Human T-Cell Leukemia Virus-I and Hematologic Malignancies in Panama

PAUL H. LEVINE, MD* WILLIAM C. REEVES, MD, MSPH† MARINA CUEVAS, MT†
JUAN RAMON AROSEMEÑA, MD‡ ELAINE S. JAFFE, MD§ W. CARL SAXINGER, PHD,‡
MARIA ALTAFULLA, MD∥ JUDITH DE BERNAL, PHD∥ HERIBERTO ESPINO, MD∥ BARKLAY RIOS, MD∥
HARRY XATRUCH, MD∥ MARJorie BARNETT, MS∥ JAMES DRUMMOND,∥
STEVE ALEXANDER, PHD∥∥ AND WILLIAM BLATTNER, MD*§

Serum samples were obtained in a 2-year period (November 1, 1984–December 31, 1986) from 136 Panamanian patients with hematologic malignancies identified by a population-based registry designed for studies investigating human T-cell lymphotropic virus (HTLV)-I. Only three patients had clinical and serologic findings of HTLV-I-associated adult T-cell leukemia/lymphoma (ATLL). The authors conclude that although classical HTLV-I-associated ATLL occurs in the Panamanian population, it is not as prevalent as in other Caribbean populations.


HUMAN T-CELL LYMPHOTROPIC VIRUS (HTLV)-I is endemic in southern Japan and parts of the Caribbean basin and is the apparent cause of adult T-cell leukemia/lymphoma (ATLL). Several recent reports have suggested a high prevalence of antibody to HTLV-I in certain new world populations but the incidence of ATLL in these populations has not been documented. A preliminary study found HTLV-I seropositivity rates of approximately 4% among healthy individuals from Panama City and Colon. These rates are similar to those in other Caribbean areas such as Jamaica, where ATLL comprises approximately 50% of the cases of non-Hodgkin's lymphoma (NHL) in adults. Since Panama and Jamaica are similar with respect to geographic location, HTLV-I seroprevalence, but have different racial/ethnic groups, we conducted this population-based study to define the relationship between HTLV-I infection and hematologic malignancy in the Republic of Panama. We report seroepidemiologic data from all hematologic malignancies identified in the Republic between 1984 and 1986.

Materials and Methods

Identification of Cases

The Republic of Panama environmentally typifies the tropical Americas and physically and culturally links the Caribbean, Central and South America. The population is stable and composed of distinct racial and ethnic groups; the largest group is Mestizo and there is also a large black population, one segment having immigrated from the West Indies during the late 1800s and another having originated from slaves brought to Panama by Spanish colonists. The major population centers are Panama City and Colon, the metropolitan termini of the Panama Canal, and David near the Costa Rican border.

Hematologic malignancy patients were identified in a two-phase study based on the development of a population-based leukemia/lymphoma registry. In the first phase, November 1, 1984 through October 30, 1985, our registry protocol collected all new cases diagnosed at three referral hospitals: Santo Tomas and the Social Security Hospital in Panama City and Rafael Hernandez Hospital in David. In the second phase, November 1985 through December...
1986, we expanded the surveillance system to include all hematologic malignancy patients identified at government hospitals.

The additional hospitals included in the survey were as follows: (1) Panama Province, a secondary hospital in Chorrera and three tertiary care hospitals in Panama City; (2) Colon Province, a secondary hospital with Pathology and Hematology Services; (3) Coche Province, two general hospitals; (4) Veraguas Province, one general hospital; and (5) Herrera and Los Santos Provinces, six general hospitals with a modern centralized Pathology service and several Hematology Services. Surveillance encompassed 16% of all hospital beds in Panama.

For hospitals without specialized Pathology or Hematology services, we reviewed hospital discharge log books. Charts from all putative hematologic malignancy cases were reviewed by Gorgas Memorial Laboratory (GML) Division of Epidemiology staff physicians. We enrolled 136 cases over the 3-year study period. Slides from all patients were reviewed in Panama and those with NHL, with the exception of one case whose primary tumor biopsy specimen could not be located, were sent to the National Cancer Institute in Bethesda, Maryland, for additional review by one of us (E.S.J.). Typing for B-cells and T-cells was not routinely available.

**Laboratory Methods**

Five assays were utilized to detect HTLV-I antibody. All sera were tested at GML in Panama (M.C.) at a 1:10 dilution in an immunofluorescence assay (IFA) using kits kindly supplied by Dr. Daniel Zimmerman, Electronucleonics (ENI) (Columbia, MD). The IFA uses the HUT-102B HTLV-I-infected cell line and HUT-78-uninfected control. Sera positive in IFA were titered using four-fold dilutions 1:10 to 1:640; the highest positive dilution was chosen as the endpoint. All sera were also screened at a 1:20 dilution for HTLV-I antibody in a whole virus enzyme-linked immunosorbent assay (ELISA) test (Du Pont, Wilmington, DE/New England Nuclear, Boston) at National Cancer Institute-Frederick Cancer Research Facility (Frederick, MD) (J.D.) and those found positive were titered to endpoint using three-fold dilutions.

