were performed. The temperature was maintained at 74°C for 10 minutes at the end of the last cycle. To detect the 2,262-bp product, a modified temperature cycling (96°C for 30 seconds, 56°C for 15 seconds, and 74°C for 60 seconds) that has been shown to be able to produce a 2.4-kb fragment efficiently was also performed.20 DNA samples from healthy persons and reagents without DNA were used as negative controls in the PCR assay. Ten microliters of the PCR products was electrophoresed in a 2% agarose gel and stained with ethidium bromide. To further differentiate DNA from RNA template, nucleic extracts were subjected to PCR with or without a prior RT.

RESULTS
In the initial screening by EIA, 23 of the 26 samples from 13 recipients were positive. On repeat tests, 9 of the 13 initially positive samples were repeatedly positive. The mean sample/cut-off value in repeatedly reactive samples was 2.9 (range, 1.4 to 4.9) and in not repeatedly reactive samples was 1.4 (range, 1.1 to 1.9). Serial samples of the 9 recipients were then assayed, and 9 patients were found to be repeatedly positive by EIA. Among the 9 true-positive recipients, 1 was positive in the first 3 posttransfusion samples only, 4 were positive in both the pretransfusion and posttransfusion samples, and 4 seroconverted at 1 to 70 days after transfusion. One of the 4 seroconverters was transfused after June 1992, whereas the other 8 seropositive patients were transfused before the anti-HCV screening. In the family study, a total of 45 members from 6 families were tested. Only the spouse of the patient who was already infected before transfusion was positive.

By Western blot analysis, all the repeatedly reactive samples were confirmed. The 4 initially EIA-reactive patients who became EIA negative on repeat tests were seronegative by Western blot. In the first seroconverter, antibodies to p24 and gp46 appeared immediately after transfusion, whereas antibodies to other major proteins (p19, p21, and p28) appear approximately 30 days later (Fig 1A). In the second seroconverter, p19, p21, p24, p28, p38, and gp46 antibodies appeared at the first posttransfusion sample; p32 appeared 40 days later (Fig 1B). In the third patient, p19, p21, p24, p28, p32, and p38 antibodies appeared 27 days after transfusion, whereas gp46 appeared 34 days later (Fig 1C). In the fourth seroconverter, the antibody pattern was similar to that of seroconverter 3 (ie, p19, p21, p24, p28, p32, and p38 antibodies appeared at 31 days after transfusion, whereas gp46 appeared 4 weeks later; data not shown). Antibodies in the 4 seroconverters remained positive as long as 1 to 5 years after transfusion. In the transiently reactive patient, faint bands of p19, p21, p24, and p28 were seen in the first 3 posttransfusion samples (2, 4, and 6 weeks after transfusion). All antibodies disappeared 8 weeks after transfusion and remained negative after 4 years of follow-up (Fig 2). Thus, these may be caused by carry-over of HTLV antibodies. Several EIA-negative and borderline reactive samples (lanes 1 and 2, Fig 1A; lanes 2 and 3, Fig 2) displayed visible bands on Western blot analysis.

On PCR, all serologically positive patients except the "carry-over" patient were positive for HTLV sequences in the PBMCs by the use of LTR primers. Two (patients no. 1 and 3, Table 2) were also positive for mRNA product by the second primer (Fig 3). Viral sequences could also be detected in serial serum samples of 6 serologically positive patients by the LTR primer, and 2 of them were also positive for viral coding sequence of 2,262 bp by the PRX primers (Fig 3). All except the mRNA product could be detected by PCR without prior reverse transcription. Interestingly, we also observed 2 other cases (patients no. 1 and 5, Table 2) coinfected by HCV and HTLV-I and 1 superinfected by

Table 1. Clinical Data of 699 Recipients in a Prospective Study

<table>
<thead>
<tr>
<th>Reason for transfusion</th>
<th>630</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open heart surgery</td>
<td>630</td>
</tr>
<tr>
<td>Other surgery</td>
<td>51</td>
</tr>
<tr>
<td>Gastrointestinal bleeding</td>
<td>18</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>319/380</td>
</tr>
<tr>
<td>Age (ys)</td>
<td>47.6 ± 15.1 (15-84)</td>
</tr>
<tr>
<td>Donor number</td>
<td>18.6 ± 14.5 (1-97)</td>
</tr>
<tr>
<td>Whole blood</td>
<td>2.4 ± 2.8 (0-22.5)</td>
</tr>
<tr>
<td>Platelet</td>
<td>4.9 ± 7.5 (10-120)</td>
</tr>
<tr>
<td>Fresh frozen plasma</td>
<td>5.5 ± 3.0 (2-26)</td>
</tr>
</tbody>
</table>

Values for age and donor number are the mean ± SD, with the range in parentheses.

(range, 0.414 to 0.482 on our testings). Serial serum samples of a recipient before and after transfusion were tested if posttransfusion samples registered as positive.

