Infection of Human T-Cell Leukemia Virus Type I and Development of Human T-Cell Leukemia/Lymphoma in Patients With Hematologic Neoplasms: A Possible Linkage to Blood Transfusion

By Yao-Chang Chen, Chiu-Hwa Wang, Ih-Jen Su, Chung-Yi Hu, Min-Ji Chou, Tun-Ho Lee, Dong-Tsann Lin, Tze-Yuan Chung, Chen-Hui Liu, and Czao-Siung Yang

Among 354 adult patients with either hematological malignancy or aplastic anemia, eight were positive for anti-HTLV-I antibodies; six of eight had received multiple transfusions. There was an approximately 3.5-fold increase (P < .001) of HTLV-I seropositivity in the patients with hematologic disease (8 of 354, 2.23%) compared to the healthy adults older than 20 years (34 of 5252, 0.65%). Two hematological patients, one with Hodgkin’s disease and one with acute promyelocytic leukemia, were found to be positive for HTLV-I, and developed and died of adult T-cell leukemia/lymphoma (ATL) subsequently. Both were long-term survivors of the primary disease and had received multiple transfusions. The latent period from blood transfusion to onset of ATL was 6 months and 11 years, respectively. Immunocompromised patients, who are seropositive for HTLV-I, may be at increased risk for ATL compared to healthy carriers of HTLV-I, and the latent period may be shorter.

A simultaneous survey of prevalence of HTLV-I infection in the general population in Taiwan was also conducted as described elsewhere. Briefly, specimens of 7,278 inhabitants of different age groups, from 20 communities of Taiwan by stratified random sampling methods and screened for HTLV-I antibodies. An increased incidence rate of HTLV-I infection and the malignant transformation.

Identification of HTLV-I Infection

Screening for HTLV-I antibodies with an enzyme-linked immunosorbent assay (ELISA) was carried out using antigen plate (Diagnostic Biotech Research Lab Inc, Singapore) coated with disrupted HTLV-I viral particles purified from HUT-102 culture supernatant.

HTLV-I seropositivity was confirmed by Western blot analysis using Diagnostic Biotech kits. The antigen strips contain electrophoretically separated peptides of HTLV-I virion pelleted from MT-2 culture supernatant. Sera were tested at a dilution of 1:100. Immunohistochemical detection was performed with an avidin-biotin system. Serum specimens with antibodies to both p19 and p24 antigens were considered to be positive.

Detection of proviral HTLV DNA sequences in leukemic cells was performed as the confirmation study of the diagnosis of ATL. Tumor tissues from the seropositive patients and 14 HTLV-I antibody-negative individuals were sampled for Southern blot analysis of HTLV-I proviral genome. Cells from the HTLV-I-positive MT-2 cell line, and two well-documented ATL patients, were used as positive controls. Briefly, DNA was extracted with phenol/chloroform from the tumor tissue lystate. Extracted DNA was then digested with restriction endonuclease Pst-I and EcoRI, gel electrophoresed, and transferred to nitrocellulose paper by Southern blot technique. The paper was hybridized with a 32P-labelled full genomic HTLV-I probe (8.0 kb), isolated from a pMT-2 clone (Diagnostic Biotech), and exposed to Kodak (Rochester, NY) x-ray film. The resulting autoradiograms were interpreted for evidence of HTLV-I DNA sequences.

Materials and Methods

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Patients and Normals

From February 1983 to April 1987, 354 hematological patients admitted to National Taiwan University Hospital were routinely screened for HTLV-I antibodies.

A simultaneous survey of prevalence of HTLV-I infection in the general population in Taiwan was also conducted as described elsewhere. Briefly, specimens of 7,278 inhabitants of different age and sex were collected from 20 communities of Taiwan by stratified random sampling methods and screened for HTLV-I antibodies. An increased incidence rate of HTLV-I antibody with age has been
invariably found in the studies in Japan\textsuperscript{[14,21]} and Taiwan.\textsuperscript{[30]} In order to obtain an age-correspondence comparison of the HTLV-I incidence between the normal population and the hematological patients, only 5,252 health adults (over age 20 years) out of the total 7,278 persons were included as the control group in the present study.

