The Human T-Cell Leukemia/Lymphoma Virus Associated With American Adult T-Cell Leukemia/Lymphoma

By Douglas W. Blayney, Elaine S. Jaffe, William A. Blattner, Jeffrey Cossman, Marjorie Robert-Guroff, Dan L. Longo, Paul A. Bunn, Jr., and Robert C. Gallo

The human T-cell leukemia/lymphoma virus (HTLV) is a novel type-C retrovirus isolated from patients with T-lymphoproliferative malignancies. Thirteen cases of HTLV-associated malignancy from US centers were studied in detail. Ten of these cases share common clinical features and define a typical virus-associated adult T-cell leukemia/lymphoma (ATL). All ten patients presented with Ann Arbor stage IV lymphoma because of skin involvement, bone marrow involvement, or lymphomatous leptomeningitis. Lymphadenopathy occurred in 7 of 10 patients at presentation, and the malignant cells were cytologically pleomorphic. Leukemia occurred in 60% of the patients at presentation. Hypercalcemia was found initially in two-thirds of the patients, with lytic bone lesions or positive bone scans in 7 of 10. Complete remission occurred in 40%, but all have relapsed. These cases closely resemble those virus-positive cases of adult T-cell leukemia/lymphoma (ATL) reported from Japan and the Caribbean. Three additional virus-positive patients had atypical presentations and diagnoses (acute lymphocytic leukemia, Sézary’s syndrome, leukemic reticulendotheliosis), usually with less aggressive clinical courses and atypical demographic and laboratory features. Presence of HTLV serum antibodies in cases of ATL (with hypercalcemia and circulating malignant cells) appears to define a distinct clinicopathologic entity that may occur in geographic clusters.

The UNIQUE RETROVIRUS, the human T-cell leukemia/lymphoma virus (HTLV), was first isolated, identified, and characterized from a US patient with cutaneous T-cell lymphoma. HTLV is distinct from other human viruses and from animal retroviruses by studies of its nucleic acids, its major core proteins p24 and p19, and its reverse transcriptase. Seroepidemiologic studies in 1981 first demonstrated that HTLV was associated with the previously described clinical entity, adult T-cell leukemia (ATL) of Japan. Later, Catovsky et al. reported a cluster of T-lymphosarcoma cell leukemia (LCL) in Caribbean blacks that was shown to be HTLV associated. Subsequently, three additional cases of lymphoma, hypercalcemia, bone lesions, and HTLV antibodies were reported. In later serologic surveys, a distinct clinical syndrome has emerged as a “sentinel disease” for association with HTLV. We describe HTLV-associated lymphoma cases ascertained during a disease-oriented seroepidemiologic survey and treated at US institutions. Patients who had specific serum antibodies to disrupted HTLV viral particles (and most had antibodies to viral core protein p24) qualified for inclusion into the study. The characteristic clinical and pathologic disease is distinct from cutaneous T-cell lymphoma (CTCL), and common features are shared by most patients. The typical disease is apparently unusual in the US, where only one endemic focus has been tentatively identified.

MATERIALS AND METHODS

Serum and plasma samples were submitted to the authors by over 50 clinicians and investigators in the United States. Samples were solicited as part of a worldwide effort to characterize HTLV-associated disease and represented patients with lymphoreticular (particularly T cell), hematologic, and other malignancies. Only patients treated at US institutions and not Caribbean born (who were reported elsewhere) qualified for inclusion in this study. When a positive sample was found, the patient, if alive, was examined by one of us (D.W.B) and further samples were obtained for lymphocyte culture and other studies. Families of index patients were also investigated.

Serum antibodies to a disrupted whole virus preparation were detected by a modification of the solid-phase radioimmunoassay previously reported. Specimens were considered positive only of their binding to HTLV could be subsequently competed by extracts of HTLV and HTLV-producing cells, but not by extracts of other retroviruses or cells not producing HTLV, nor by fetal calf serum.

Natural antibody to the major core protein p24 of HTLV was detected by a radioimmune precipitation (RIP) assay as previously described. Specificity of the HTLV RIP p24 was demonstrated by competition experiments using preparations of disrupted retroviruses or cellular extracts and limiting dilutions of positive human sera.

