Epidemiology and Immunovirology of Human T-Cell Leukemia/Lymphoma Virus Type I-associated Adult T-Cell Leukemia and Chronic Myelopathies as Seen in France


Abstract

Seventeen patients with adult T-cell leukemia (ATL) and 21 with tropical spastic paraparesis/human T-cell leukemia/lymphoma virus type I (HTLV-I)-associated myelopathy (TSP/HAM) were observed during a 3-year period (1986–1988) in some hospitals in Paris, France. Most of them were black, originating from high-HTLV-I-endemic areas (West Indies or Africa), but a second case of TSP/HAM occurred in French Caucasians. In one case, the patient acquired the virus from a transfusion during a cardiac transplantation. Most of the ATL cases were diagnosed as acute leukemia or lymphoma, with a proliferation of CD2+, CD3+, CD4+, CD8-, DR-, and CD25+ lymphoid cells. Only three cases were diagnosed as smoldering ATL. All of the TSP/HAM cases exhibited a spastic paralysis with a chronic and slow evolution. A high HTLV-I antibody titer was observed in serum and cerebrospinal fluid, with a high HTLV-I antibody index and specific HTLV-I immunoglobulin = oligoclonal bands. In TSP/HAM, a high percentage of DR-expressing cells (15 to 40%) was found, with a slightly elevated CD4/CD8 ratio. This was associated with the presence of 1 to 10% abnormally shaped nuclei in lymphoid cells and a polyclonal integration of HTLV-I proviruses in these peripheral blood mononuclear cells. On the contrary, a clonal integration was always found in the ATL malignant cells (leukemic, lymph node, and cutaneous infiltrate). Long-term interleukin 2-dependent T-cell lines (CD2+, CD3+, CD4+, and WT31+) with activated T-cell markers (CD25+ and DR+) producing HTLV-I were thus established from ATL and TSP/HAM peripheral blood mononuclear cells.

Introduction

HTLV-I, a type C retrovirus discovered in 1980 (1), is considered as the etiological agent of ATL (2, 3). This lymphoproliferative malignancy occurring in HTLV-I high endemic areas (Japan, Caribbean, South America, and Africa) is characterized by a clonal expansion of CD4-positive lymphocytes and a monoclonal integration of HTLV-I provirus in the tumoral cells (4). This retrovirus, transmitted mainly by breast feeding from mother to child, by sexual route from men to women, or by blood transfusion, has also been linked in 1985 (5) with a chronic progressive myelopathy frequent in viral endemic areas and referred to as TSP or HAM (6–8). We present here an update of our studies concerning the epidemiological and immunovirological aspects of these two diseases in France, a low endemic area for HTLV-I.

Materials and Methods

Patients. All the patients seen in St. Louis Hospital, Paris, France, with an ATL diagnosis were studied during a 36-mo period (1986–1988) as well as ATL cases diagnosed in other hospitals in Paris during the same period were included in this study. Furthermore, at the Pitié-Salpêtrière in Paris, site of the largest neurological department in France, study of most of the patients with CPM was carried out during the same period. Fifty cases of CPM were thus registered, among whom 21 representing a relatively homogenous group exhibited HTLV-I antibodies in both their serum and CSF.

HTLV-I Antibody Assays and CSF Studies. Three tests were used to detect HTLV-I antibodies: (a) ELISA (Dupont, Wilmington, DE); (b) indirect IF assay, using the HUT-102 cell line as the source of HTLV-I antigen; and (c) PA (Fujirebio, Inc., Japan). Serial 2-fold dilution of serum or CSF was used to determine the titers of HTLV-I antibodies. For Western blot confirmation, we used the available preblotted Dupont Western blot kits, with serum diluted 1:200 and CSF diluted 1:20. In some cases, RIPA was used as previously described (9). Two indicators of intrathecal IgG production were used: the IgG index (10) and the BBB IgG synthesis (9). Detection of the CSF IgG oligoclonal band pattern was performed as previously described (9, 10).

Morphological and Surface Markers Studies. Cell morphology was analyzed by light microscopy on May–Grünwald-Giemsa-stained PBMC. The surface phenotype of the cells was determined by a standard method of indirect immunofluorescence and cytofluorograph reading, using a large panel of MoAbs that recognize T-associated or T-restricted antigens (CD2, CD3, CD4, CD7, and CDS) and B-restricted antigens (CD19 and CD20). Other MoAbs were also used (CD25, CD33, CD41, anti-Class II molecule DR, and WT31).