Statistics. The significance of difference in HTLV-I seropositive rate between hematological patients and general populations was tested by goodness of fit chi-square test, using the positive rate in the general populations to calculate the expected positive number of the hematological patients.

RESULTS

The prevalence rate of HTLV-I infection in healthy adults and in hematological patients is shown in Tables 1 and 2. There was no difference in age and sex between the two groups. The details of the results in the healthy inhabitants were published in another article.\textsuperscript{[30]} Eight of 354 (2.23%) adult patients with either hematological malignancy or aplastic anemia were found to be asymptomatic carriers, compared with 34 of 5252 (.65%) healthy adults. This difference represents a 3.5-fold-increase in HTLV-I seropositivity in the patient group ($P < .001$).

Clinical profiles of the eight hematological patients with HTLV-I antibodies are listed in Table 3. Six of eight hematological patients had multiple platelet and/or granulocyte transfusions. Among the 346 hematological patients seronegative for HTLV-I, 177 (51.2%) also had available history of blood transfusion. We were unable to calculate the exact numbers or units of blood products transfused in every patient, because some received their previous transfusions at many local hospitals and were difficult to trace back. Two hematological patients later developed ATL. Both patients were long-term survivors of the primary hematological malignancy, and both were multi-transfused. In contrast, none of the 34 HTLV-I seropositive healthy carriers developed ATL over a similar time period. The difference was statistically significant (0/34 v 2/8, $P = .03$) by Fisher's exact test.

Case Reports

Both case A and case B were Chinese who were lifetime residents of Taiwan. Both were married and were restrictively heterosexual. There was no history of intravenous drug use and no travel history outside of Taiwan.

Case A. In 1971, a 49-year-old man presented with slow-growing neck masses and the gradual development of compression symptoms. Excisional biopsy in 1979 revealed Hodgkin's disease of lymphocyte predominant type, with typical Reed-Sternberg cells (Fig 1A). Positive reaction of Reed-Sternberg cells to Leu M1 monoclonal antibody (Beckton-Dickinson, Mountain View, CA) was demonstrated using the avidin-biotin complex immunoperoxidase method\textsuperscript{[22]} (Fig 1B). Para-aortic lymphadenopathy was demonstrated by lymphangiography. After receiving six courses of COPP (cyclophosphamide, vincristine, prednisolone, and procarbazine), he achieved complete remission lasting for 2\2 years.

He underwent further chemotherapy including bleomycin-COPP, ABVD (adriamycin, bleomycin, vinblastine, and DTIC), and BCNU-COPP and Mantle irradiation for recurrent disease from March 1982 to July 1984, with complete temporary response. In July 1984, Hgb was 8.0 gm/dL and WBC $6.7 \times 10^9/L$, with normal differentials. Bone marrow studies revealed erythroid hypoplasia secondary to previous chemotherapy. On July 1984, he had his first blood transfusion. Although the bone marrow studies revealed no evidence of lymphoma or lymphocytosis, reenlargement of para-aortic lymph nodes was demonstrated by computed tomography scanning. The abdominal lymph nodes responded to chemotherapy with BCNU-COPP. The patient developed pneumonia, however, and received multiple blood transfusions for granulocytopenia and thrombocytopenia.

In December 1984, he was found to have mild lymphocytosis approximately 150 days after his first blood transfusion. WBC was $12.7 \times 10^9/L$, with 48% lymphocytes. In April 1985, he developed generalized erythromelalgia over the face and neck area. Hgb was 15.9 gm/dL. WBC was $17.5 \times 10^9/L$ with 75% lymphocytes. The platelet count was 176 x

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of Cases</th>
<th>No. Positive</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin's disease</td>
<td>21</td>
<td>1</td>
<td>4.76</td>
</tr>
<tr>
<td>Non-Hodgkin's lymphoma</td>
<td>156</td>
<td>2</td>
<td>1.28</td>
</tr>
<tr>
<td>CLL</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ALL</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CML</td>
<td>45</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AML</td>
<td>62</td>
<td>3</td>
<td>4.84</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>36</td>
<td>2</td>
<td>5.56</td>
</tr>
</tbody>
</table>

Abbreviations: CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia.

