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. 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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TRANSFUSION-TRANSMITTED HTLV-I INFECTION

confirmed by a commercial immunoblot analysis according to the manufacturer’s instructions (Cambridge Biotech, Worcester, MA). Samples registered as positive.

TAC TTG GCC GT, antisense) and LTRl (nucleotide 401-425, 5’ AGT CGC CGG TAC T7G GCC GT, antisense) and LTR1 (nucleotide 401-425, 5’ ATC CAC GCC GGT TGA GTC GCC TCC 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 taxI/rexl gene. The outer primers were RPX2 (nucleotide 7066-7047, 5’ GTA GCC GGG CCG AAC ATA GT, antisense) and RPX1 (nucleotide 4721-4740 AAG CGC TAG TTC TGC CCA GT, sense). The inner primers were RPX4 (nucleotide 7004-6985, 5’ AAC AGC TAG ACT GGG TAT CC, antisense) and RPX3 (nucleotide 4743-4762, 5’ ATC CGG TGG AGA CTG ATC A, sense).

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 mL of serum or PBMCs were extracted from 10 mL of EDTA blood and reverse transcribed (RT), as described previously. Nucleic acids from 20 mL of serum were then subjected to a nested PCR in a 50-L mixture containing 10 nmol/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. 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 7 to 97 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 testing 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 (nos. 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) coinfect 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>
<th>51</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open heart surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal bleeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>319/380</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>47.6 ± 15.1 (15-84)</td>
<td></td>
</tr>
<tr>
<td>Donor number</td>
<td>18.6 ± 14.5 (1-97)</td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>2.4 ± 2.8 (0-22.5)</td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td>2.4 ± 2.9 (0-19.5)</td>
<td></td>
</tr>
<tr>
<td>Fresh frozen plasma</td>
<td>4.9 ± 7.6 (0-120)</td>
<td></td>
</tr>
</tbody>
</table>

Values for age and donor number are the mean ± SD, with the range in parentheses.
Fig 1. (A) Western blot analysis and PCR results of serial samples of seroconverter 1. Lane 1, HTLV-1-positive control; lane 0, pretransfusion sample; lane 1, a sample taken 21 days after transfusion; lane 2, a sample taken 35 days after transfusion; lane 3, a sample taken 70 days after transfusion; lane 4, a sample taken 130 days after transfusion; lane 5, a sample taken 180 days after transfusion; lane II, HTLV-II-positive control. (B) Seroconverter 2. Lane 0, pretransfusion sample; lane 1, a sample taken 48 days after transfusion; lane 2, a sample taken 60 days after transfusion; lane 3, a sample taken 88 days after transfusion; lane 4, a sample taken 95 days after transfusion; lane 5, a sample taken 105 days after transfusion; lane 6, a sample taken 180 days after transfusion; lane 7, a sample taken 2 years after transfusion; lane II, HTLV-II-positive control. (C) Seroconverter 3. Lane 0, pretransfusion sample; lane 1, a sample taken 13 days after transfusion; lane 2, a sample taken 27 days after transfusion; lane 3, a sample taken 40 days after transfusion; lane 4, a sample taken 61 days after transfusion; lane 5, a sample taken 90 days after transfusion; lane 6, a sample taken 120 days after transfusion; lane 7, a sample taken 180 days after transfusion; lane II, HTLV-II-positive control.

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 been since 1984, although it is not very complete. This screening has shown to significantly reduce to HTLV transmission via transfusion in Japan.

The seroprevalence of HTLV-III 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 × 18.6) or 0.08% per unit cellular blood product (4/699 × [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

### 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>
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<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>3</td>
<td>47/F</td>
<td>1992</td>
<td>PLT × 6U</td>
<td>Seroconversion</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>40/F</td>
<td>1988</td>
<td>WB × 4U</td>
<td>Transiently reactive</td>
<td>No</td>
</tr>
<tr>
<td>5*</td>
<td>34/F</td>
<td>1992</td>
<td>FFP × 3U</td>
<td>Before</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>71/F</td>
<td>1990</td>
<td>WB × 4U</td>
<td>Before</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>41/F</td>
<td>1990</td>
<td>WB × 4U</td>
<td>Before</td>
<td>Yes</td>
</tr>
<tr>
<td>8</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.

Antibody to p24 antigen is most frequently detected earlier than antibody to p19 antigen. We found that antibodies to most antigens were detectable at the first seroconverted serum sample in the 3 seroconverters except antibodies to gp46 and p32. In the other seroconverter, gp46 and p24 appeared earliest. The early appearance of antibodies in the first seroconverter could be caused by carry-over of donor antibodies. In the patient with HTLV antibody carry-over, antibodies to p19, p21, p24, and p28 were detected immediately after transfusion. However, all antibodies lasted for 8 weeks after transfusion. Because the donor of this "carry-over" patient was not identified, we were unable to investigate the HTLV-I infection status in the donor. Therefore, we could not correlate the absence of antibodies to p32, p38, and gp46 to the noninfectivity in the recipient.

A previous study suggests that HTLV-I is transmitted only by cellular components. In our 4 cases of seroconverters, the data are in agreement with this concept. However, we detected HTLV-I sequences in serum and plasma by nested PCR. The source of the viral nucleic acid could be from the cellular components contaminated because of incomplete separation. Actually, detection of HTLV-I sequences in cell-free samples has also been reported before. These findings could have important epidemiologic implications irrespective of the source of viral sequences. Nevertheless, the extreme sensitivity of the nested PCR could have detected viral doses lower than that required in in vivo infection. Therefore, more clinical observations are needed to establish the significance of the HTLV-I positivity in cellular-free specimens.

In our series, 3 healthy carriers (2 index patients and 1 spouse) were positive for mRNA by nested RT PCR. Because a visible band was not visualized after the first PCR round, the expression level should be very low. This finding was in agreement with a previous report in which HTLV mRNA expression has been detected by RT PCR but not by Northern blot in PBMCs of patients with TSP/HAM.

Detection of the conserved LTR sequence by nested PCR was especially useful in the diagnosis of current HTLV infection. We found that all serologically positive patients were PCR positive in PBMCs except the 1 patient with transient seroreactivity. Because HTLV-I was not detected in the patient and antibodies disappeared shortly after infection, infection was considered to not be established. Superinfection of HCV has been shown to inhibit HBV replication. Therefore, although further studies are needed, coinfection of HCV in this patient could play a role in the abortion of HTLV-I infection.

Previous in vitro study has shown the inhibitory effect of interferon on HTLV replication. However, a 4-week course of interferon treatment seemed ineffective in eradicating HTLV in PBMCs in our patient. Interferon treatment also showed no evident effect on the antibody patterns.

A previous study in Taiwan has shown a latent period to develop human T-cell leukemia/lymphoma from 6 months to 11 years in an immunocompromised host. However, in the current observation, all the patients were well at least 1 to 5 years after infection. This could be caused by a slow
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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