Molecular Hybridization. High-molecular-weight DNA was extracted from uncultured PBMC from TSP/HAM and ATL patients, (6) lymphoid cells of ATL lymph nodes, and (c) cutaneous biopsy of the three smoldering ATL cases. The Southern blot analysis was performed as previously described (11), using different HTLV-I radiolabeled probes (kindly provided by M. Yoshida, Cancer Institute, Tokyo, Japan).

Isolation and Characterization of HTLV-I. PBMC of TSP/HAM and ATL patients were isolated by standard procedures and cultured after initial stimulation by phytohemagglutinin in RPMI medium, with 10% of interleukin 2 as previously described (12, 13). HTLV-I antigens were detected in these cultured cells by an IF test, using human polyclonal (sera and CSF from TSP and ATL cases) and mouse monoclonal antibody anti-p19/p24 (Dupont). Positive control cells included HUT 102 and MT2 bearing HTLV-I. Uninfected CEM cells served as negative controls. Electron microscopy was regularly done on these cultured cells using standard procedures.

Results

Seventeen patients with ATL and 21 patients with TSP/HAM were observed during this 3-yr survey in some hospitals in Paris, France. While 11 of 17 ATL cases were men, a female...
Table 1 Geographical origin of ATL and TSP/HAM patients as observed in some hospitals in Paris, France, between 1986 and 1989

<table>
<thead>
<tr>
<th>Place of birth</th>
<th>ATL</th>
<th>TSP/HAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>French West Indies</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Martinique</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Guadeloupe</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>French Guiana</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Haiti</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Ivory Coast</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gabon</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Morocco</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Zaire</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central African Republic</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Senegal</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mauritania</td>
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<td></td>
</tr>
<tr>
<td>Peru</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>21</td>
</tr>
</tbody>
</table>

predominance (15 of 21 cases) was observed in TSP/HAM. As seen in Table 1, all the ATL cases occurred in black patients originating either from the Caribbean area (French West Indies and French Guiana) or African countries. A similar origin was found in the majority of TSP/HAM patients. However, we observed 2 French Caucasian cases, one being a 41-yr-old man who acquired HTLV-I from a transfusion during a cardiac transplantation (14) and the second being a woman who probably acquired the virus from her husband, a French Caucasian man found HTLV-I seropositive without a known risk factor for such an infection.

Most of the ATL cases were diagnosed as acute leukemia or lymphoma with a high white blood count, peripheral lymph node enlargement, hepatosplenomegaly, and hypercalcemia. Three patients had a smoldering ATL, with specific lymphoid cutaneous lesions and a few PBMC with abnormal nuclei or typical ATL features.

All the TSP/HAM patients exhibited a spastic paraparesis or paraplegia with sphincter disturbances and minimal sensory loss. A chronic but slow evolution without neither remission nor attack was regularly observed, without personal or familial neurological history. The spinal imaging (myelography or spinal magnetic resonance imaging) was normal in all patients. Evoked potential studies showed an abnormal central conduction in 14 patients of the 17 TSP studied, and brain magnetic resonance imaging showed a high signal lesion on T2 sequences in 9 cases of the 16 studied. None of the 21 TSP/HAM patients developed an ATL during the time of the survey.

Specific HTLV-I antibodies were detected in the sera of all ATL cases but one and in all the sera and CSF of the TSP/HAM patients by ELISA, IF, and PA. By Western blot, reactivities against gag-encoded proteins p19, p24, and Pr53 were regularly detected in the sera and CSF of TSP patients, while detection of env-encoded gp46 and gp62, although found in most of the sera and CSF, was easier using RIPA than Western blot. Both sera and CSF of TSP/HAM patients exhibited HTLV-I antibodies in a pattern qualitatively undistinguishable from that of patients with ATL. The only difference was a quantitative one (significantly higher titers were observed in TSP/HAM sera than in ATL sera). The intensity of the Western blot reaction in serum at the same dilution of 1:200 was always higher with a clearer resolution in TSP/HAM serum than in ATL serum, for which the Western blot reaction was eventually faint and difficult to analyze. No HTLV-I antibody was detected in the CSF samples of the four ATL cases studied. Intrathecal IgG synthesis was present in 20 of 21 TSP/HAM cases, with an elevated IgG index (>0.70) and/or an elevated BBB synthesis (>3.5 mg/day). Furthermore, IgG oligoclonal bands were found in 19 of 21 TSP/HAM CSF samples, and the HTLV-I specificity of some of them was demonstrated by immunoblot and absorption studies (10). In contrast, neither intrathecal IgG synthesis nor IgG oligoclonal bands were found in the CSF/serum samples of the 4 ATL cases studied.