Table 1. Age-Sex-Specific Anti-HTLV-I Antibody-Positive Rates

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Males</th>
<th>Females</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Tested</td>
<td>No. Positive</td>
<td>Rate (%)</td>
<td>No. Tested</td>
<td>No. Positive</td>
</tr>
<tr>
<td>20-39</td>
<td>885</td>
<td>2</td>
<td>.23</td>
<td>1274</td>
</tr>
<tr>
<td>40-59</td>
<td>903</td>
<td>5</td>
<td>.55</td>
<td>1005</td>
</tr>
<tr>
<td>60+</td>
<td>710</td>
<td>7</td>
<td>.99</td>
<td>475</td>
</tr>
<tr>
<td>Subtotal</td>
<td>2498</td>
<td>14</td>
<td>.56</td>
<td>2754</td>
</tr>
<tr>
<td>Total</td>
<td>34/5252 = .65%</td>
<td>$P &lt; .001$</td>
<td>8/354 = 2.23%</td>
<td></td>
</tr>
</tbody>
</table>
The lymphocytes had the characteristic morphology (Fig 1C) and surface markers of ATL (Tac+, OKT3+, T4+, T11+, T6-, T8-, T10-). The disease was partially responsive to the use of alkylating agents.

Serological tests for HTLV-I using ELISA and Western blot analysis were negative in October and December 1983, and also in July 1984, 1 week before his first transfusion (Fig 2). All other members of his family, including his wife who had travelled twice to Japan, were seronegative (Fig 1). Retrospective examination of HTLV-I in the four donors and platelet- and granulocyte-transfusions from four paid donors was negative. The three volunteer donors could not be reached for further testing (see text and Fig 2 for the details).

The patient’s serum was negative for human immunodeficiency virus (HIV) by ELISA and Western blot analysis. Before development of ATL, he received red cell transfusions from three volunteer donors of Taipei Blood Center; and platelet- and granulocyte-transfusions from four paid donors. Retrospective examination of HTLV-I in the four paid donors was negative. The three volunteer donors could not be reached for further testing for HTLV-I antibody, however.

The diagnosis of ATL was confirmed by the detection of proviral genome in his circulating lymphocytes using Southern blot analysis (Fig 3). Also, type C retrovirus particles were demonstrated on cultured leukemic cells (data not shown).

The patient developed multiple bacterial and fungal infections and died of sepsis in November 1986, 22 months after the development of lymphocytosis, 15 years after the presentation of Hodgkin’s disease. At autopsy, features of Hodgkin’s disease were demonstrated involving lymph nodes, spleen, liver, bone marrow and adrenals; ATL of small cell type were observed on skin, lymph nodes, liver, spleen, bone marrow, adrenals, kidneys, and lungs.

**Case B.** In October 1973, a 35-year-old Chinese man was diagnosed with acute promyelocytic leukemia. The patient achieved long-term complete remission after chemotherapy combined with multiple transfusions of red cells and platelets.

In October 1984, 22 months following the incidental detection of seropositivity for HTLV-I, he presented with an epigastric mass, cervical lymphadenopathy, and several cutaneous maculopapular lesions. Computed tomography scanning of the abdomen showed enlarged para-aortic nodes. There was no lymphocytosis. Histopathological features and surface marker studies of the biopsied lymph node and skin verified the diagnosis of adult T-cell lymphoma, which was confirmed by Western blot analysis (Fig 2) and by the detection of proviral genomes in the patient’s lymphocytes (Fig 3). The lymphoma was unresponsive to chemotherapy. He soon developed hypercalcemia and died 4 months after the initial presentation of lymphadenopathy. Familial studies of HTLV-I in 1984 showed that the wife was weakly sero-positive, while a baby born in 1982 was sero-negative, suggesting possible horizontal transmission of the retrovirus from her husband. Screening for HIV was also negative.