All sera positive by ELISA or IFA were tested independently, under code, in three additional assays. A competitive inhibition assay measuring ability to block (more than 50%) a reaction between heterologous specific HTLV-I antibody was performed at the National Institutes of Health (Bethesda, MD) (W.C.S.); a radioimmunoassay to detect antibody against the HTLV-I p24 antigen was performed at National Cancer Institute-Frederick Cancer Research Facility (J.D.), and a Western blot was performed at Biotech Research Laboratories, Rockville, MD (J.A.) using HTLV-I lysate prepared from singly banded virus from the culture supernatant of HUT 102 cells.

**Results**

One hundred thirty-six patients were enrolled in the study. Eighty-eight (64.7%) had lymphoproliferative diseases (Table 1) and 45 (33.1%) had myeloproliferative diseases. Three of the lymphoproliferative disease patients presented with clinical and pathologic features compatible with the diagnosis of ATLL (see "Case Reports" and "Discussion"). The HTLV-I antibodies were detected by at least one screening assay and one confirmation assay in ten patients (7.4%) (Table 2). Antibody rates varied by diagnostic category. Using Western blot as the standard for seropositivity, seven (8.0%) of lymphoproliferative malignancy patients (including all three with ATLL, one with chronic lymphocytic leukemia (CLL), one with multiple myeloma, one with acute lymphocytic leukemia (ALL), and one with an unclassified lymphoid malignancy) were seropositive contrasted with one (2.2%) of the myeloproliferative disease patients. The highest antibody titers in the confirmation assays were found in the sera of patients with ATLL and chronic lymphocytic leukemia (Table 2) and Western blot patterns obtained from these sera were typical for HTLV-I antibody (Fig. 1). The single confirmed positive ALL case was aged 16 years and lacked any clinical manifestations of ATLL. One patient (CA124) with lymphoproliferative malignancy was HTLV-I seropositive as confirmed by Western blot, and had features of ALL and NHL, but could not be classified as a specific clinical entity.

**Case Reports**

**CA1030**

This 62-year-old Mestizo woman, a resident in Panama for her entire life, presented to the Panama City Social Security Hospital in April 1985 with a 2-month history of "inflamed" hands. On physical examination, she was noted to have two soft coin-shaped nodules with central erythema on the anterior portion of her right leg, a single hard painless skin lesion on the
TABLE 2. Study Subjects With HTLV-I-Positive Serologic Confirmation Assays

<table>
<thead>
<tr>
<th>Case</th>
<th>DX</th>
<th>ENI (titer)</th>
<th>Dupont ELISA (titer)</th>
<th>Competition assay (titer)</th>
<th>p24 RIA (titer)</th>
<th>W. blot* (2+ or 3+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 30</td>
<td>ATLL (malignant lymphoma, diffuse, large cell type)</td>
<td>1:160</td>
<td>+1:28, 186</td>
<td>+1:2296</td>
<td>+1:1, 560</td>
<td>All bands</td>
</tr>
<tr>
<td>CA 57</td>
<td>ATLL (malignant lymphoma, diffuse, mixed small and large cell type)</td>
<td>≥1:640</td>
<td>+1:39, 189</td>
<td>+1:76, 192</td>
<td>+11, 262</td>
<td>All bands</td>
</tr>
<tr>
<td>CA 92</td>
<td>ATLL (non-Hodgkin’s lymphoma)</td>
<td>1:640</td>
<td>+1:2806</td>
<td>+1:270, 000</td>
<td>+1:240</td>
<td>All bands except p15</td>
</tr>
<tr>
<td>CA 124</td>
<td>Lymphoid malignancy, not classified</td>
<td>1:640</td>
<td>+1:750</td>
<td>+1:4549</td>
<td>Neg.</td>
<td>All bands except p15</td>
</tr>
<tr>
<td>CA 136</td>
<td>Chronic lymphocytic leukemia</td>
<td>1:640</td>
<td>+1:9395</td>
<td>+1:69, 480</td>
<td>+1:2167</td>
<td>All bands</td>
</tr>
<tr>
<td>CA 118</td>
<td>Hodgkin’s disease</td>
<td>1:160</td>
<td>Neg</td>
<td>Neg</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CA 43</td>
<td>Multiple myeloma</td>
<td>1:160</td>
<td>+1:1044</td>
<td>ND</td>
<td>Neg</td>
<td>All bands except p15</td>
</tr>
</tbody>
</table>