In the studied cases, the leukemic cells of ATL were always CD2+, CD3+, CD4+, CD8−, CD19−, and CD20−, with a high expression of activated T-cell markers CD25+ and HLA DR+. In the PBMC of smoldering ATL cases, the phenotypic analysis was normal or showed only a slight increase of HLA DR expression. In TSP/HAM patients, the majority of the T-cells were CD4+, and the CD4/CD8 ratio was slightly elevated in most of the cases. The percentage of B-cells (CD20+) was low, as well as the percentage of the monocyte population (CD33+). The major finding was the presence of a high percentage of DR+ cells ranging from 15 to 40%, which largely exceeds that of B-cells and monocytes in all the patients, indicating that DR-espressing cells were present in these patients. The percentage of CD25+ cells was low (range, 1 to 15%). In these TSP/HAM, lymphoid cells with an abnormally shaped nucleus were detected in 5 to 20% of the total lymphoid cells in all the patients. Typical ATL-like cells with foliated nuclei and hyperbasophilic cytoplasm were detectable in most of the patients’ WBC, representing 1 to 5% of the total lymphocytes.
A clonal integration of one, two, or three HTLV-I proviruses, often defective, was demonstrated by Southern blot analysis (Fig. 1) in the tumorous cells (leukemic, lymph node, or lymphoid cutaneous infiltrate) of the 8 ATL cases, where DNA was available for molecular studies.

Furthermore, a T-cell receptor \( \beta \) and/or \( \gamma \) gene rearranged pattern was observed in these 8 cases, indicating a T-cell clonality of the lymphoid proliferation. In contrast, a polyclonal integration of HTLV-I proviruses was detected in the PBMC of all the 14 TSP/HAM cases studied. By dilution experiments, we could estimate that this HTLV-I random integration was present in 3% to 15% of the PBMC of these TSP/HAM patients. HTLV-I antigens could never be detected in uncultured PBMC of TSP/HAM nor in leukemic cells of ATL using IF, but after short-term culture in the presence of 10% IL2, specific HTLV-I antigens were easily detectable by IF in lymphoid elements of 6 of the 7 cultured ATL/PBMC and in 13 of 15 TSP/HAM cultured PBMC. Furthermore, the presence of C-type retroviral particles was seen in the extracellular spaces by electron microscopy in these ATL and TSP/HAM cultures. Long-term IL2-dependent (5 to 10% for optimal growth) T-cell lines (CD2+, CD3+, CD4+, and WT31+) with activated T-cell markers (CD25+ and DR+) producing HTLV-I were established in 4 cases of ATL and in 10 cases of TSP/HAM. In 5 cases of TSP/HAM cell lines, expression of CD8 was detected in a significant number of cells. The pattern of fluorescence staining using either polyclonal or monoclonal HTLV-I antibodies was similar in TSP/HAM and ATL cell lines, while slight differences in high-molecular-weight peptides were detected by Western blot, when comparing TSP/HAM- and ATL-derived cell lines (12, 13).

**Discussion**

These preliminary results indicate that patients with an ATL or a TSP/HAM seen in France usually originate from HTLV-I highly endemic areas (Footnote 4; Refs. 14 and 15). This is comparable to the results of Catovsky et al. (16) and Greaves et al. (17) for ATL and Cruickshank et al. (18) for TSP/HAM, whose patients studied in London originated from Jamaica and the British West Indies. However, some cases of ATL and TSP/HAM seen in Italy suggest the presence of microfoci of HTLV-I-endemic areas in continental Europe (19).

The main immunological and molecular characteristics of ATL and TSP/HAM are summarized in Table 2. While, as noted above, monoclonal integration is the rule in tumoral cells of ATL patients studied in different parts of the world (3, 4, 20, 21), polyclonal integration is regularly observed in PBL of TSP/HAM (11, 21, 23). The main and constant hematological and immunological disorders in TSP/HAM patients, despite their geographical origin, are the presence in PBMC of 1 to 20% of morphologically abnormal lymphoid cells, either with a mishaped nucleus or with typical ATL cells (multifoliated nucleus). This feature is associated with an elevated CD4/CD8 ratio and with the presence of T-activated cells (TAC+ and DR+) (11, 24, 25).