1-2-Aminoethylisothiouronium (AET) bromide-treated sheep erythrocyte rosette formation, monoclonal antibody staining, and flow cytometry were performed as described previously. Murine monoclonal antibodies included OKT4, OKT8, OKT11 (Ortho Diagnostics, Raritan, NJ) and Leu-1, Leu-2a, Leu-3a, and Leu-4 (Becton Dickinson, Sunnyvale, CA).

RESULTS

Thirteen patients met the inclusion criteria and are the subject of this report. Ten of the 13 represent a...
common clinical entity; their major features are listed in Table 1. The patients were relatively young (mean age 32 yr) and presented with diffuse intermediate or high-grade lymphomas.\(^5\) Two distinguishing features of HTLV-associated lymphoma include malignant cells in the peripheral blood (uniformly present) and hypercalcemia (present in all but one patient). Other common features include lymphadenopathy, bone marrow involvement, hepatosplenomegaly, skin lesions, and lytic bone lesions. All patients would be classified as stage IV in the Ann Arbor classification,\(^16\) based on skin lesions (50%), bone marrow involvement (55%), CNS involvement (30%), or pathologically proven liver involvement or tumor cells in ascites fluid (20%).

Lymph node biopsy tissue from all patients was reviewed in the Laboratory of Pathology, NCI, and classified according to the working formulation.\(^15\) Nine patients had diffuse lymphomas of intermediate or high grade. There were five patients with diffuse immunoblastic lymphoma (including the initial patient who was previously classified as CTCL and from whom HTLV was first isolated\(^17\)), three patients with diffuse mixed (small and large cell) lymphoma, and one with diffuse large cell lymphoma. One patient had a T-cell lymphoma that was unclassifiable in the working formulation, but was classified in the Rappaport scheme\(^8\) as diffuse poorly differentiated lymphocytic lymphoma with T-lymphocyte surface markers.\(^19\) Nuclear pleomorphism was generally evident, but was not as conspicuous a feature as generally reported in ATL.\(^20\) In two-thirds of the patients with cutaneous lymphoma, epidermal involvement was seen, making a distinction from mycosis fungoides and Sézary’s syndrome on purely morphological grounds difficult. Currently, no cytologic or histologic features are pathognomonic for HTLV association, although presence of highly pleomorphic and polylobed lymphoid cells in the peripheral blood is highly characteristic, if such cells are present.

Results of serologic studies and tumor lymphocyte surface marker studies on these 10 patients are shown in Table 2. All patients had serum antibodies to a disrupted preparation of whole virus. Antibody binding was not competed by non-virus-producing cells, but was competed by HTLV-producing cells. Serum antibodies to the viral core protein p24 were also present. Malignant cells, when available for study, had surface markers characteristic of T cells. All patients studied in our laboratory or by their referring physicians had malignant cells with pan-T-cell (AET-treated sheep erythrocyte rosette, Leu-1, Leu-4, or OKT11) surface markers. Further, the cells also expressed the “helper” cell phenotype (Leu-3a or OKT4) and did not have the “suppressor” surface marker (Leu-2a or OKT8).

Of the 10 typical patients, all four mainland US-born cases were black and came from 1 of 3 southeastern states. Two patients were born in South America (Brazil and Ecuador) and were referred to US institutions for their treatment. Additional typical patients were born in Japan (and emigrated from the HTLV endemic region 9 yr before developing lymphoma), Israel, and Alaska. In contrast to Japanese ATL, only about one-half of our typical patients had rural birthplaces. Adult occupations were predominately indoor (e.g., schools, factories, or hospitals). Three of the patients had outdoor occupations, including farming and construction.