Western blot analysis. All the samples positive by EIA were confirmed by a commercial immunoblot analysis according to the manufacturer’s instructions (Cambridge Biotech, Worcester, MA). A distinct band that presented at p24, p46, or p21env (a recombinant gp21) was interpreted as positive. No viral-specific band that was present was interpreted as negative. A viral-specific band that presented but whose pattern did not meet the criteria for a positive result was interpreted as indeterminate.

Polymerase chain reaction (PCR). Serial serum samples of the anti-HTLV-IV-positive patients were tested for viral RNA and proviral DNA by a nested PCR using two independent primer pairs. The first primer pair was from the 5' long terminal repeat (LTR) region. The outer primers were LTR1R (nucleotide 691-672, 5' ATG CGC CGG TAC TTG GCC GTT, antisense) and LTR1 (nucleotide 401-425, 5' ATC CAC GGT TGA GTC GCC TTC T, sense). The inner primers were LTR2R (nucleotide 645-625, 5' TGA AAG GGA AAG GGG TGG AAC, antisense) and LTR2 (nucleotide 439-460, 5' TGT GGT GCC TCC TGA ACT GGG T, sense). The expected product was 206 bp. The second primer pair was from the splicing junction of tax1/ex1 gene. The outer primers were RPX2 (nucleotide 7066-7047, 5' GTA GCC GGG CGC AAC ATA GT, antisense) and RPX1 (nucleotide 4721-4740 AAG CGG TAG TTC TGC CCA GT, sense). The inner primers were RPX4 (nucleotide 7004-6985, 5' AAC ACG TAC ACT GGG TAT CC, antisense) and RPX3 (nucleotide 4743-4762, 5' ATC CGG TGG AGA CTC CTC A, antisense). The expected products amplified from viral RNA and proviral DNA were 2,262 bp in length, but the spliced mRNA was of 145 bp. Nucleic acid was extracted from 100 μL of serum or PBMCs were extracted from 10 mL of EDTA blood and reverse transcribed (RT), as described previously.16 Nucleic acids from 20 μL of serum were then subjected to a nested PCR in a 50-μL mixture containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 0.01% gelatin, 2.5 U recombinant Taq DNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT), 200 μmol/L of dNTP, 0.6 μmol/L of each outer primer, and 5 μL of the cDNA mixture. Forty cycles of 96°C for 30 seconds, 56°C for 15 seconds, and 74°C for 30 seconds were performed. The temperature was maintained at 74°C for 10 minutes at the end of the last cycle. To detect the 2,262-bp product, a modified temperature cycling (96°C for 30 seconds, 56°C for 15 seconds, and 74°C for 60 seconds) that has been shown to be able to produce a 2.4-kb fragment efficiently was also performed.20 DNA samples from healthy persons and reagents without DNA were used as negative controls in the PCR assay. Ten microliters of the PCR products was electrophoresed in a 2% agarose gel and stained with
HCV (patient no. 9, Table 2). The latest patient with hepatitis C (patient no. 9, Table 2) who received interferon α-2b 3MU three times weekly for 4 weeks in an attempt to prevent chronicity of hepatitis C was positive for HTLV DNA sequence by both primer sets before the treatment. His PBMCs remained positive for HTLV genome 1 year after cessation of the treatment. For patient no. 5 (Table 2), the HTLV-I infection did not occur, but hepatitis C persisted, as shown by the persistence of HCV RNA in serum and the elevation of ALT.

A total of 45 family members from 6 families (3 of the seroconverters and 3 of the pretransfusion-positive patients) were tested for anti-HTLV-I in plasma and PCR in PBMCs. There were 6 spouses, 4 parents, 7 siblings, and 28 children of the index cases. Only 1 spouse was positive by serology and PCR. The spouse was also positive for mRNA sequences. The others were negative in both tests.

All 3 subjects (2 index patients and 1 spouse) who were positive for mRNA product after nested PCR showed no visible band after first round of PCR.

All the infected subjects were clinically well and there was no evidence of TSP/HAM or adult T-cell leukemia/lymphoma during the follow-up period.

**DISCUSSION**

HTLV-I is a retrovirus distantly related to HIV and closely related to HTLV type II (HTLV-II).21 The infection is endemic in Southwestern Japan, the Caribbean, intertropical Africa, and some regions of South and Central America and
of Melanesia. Transmission of HTLV-I occurs through the transfusion of cellular components, sexual contact, breast feeding, and intravenous drug abuse. HTLV is causally associated with adult T-cell leukemia and with a progressive myelopathy. Therefore, despite the fact that the natural course of HTLV carriers is not exactly documented, transmission of this virus by transfusion has raised serious concerns. To reduce this infection, screening of HTLV has begun in the United States. In France, screening of endemic zones such as the French West Indies has begun since 1984, although it is not complete. This screening has shown to significantly reduce HTLV transmission via transfusion in Japan.