Myeloproliferative malignancies

<table>
<thead>
<tr>
<th>Case</th>
<th>Disease</th>
<th>ENI (titer)</th>
<th>Dupont ELISA (titer)</th>
<th>Competition assay (titer)</th>
<th>p24 RIA (titer)</th>
<th>W. blot* (2+ or 3+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 122</td>
<td>Acute myelocytic leukemia</td>
<td>1:640</td>
<td>+1:4505</td>
<td>+1:22, 211</td>
<td>Neg</td>
<td>p19, p24, p36</td>
</tr>
<tr>
<td>CA 85</td>
<td>Acute monocytic leukemia</td>
<td>1:160</td>
<td>+1:145</td>
<td>+1:2623</td>
<td>Neg</td>
<td>p19</td>
</tr>
</tbody>
</table>

DX: diagnosis; ELISA: enzyme-linked immunosorbent assay; RIA: radioimmunoassay; W. Blot: Western blot; ND: not done; ATLL: adult T-cell leukemia/lymphoma; HTLV-I: human T-cell leukemia virus; Neg: negative; ENI: Electronuclonics (Columbia, MD); IFA: immunofluorescence assay.

* HTLV-I antibody bands identified were p15, p19, p24, p26, p28, p32, p36, p42, gp46, and p53. p19 and p24 bands were required for an individual to be classified as seropositive for HTLV-I.

posterior surface of her right leg measuring 6 × 8 cm, hepatosplenomegaly, and bilateral leg edema. Two 4 × 4 cm hard coin-shaped lesions with erythematous borders and pale centers were also noted on the left leg. Hematologic exam was unremarkable. A skin biopsy specimen was interpreted as malignant lymphoma, diffuse, large cell type, non-epidermotropic Stage IV. Chest radiographs revealed scattered opacities in both lungs. A brief remission was induced with vincristine, cyclophosphamide, and Adriamycin (doxorubicin), but the patient died with a recurrent tumor 5 months after diagnosis. Serologic studies showed strong reactivity to HTLV-I (Table 2, Fig. 1).

CA 092

This 67-year-old black woman, a lifelong resident of the Colon/Panama City area, was first seen at Social Security Hospital, October 16, 1985 with a 2-month history of anorexia, weight loss, and cervical, inguinal, and axillary painless lymphadenopathy. Approximately 2 to 3 weeks before admission, a rash was noted over the extremities and the posterior cervical lymph node region. On initial physical examination, hepatosplenomegaly was apparent. Initial laboratory tests were unremarkable except for a leukocyte count of 17,200/mm³ with 41% neutrophils and 57% lymphocytes. A cervical lymph node biopsy was obtained which showed malignant lymphoma, diffuse, large cell type, compatible with ATLL (Fig. 2). Immunologic studies confirmed a T-cell lymphoma. Treatment was initiated with cyclophosphamide, Adriamycin, vincristine, and bleomycin. Although axillary lymph nodes diminished in size, cervical, and inguinal lymphadenopathy persisted. The patient developed esophageal candidiasis and died within 5 months after diagnosis with septicemia and NHL. Serologic studies showed high antibody titers to HTLV-I (Table 2, Fig. 1).

Discussion

Since the isolation of HTLV-I in 1980 and its linkage to an unusual human T-cell leukemia/lymphoma,9,10 a number of groups have investigated the geographic distribution of the virus and its clinical consequences. Unlike previously isolated human viruses with apparent oncogenic sequelae,11-14 HTLV-I is striking because of the geographically focal nature of its infection and the apparent
restriction of transmission to contact with infected cells. An additional problem in evaluating the seroepidemiology of HTLV-I is that unlike Epstein-Barr virus (EBV) and hepatitis virus, there is no standardized serologic assay that is used internationally, thereby producing markedly discrepant results in similar populations under study. Because of the appearance of antibody cross-reacting with HTLV-I in a significant percentage of the Panama population, we have used the National Hematologic Malignancy Registry to investigate the proportion of hematologic malignancies in Panama that are potentially due to HTLV-I.

Since 1982, cancer has been a reportable disease on a national level and we have used the registry to confirm our registration of all hematologic malignancies. Our basic source of case ascertainment has been to use the social security and government hospitals throughout the country which are referral centers for all cases of cancer. As noted above, our registry covers 76% of all hospital beds in Panama, and although it is possible that our surveillance system may not have identified all cases of ATLL in the country, it is unlikely that we have a major bias in our representation of the proportion of NHL patients with ATLL. The three lymphoma patients we describe with high titers of antibody to HTLV-I support the likelihood that this oncogenic virus is present in Panama and confirm the oncogenicity of this virus in native-born Latin Americans. Of further interest is the contrast in leukemia/lymphoma patterns in Panama and nearby Jamaica. In Jamaica, 50% of the NHL patients are reported to have HTLV-I-related ATLL whereas only three of 40 (7.5%) NHL patients in Panama had biological and pathologic signs as well as antibody titers compatible with ATLL.