### Table 1. Typical HTLV-Associated Lymphoma/Leukemia: US Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
<th>Place of Birth</th>
<th>Diagnosis†</th>
<th>Leukemia†</th>
<th>Hypercalcemia</th>
<th>Bone Marrow Involved†</th>
<th>Lymphadenopathy†</th>
<th>Initial Skin Lesions</th>
<th>Initial Hepatosplenomegaly</th>
<th>Lytic Bone Lesions</th>
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</thead>
<tbody>
<tr>
<td>C.R.</td>
<td>28</td>
<td>M</td>
<td>Black</td>
<td>Alabama</td>
<td>DIBL</td>
<td>+/+/+</td>
<td>+/+/+</td>
<td>+/+/+</td>
<td>+/+/+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>W.A.</td>
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<td>M</td>
<td>Black</td>
<td>Georgia</td>
<td>DML</td>
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<td>+/+/+</td>
<td>+/+/+</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J.S.</td>
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<td>M</td>
<td>White</td>
<td>Ecuador</td>
<td>DML</td>
<td>-/+/-</td>
<td>+/+/+</td>
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<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>M</td>
<td>White</td>
<td>Israel</td>
<td>DCL</td>
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<td>+/+/+</td>
<td>-/+/-</td>
<td>-/+/-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>P.L.</td>
<td>27</td>
<td>F</td>
<td>Black</td>
<td>Florida</td>
<td>DML</td>
<td>+/+/+</td>
<td>-/+/-</td>
<td>+/+/+</td>
<td>-/+/-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>L.J.</td>
<td>46</td>
<td>M</td>
<td>Black</td>
<td>US</td>
<td>DPDL-T</td>
<td>+/+/+</td>
<td>+/+/+</td>
<td>+/+/+</td>
<td>-/+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S.D.</td>
<td>49</td>
<td>F</td>
<td>Japanese</td>
<td>S.W. Japan</td>
<td>DIBL</td>
<td>+/+/+</td>
<td>-/†</td>
<td>+/+/+</td>
<td>-/†</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>O.B.</td>
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<td>F</td>
<td>Black</td>
<td>Georgia</td>
<td>DIBL</td>
<td>-/+/-</td>
<td>+/+/+</td>
<td>-/+/-</td>
<td>+/+/+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M.A.</td>
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<td>F</td>
<td>White</td>
<td>Brazil</td>
<td>DIBL</td>
<td>+/+/+</td>
<td>-/+/-</td>
<td>NP§</td>
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<tr>
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<td>76</td>
<td>M</td>
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<td>DIBL</td>
<td>-/+/-</td>
<td>NP†</td>
<td>+/+/+</td>
<td>+/+/+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

†DIBL: diffuse immunoblastic lymphoma; DML: diffuse mixed lymphoma; DCL: diffuse large cell lymphoma; DPDL-T: diffuse poorly differentiated lymphomas with T-cell markers (not classified in working formulation).
‡Manifestation expressed as present initially/present during clinical course.
§NP, not performed: "†" denotes patient still alive and being actively treated and has not exhibited manifestations thus far.
¶Defined as lymphoma cells present in dermis or epidermis. Patient U.K. had parapsoriasis on biopsy.
Three of the 13 patients do not fit into the typical syndrome (Table 3). One patient, a 49-year-old white merchant marine seaman, has classic exfoliative erythroderma and circulating Sézary cells. He is the third patient from whom HTLV was isolated and has survived since diagnosis in 1978. Cultured malignant T cells express HTLV, and his serum contains antibody to HTLV antigens.21 A second “atypical” patient, a 37-yr-old black man, had terminal deoxynucleotidyl transferase (TdT) positive acute lymphoblastic leukemia and serum antibodies to HTLV. The third “atypical” patient has leukemic reticuloendotheliosis (hairy cell leukemia) and tartrate-resistant acid phosphatase (TRAP) positive cultured T cells that express HTLV-II.22,24 He also has serum antibodies to HTLV and has had prolonged survival since splenectomy in 1976.

DISCUSSION

In US patients, typical HTLV-associated lymphoma is a distinct subset of the so-called “peripheral T-cell lymphomas”25 and is best described as the clinicopathologic entity of ATL. Typical features of the US HTLV-associated ATL cases usually include hypercalcemia, leukemia at presentation or during the clinical course, bone marrow involvement, lytic bone lesions, and skin involvement. Bone lesions often do not contain lymphoma cells, but are areas of fibrosis and increased osteoclastic activity.9,19 While US-born patients with the typical disease are usually black, patients born in some other areas of the world are white.

Several features of HTLV-associated lymphoma deserve the attention of clinicians. In addition to hypercalcemia, which may be difficult to diagnose and treat,8 involvement of the central nervous system occurs in many patients and is manifested as lymphomatous leptomeningitis. During the initial staging evaluation of all HTLV-associated patients, the cerebrospinal fluid cytology should be evaluated so that appropriate treatment can be given. Repeated lumbar punctures and cytologic examinations are often necessary to establish the diagnosis of lymphomatous leptomeningitis. Furthermore, opportunistic infections are also a common complicating factor. Often these infections occur in patients treated with corticosteroids for refractory hypercalcemia.