The seroprevalence of HTLV-II infection in a previous study in Taiwan is estimated to be 0.5%, which is higher than that reported in a multicenter survey of the US blood donors. In our study, we estimated that the risk of HTLV-I infection from transfusion in Taiwan was approximately 0.04% per blood donor (4/699 X 18.6) or 0.08% per unit cellular blood product (4/699 X [2.4 + 2.4 + 4.9]). Therefore, the infection rate of blood donors seemed lower than the community-based population in the same area and is slightly higher than the rate given in the report from the United States. Therefore, screening of anti-HTLV is indicated to reduce the risk of this infection in Taiwan.

In this study, we found that 3 of the 4 patients positive for anti-HTLV before transfusion had a previous history of transfusion. In addition, 3 of the 9 HTLV-seropositive patients had HCV coinfection or superinfection. The results suggested that transfusion was an important risk factor of HTLV-I infection in Taiwan. Screening of anti-HCV could probably reduce the risk of HTLV infection because some donors would be at high risk of infection by both viruses from a previous history of transfusion. However, because the infection risk was very low, it was difficult to demonstrate a significant reduction of HTLV infection after anti-HCV screening.

Intrafamilial transmission has been reported through breast feeding and sexual contact; nevertheless, there could be other potential routes of intrafamilial transmission. In our series, family members of all the seroconverters were free of HTLV-I infection by serology and PCR. In the 3 patients infected before this transfusion, only 1 spouse was positive for HTLV-I. Because the index patient was seropositive before transfusion, the exact infection period was not known, and the spouse could have been infected before the index patient. Nevertheless, the frequency of intrafamilial transmission was low in our study.

The major antigenic structural genes of HTLV are core (also termed gag), composed of p24 and p19, and envelope

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**Table 2. Clinical Data of Nine Seropositive Recipients**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)/ Sex</th>
<th>Date of Transfusion</th>
<th>Component</th>
<th>Anti-HTLV</th>
<th>Previous Transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>56/M</td>
<td>1988</td>
<td>WB × 1U</td>
<td>Seroconversion</td>
<td>Yes</td>
</tr>
<tr>
<td>2†</td>
<td>47/F</td>
<td>1992</td>
<td>FFP × 3U</td>
<td>Seroconversion</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PRBC × 2U</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PLT × 6U</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FFP × 9U</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PRBC × 6U</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PLT × 6U</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>35/M</td>
<td>1991</td>
<td>WB × 1U</td>
<td>Seroconversion</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>69/F</td>
<td>1992</td>
<td>FFP × 9U</td>
<td>Seroconversion</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PRBC × 5U</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PLT × 12U</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5*</td>
<td>40/F</td>
<td>1988</td>
<td>WB × 4U</td>
<td>Transiently reactive</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>34/F</td>
<td>1992</td>
<td>FFP × 3U</td>
<td>Before</td>
<td>Yes</td>
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<tr>
<td>7</td>
<td>71/F</td>
<td>1990</td>
<td>WB × 4U</td>
<td>Before</td>
<td>No</td>
</tr>
<tr>
<td>8†</td>
<td>41/F</td>
<td>1990</td>
<td>WB × 4U</td>
<td>Before</td>
<td>Yes</td>
</tr>
<tr>
<td>9†</td>
<td>37/M</td>
<td>1992</td>
<td>FFP × 6U</td>
<td>Before</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Abbreviations: WB, whole blood; FFP, fresh frozen plasma; PRBC, packed red blood cell; PLT, platelet.

* Coinfected with acute hepatitis C.
† Transfused after anti-HCV screening.
‡ Superinfected with HCV and treated with intron-A 300 MU twice weekly for 4 weeks.
Fig 3. PCR products of a patient infected with HTLV. (A) Lane M, \( \phi x174/Hae \) III; lane 1, PCR product in PBMCs using RPX primer and long extension time cycling without RT; lane 2, PCR product in PBMCs using RPX primer and short extension time cycling and RT; lane 3, PCR product in PBMCs using LTR primers; lane 4, a negative control sample. (B) Lane M, \( \phi x174/Hae \) III; lane 1, PCR product in plasma using RPX primer and long extension time cycling without RT; lane 2, PCR product in plasma using RPX primer and short extension time cycling and RT; lane 3, PCR product in plasma using LTR primers; lane 4, a negative control sample.
disease process in immunocompetent hosts. Although we could not detect any evidence of TSP/HAM in these carriers, HTLV-related neuropathy has been noted in Taiwan. Therefore, reducing the blood-borne transmission in this area is warranted.

It is concluded that approximately 0.6% of transfusion recipients were infected by HTLV-I in Taiwan, and that the frequency of intrafamilial transmission is low in HTLV-I carriers.

ACKNOWLEDGMENT

The authors are indebted to Prof J.L. Sung for initiating the prospective study of posttransfusion hepatitis in Taiwan.

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