**DISCUSSION**

ATL is a HTLV-I-related disease with characteristic geographical distribution. It has been very difficult to explain how this virus became established in a very limited area of southwestern Japan. It is interesting that ATL is seldom found in some neighboring countries of Japan, such as Mainland China and South Korea. In contrast, ATL is not uncommon in Taiwan. Results from various independent epidemiological studies disclose that approximately 0.5 to 1.0% of inhabitants in Taiwan are HTLV-I seropositive. It is hard to speculate the reasons of the presence of HTLV-I in Taiwan v Mainland China because data on the origin of HTLV-I are fragmentary and controversial. Hinuma considers that the original ATL carriers were the ancient, earliest inhabitants of Japan, while Gallo speculates that the virus originated in central Africa and spread with Africans via the slave trade to American, or via Portuguese seamen to the southwestern coastal area of Japan. Historically, Taiwan is remarkable for heavy contact with Portuguese in the 16th century, and also for 50 years’ occupation by the Japanese in the early 20th century. It might be important to point out that the Taiwan aborigines, previously considered to be free of HTLV-I infection in a smaller survey, were now shown to have similar carrier rate compared to the Taiwan Chinese. Distinctive geographical, historical, and ethnic background of Taiwan should all be taken into consideration in proposing the possible routes of HTLV-I invasion into Taiwan.
The present study demonstrates that, in Taiwan, the prevalence of HTLV-I infection is .65% in the normal adult population and 2.23% in adult patients with hematological disorders (including hematological malignancy and aplastic anemia), further confirming the findings of Shimoyama et al. in Japan and Minamoto et al. in the United States, that there is an approximately three- to fourfold increase of HTLV-I seropositivity in patients with hematological disor-
tumor DNA was digested with Pst I and hybridized with 32P-labeled HTLV-I full genome (8.0 kb) probe. Lane 1 was the undigested HTLV-I DNA in pMT-2 clone (Biotech, Maryland); lane 2 (culture cells from HTLV-I-positive MT-2 cell line), lanes 3 and 6 (two ATL patients) represented positive controls. Case B (lane 5) and lane 2 had detectable internal fragments (2.5 kb, 1.8 kb, and 1.2 kb) of HTLV-I provirus DNA. In case A (lane 4), only a 2.5 kb fragment was observed. Such an abnormal restriction pattern of the provirus was observed by Yoshida et al in approximately 10% of ATL. Lane 7 was HTLV-I-negative T-cell leukemia/lymphoma as negative control.

Fig 3. Southern blot hybridization analysis of HTLV-I provirus in tumor DNA extracted from tumor cells of case A and case B. The tumor DNA was digested with Pst I and hybridized with 32P-labeled HTLV-I full genome (8.0 kb) probe. Lane 1 was the undigested HTLV-I DNA in pMT-2 clone (Biotech, Maryland); lane 2 (culture cells from HTLV-I-positive MT-2 cell line), lanes 3 and 6 (two ATL patients) represented positive controls. Case B (lane 5) had detectable internal fragments (2.5 kb, 1.8 kb, and 1.2 kb) of HTLV-I provirus DNA. In case A (lane 4), only a 2.5 kb fragment was observed. Such an abnormal restriction pattern of the provirus was observed by Yoshida et al in approximately 10% of ATL. Lane 7 was HTLV-I-negative T-cell leukemia/lymphoma as negative control.

The two patients reported herein initially had hematological neoplasms, acquired HTLV-I infection, and subsequently developed ATL and died of ATL. Many investigators agree that transfusion of HTLV-I seropositive blood from the unscreened donor is probably the most important source of viral transmission in these patients. In our studies, such a route of transmission was highly possible, although we were unable to document that the two patients were exposed to the HTLV-I seropositive blood when they received component therapy for their primary hematological disorders. The first patient was seronegative for anti-HTLV-I antibody until one week before his first blood transfusion. Although the possibility of HTLV-I infection prior to transfusion could not be definitively excluded in case B, the chance seemed very rare, and the development of ATL suggests that certain triggering mechanisms might be related to his hematological disorders.