Furthermore, evidence of intra-CNS HTLV-I viral activity, only seen in TSP/HAM, comes from the regular observation of an elevated HTLV-I antibody index (9), IgG oligoclonal bands in CSF with HTLV-I specificity (10), and the presence of HTLV-I isolated in vitro from CSF cells (26, 27).

The question of the identity of HTLV-I associated with either ATL or TSP/HAM is critical. Molecular characterization of HTLV-I isolates from PBL or CSF lymphocytes of patients with either ATL or TSP/HAM from different geographical areas showed very similar molecular characteristics suggesting that, in contrast to HIV-1 or 2, HTLV-I is quite stable. Howver, up to now, only a few complete nucleotidic sequences have been established (28-30), and further characterization of various isolates is needed. Slight differences have been observed in

**Table 2 In vivo immunovirological aspects of HTLV-I and associated diseases (ATL, TSP/HAM)**

<table>
<thead>
<tr>
<th>Mode</th>
<th>Site</th>
<th>% cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute type</td>
<td>Leukemic cells</td>
<td>100</td>
</tr>
<tr>
<td>Smoldering type</td>
<td>Leukemic cells</td>
<td>100</td>
</tr>
<tr>
<td>TSP/HAM</td>
<td>Leukemic cells</td>
<td>85</td>
</tr>
<tr>
<td>Intermediate state</td>
<td>Leukemic cells</td>
<td>100</td>
</tr>
<tr>
<td>Healthy carrier state</td>
<td>Leukemic cells</td>
<td>100</td>
</tr>
</tbody>
</table>

*PCR, polymerase chain reaction; TCR, T-cell receptor.*

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the molecular weight of the env gene product in TSP- versus ATL-derived cell lines (12, 13). Since the MuLV neuropathogenicity appears to be related to minor nucleotide sequence differences observed at the 3’ end of the polymerase gene and within the env gene region (31), these slight differences and the observed minor nucleotide sequence variations between isolates could be of importance in the pathogenesis of the associated diseases. Of further interest is the fact that certain env determinants of MuLV appear to control the interaction between the viral envelope glycoproteins and the brain cell receptors (32).

The recent observation by Gout et al. (14) of the rapid development of a subacute myelopathy (undistinguishable from a typical TSP/HAM), following an HTLV-I-contaminated transfusion in a French Caucasian cardiac transplant patient, provides conclusive evidence that HTLV-I is the etiological agent of the pyramidal syndrome named TSP/HAM. The pathogenic pathway by which HTLV-I induces either ATL or TSP/HAM could differ. Transactivation of IL-2 receptor by the HTLV-I tax gene seems to represent an early and essential step in leukemogenesis. A second or third genetic event would be required for the development of an ATL, in a manner comparable to Burkitt’s lymphoma pathogenesis (33).

For TSP/HAM pathogenesis, different possibilities could be proposed (34): (a) direct viral invasion of the nervous system, with viral tropism for either neurons, glial cells, and/or astrocytes bearing HTLV-I receptors; (b) a cell-mediated immune reaction in the central nervous system due to the presence of T-cells infected by HTLV-I in the CSF or the CNS parenchyma; (c) an antibody-mediated damage to CNS tissue by cross-reacting antibody directed against HTLV-I and some epitopes of the CNS; and (d) a neurotoxic effect of some HTLV-I-induced proteins released by infected T-cells or by endothelial cells within the brain.

Our initial observation (5, 6, 35) that TSP/HAM patients might exhibit systemic manifestations related to autoimmune or lymphoid malfunctions, such as Gougerot-Sjögren syndromes, alveolar lymphocytosis, mononuclear gammopathy, etc., raises the question of an extended multipathogenic potential of HTLV-I. ATL is endemic in southern Italy: detection of the first infectious cluster in a white patient, 1990.

References


Epidemiology and Immunovirology of Human T-Cell Leukemia/Lymphoma Virus Type I-associated Adult T-Cell Leukemia and Chronic Myelopathies as Seen in France

A. Gessain, O. Gout, F. Saal, et al.


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