The role of HTLV in cutaneous T-cell lymphomas (CTCL, mycosis fungoides/Sézary’s syndrome) is currently unclear. At least one patient with classic mycosis fungoides/Sézary’s syndrome has HTLV serum antibodies and HTLV produced from his cultured T cells. A serologic survey of 252 patients with CTCL uncovered no other patients with serum antibodies to HTLV.27 These results are in contrast to surveys of Japanese ATL, where over 90% of patients had HTLV

Table 2. HTLV Antibody Determinations and Cell Surface Marker Studies in US Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Antibodies to Disrupted HTLV</th>
<th>Antibodies to HTLV p24</th>
<th>Pan-T* Surface Markers</th>
<th>“Helper”* Surface Markers</th>
<th>“Suppressor”* Surface Markers</th>
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</thead>
<tbody>
<tr>
<td>C.R.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>W.A.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>J.S.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>U.K.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>P.L.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>L.J.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>S.D.</td>
<td>+</td>
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<td>NP</td>
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<tr>
<td>O.B.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NP</td>
<td>NP</td>
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<tr>
<td>M.A.</td>
<td>+</td>
<td>NP†</td>
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<tr>
<td>J.N.</td>
<td>+</td>
<td>+</td>
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<td>NP</td>
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</table>

*Pan-T surface markers include Leu-1, Leu-4, OKT11, and agglutination with sheep erythrocytes; “helper” surface markers include OKT4 and Leu-3a; “suppressor” surface markers include OKT8 and Leu-2a; see Materials and Methods.
†Not performed; fresh or frozen cells not available for assay.

Surface marker studies for C.R. reported in reference 17; for L.J. in reference 19; and for S.D. in reference 35.

Increased osteoclastic activity.9,19 While US-born patients with the typical disease are usually black, patients born in some other areas of the world are white.

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antibodies,38,29 and US cases of peripheral T-cell lymphoma, where 10% had HTLV antibodies.39 Furthermore, CTCL has not been reported to occur in geographic clusters, suggesting different etiologic mechanisms for ATL and CTCL. Studies using molecular biologic techniques and other serologic assay systems31 might strengthen the association of HTLV with classic CTCL, but currently, the association must be regarded as unusual. Furthermore, our preliminary serologic surveys, which included patients with both acute lymphocytic leukemia and leukemic reticuloendotheliosis,27,29 have not uncovered other patients with these diseases who have HTLV antibodies. Consequently, our three “atypical” patients may represent one or more of the following: (1) coincident malignant disease and HTLV infection; (2) superinfection of previously transformed cells with HTLV; (3) a true etiologic association of HTLV with a small fraction of cases that may have other, more frequently occurring causes; (4) a more frequent association that is not detectable by immunologic techniques because of limited virus replication in the host.

Leukemia/lymphomas associated with HTLV are diseases of mature thymic-derived lymphocytes (T cells). The disease most strongly associated with HTLV by serologic, epidemiologic, and virologic criteria is ATL. ATL was extensively characterized in Japan on clinical, immunologic, and epidemiologic grounds32 and is endemic in Kyushu and Shikoku. The disease most strongly associated with ATL was extensively characterized in Japan on clinical, immunologic, and epidemiologic grounds32 and is endemic in Kyushu and Shikoku. The virus-like particle seen by Japanese investigators and tested in our laboratory,28 and HTLV has been recently isolated from Japanese cases.22

These 10 typical cases of American ATL demonstrate that HTLV-associated lymphoma, as it occurs in the United States, represents a distinct clinical entity. Native-born patients referred to the authors have been young, black, and born in southeastern US. Pathologic characteristics of the lymphomas are diffuse, and they are of the intermediate or high grade.15 Clinical features include some combination of leukemic transformation, hypercalcemia, lytic bone lesions, and hepatosplenomegaly. Lymphomatous leptomeningitis and opportunistic infections, which can be subtle features, are rapidly fatal if not appreciated and treated. Overlap between American, Japanese, and Caribbean ATL is apparent from the clinical, pathologic, and virologic information presented here.

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REFERENCES

12. Robert-Guroff M, Fahey KA, Maeda M, Nakao Y, Ito Y,
The human T-cell leukemia/lymphoma virus associated with American adult T-cell leukemia/lymphoma

DW Blayney, ES Jaffe, WA Blattner, J Cossman, M Robert-Guroff, DL Longo, PA Jr Bunn and RC Gallo