Case A presented features of both Hodgkin’s disease and ATL. Traditionally, Reed-Sternberg cells are regarded as pathognomonic for Hodgkin’s disease. Recently, this concept has been challenged because giant cells resembling Reed-Sternberg cells have been reported in peripheral T-cell lymphoma including ATL. In case A, the diagnosis of Hodgkin’s disease was made based on the presence of Reed-Sternberg cells and its expression of Leu-M1. Several investigators have proposed that Leu M1 represents a useful immunodiagnostic marker of Hodgkin’s disease. As Wieczorek et al recently reported that Leu-M1 might not be specific in distinguishing Hodgkin’s disease from other lymphoid proliferative disorders, the expression of Leu M1 in case A does not definitively diagnose Hodgkin’s disease. The absence of the HTLV-I proviral genome in the tissues obtained in 1979 is uncertain. However, the patient was seronegative for HTLV-I before his first blood transfusion. Although seronegative HTLV-I has been rarely reported, sero-negative ATL mimicking Hodgkin’s disease has been described recently. Seronegativity of duration longer than 10 years, as in case A, would be extremely unlikely. Second, he presented lymphadenopathy 13 years prior to the appearance of cutaneous lesions; this is unusual even in smoldering ATL, in which skin lesions are almost the earliest manifestation, while lymphadenopathy suggests disease acceleration. A long-term preleukemic state, characterized by an insidious onset and appearance of abnormal T lymphocytosis (10% to 40%) in the peripheral blood without clinical symptoms was also not suggested by the course of case A. Third, before 1984, the patient’s response to Hodgkin’s disease-directed treatment with complete remission lasting 2 years is unusual for ATL. His course suggests that he had Hodgkin’s disease since 1971, and did not have ATL before July 1984 at least.

In case B, the link between blood transfusion and HTLV-I transmission was not directly demonstrated. In addition, presence of HTLV-I seropositivity in his wife might initiate a question that he could have been infected by his wife prior to his transfusion. However, we considered that the possibility of such a chance would be extremely unlikely based on the following reasons. First, prior studies in monkeys and in the familial occurrence of ATL suggest that the horizontal transmission of HTLV-I among the spouse is essentially from husband to wife. Second, none of the parents and the siblings of the wife was seropositive for HTLV-I, indicating that she was not infected via the vertical transmission from her parents; besides, she had no prior history of blood transfusion. Hence, the most likely source of infection was from case B. Third, the ELISA test was only weakly positive in the patient’s wife, and the baby born in 1982 was seronegative for HTLV-I, suggesting that she was probably newly infected with HTLV-I. It is thus feasible to speculate that case B was most likely infected with HTLV-I via blood transfusion in 1973, and his wife was later infected from him.

The present study suggests that patients with hematologi-
cal malignancy who are carriers of HTLV-I may be at increased risk for ATL. Although the etiologic factors contributing to the development of ATL are still unknown, host immunity may be important. An intact immune system might prevent or delay the malignant transformation. In the present series two of eight hematological patients developed ATL, while in the same time duration, no ATL was observed among the 34 HTLV-I healthy carriers. The difference is statistically significant by Fisher’s exact test. Also, in comparison to the estimated annual incidence of 1/300 to 1,600 per virus carriers over 40 years of age, the tendency of transformation in the immunocompromised patients increases remarkably. In addition, the latent period from possible primary viral infection to malignant transformation in case A is much shorter than previously reported periods of up to 10 to 20 years. Thus, the immunocompromised HTLV-I carrier may be at increased risk for ATL. We can expect to see similar such patients in the near future; long-term follow-up in this group of patients is imperative.

The present report suggests that serious consideration must be given to prevent HTLV-I infection via transfusion in immunocompromised patients. Even in nonendemic areas such as Taiwan, routine serological screening for HTLV-I antibody in blood donors is indicated to permit deferral of blood product donations by asymptomatic HTLV-I carriers.

ACKNOWLEDGMENT

We thank Dr Myron E. Essex and Dr Robert C. Gallo for helpful opinions and encouragements; and Fu-Dun Chen for the preparation of the manuscript.

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