The reason for the relative paucity of ATLL cases in Panama in view of a prevalence of antibody comparable to that in Japan and Jamaica is unknown but should be the subject of intensive research. Among the possible explanations are the following: (1) the existence of a variety
of viral strains with similar antigens but varying pathogenicity; (2) a variation in degree or type of environmental factors which result in different patterns of disease despite comparable virus exposure; (3) differences in genetic susceptibility; and (4) problems in case ascertainment. Regarding strain differences, isolates from Panama are currently being obtained with attempts to further characterize biologic and molecular behavior in parallel with isolates obtained from Japan and Jamaica. Little has been done to examine environmental exposures to possible oncogenic cofactors or the contribution of genetic determinants. The only studies of genetic susceptibility to ATLL that have thus far been reported, to our knowledge, are suggestive of a genetic predisposition but also note that the genetic markers evaluated (human leukocyte antigens [HLA] antigens) may have been modified by the virus.

As in other geographic locales, particularly in areas nonendemic for ATLL, case ascertainment continues to be a problem. In addition to the likelihood that some patients with ATLL did not reach the tertiary care hospitals that were our major source of patients, the varying clinical and pathologic features of ATLL complicate the diagnosis of this disease. In our series, one young patient initially diagnosed as having ALL died within 2 months of diagnosis of progressive disease despite aggressive therapy with cytosine arabinoside, Adriamycin, methotrexate, and prednisone. With the exception of the absence of antibody detectable by the p24 RIA, his serologic pattern was comparable to that observed in our patients with classical ATLL. In the absence of T-cell markers and pathologic material documenting lesions compatible with ATL, however, we could not definitively classify this patient's malignancy. In spite of the problems of ascertainment and disease classification, however, the proportion of non-Hodgkin's lymphoma patients with features compatible with ATLL confirms that Panama is not a country with a high incidence of ATLL.

Although HTLV-I appears to be etiologically related to many cases of ATLL, there are also a number of reports of non-HTLV-I-related ATL. The designation as to whether a patient has an HTLV-I-related malignancy, is not always readily determined since serologic findings and not the detection of viral genome in the tumor cells has been the criterion used in most studies. For example, Williams and associates reported two cases of HTLV-I-associated lymphoproliferative disease in Nigeria but only one patient with clinically typical ATL had HTLV-I sequences in his tumor cells whereas the other, with CLL, did not. Based on the report by Mann et al. suggesting an indirect role for HTLV-I in the pathogenesis of certain cases of CLL, the high antibody titers to HTLV-I in the patient with CLL in this study may be a reflection of the same process. The evaluation of the role of HTLV-I in disease pathogenesis based solely on the presence or absence of antibody could be particularly misleading in endemic areas because of the prevalence of antibody in the healthy population. As recently reviewed, there is no assay for HTLV-I antibody that has universal acceptance as a "gold standard" but for the purposes of this analysis, we required the presence of a positive Western blot as our confirmatory test.

If one is limited to antibody serologic study, one should look for elevated titers in screening assays and confirm these results with Western blot. Using this approach in our study, cutaneous involvement appears to be an important feature of HTLV-I-associated lymphoma, and was more consistent in our patients than in other reports. In reports from the United States, another nonendemic area for ATLL, two series showed a variety of clinical and pathologic features, thus emphasizing the difficulty in making a diagnosis unless there is a high index of suspicion of the possibility of ATLL in the population. The pathologic distinction of ATLL from mycosis fungoides/Sezary syndrome (MF/SS) appears especially problematic. Epidermotropism is a classical feature of MF/SS but is seen in approximately two thirds of ATLL patients with cutaneous disease. Features useful in the differential diagnosis include a more monomorphic cellular infiltrate in ATLL. In the current series of those patients with high antibody titers for HTLV-I who underwent skin biopsy, all had nonepidermotropic lymphoma, more readily permitting a distinction from MF/SS.

Information on the mechanisms determining the outcome of infection with HTLV-I is still in the early stages. As with EBV, it is apparent that most infected individuals will not develop signs or symptoms attributable to the virus. Also as with EBV, there is increasing evidence that HTLV-I may be etiologically related to nonmalignant disease, particularly chronic progressive spastic paraparesis. It is possible that the information gained from two decades of research involving EBV, the first reported human oncogenic virus (see Epstein and Achong and Levine et al. for recent reviews), which include evidence of different viral strains, environmental cofactors, and the role of genetics in host response, will be applicable to studies of HTLV-I. Current studies in Panama, which include isolation of virus from seropositive individuals and an intensive study of patients with neuropathies, may help to determine the relative importance of viral differences and host response in determination of disease patterns in patients with HTLV-I antibody.

REFERENCES