In this report we describe an Italian patient with chronic T cell leukemia whose proliferating cells were mature T lymphocytes with a helper phenotype (T helper phenotype chronic lymphocytic leukemia, or Thp-CLL). Unlike other reported cases of Thp-CLL, fresh leukemic cells from this patient were positive with the anti-Tac monoclonal antibody, which recognizes the receptor for interleukin-2 (IL-2). Thus, the phenotype of these cells was similar to that expressed by Japanese patients with adult T cell leukemia (ATL) (OKT3+, OKT4+, OKT8-, Tac+). However, the Italian patient had Thp-CLL, not ATL, since his cells, unlike ATL cells, lacked human T cell leukemia virus (HTLV-I)-related DNA sequences. The Tac receptor, which appears to be modulated in vitro by the anti-Tac antibody, was biologically inactive, since the patient’s cells did not respond in vitro to IL-2. In addition, they also failed to demonstrate in vitro functional activities. The clinical course was aggressive, as usual, for both Thp-CLL and ATL. Taking advantage of the description of this case, some similarities and differences between Thp-CLL and ATL are discussed, focusing on the importance of screening of HTLV-I in the differential diagnosis.

MATERIALS AND METHODS

Case Report

S.B., a 68-year-old Italian man came to us in September 1982. Seventeen months earlier, CLL had been diagnosed. At the time of diagnosis (April 1981), laboratory data showed a WBC count of 56,000/μL, with 90% lymphocytes, 8% granulocytes, and a platelet count of 360,000/μL. Hemoglobin (Hb) was 15 g/dL. Other routine blood biochemical evaluations (including Coombs’ tests) were normal. Bone marrow was infiltrated by 85% mature-appearing lymphocytes. He had moderate spleen size, remarkable liver enlargement, and generalized lymphadenomegaly. Tonsils were also enlarged and biopsy showed infiltration of mature lymphocytes. WBC count increased rapidly to 90,000/μL and treatment with chlorambucil and prednisone (PD) was initiated without affecting the clinical course or the WBC count (80,000 in December 1981). The patient was then treated with COP (cyclophosphamide, vincristine, and PD); during this therapy his WBC count rose to 170,000/μL, and leukopheresis was performed. Because of unsuccessful COP treatment, he later (May 1982) received bleomycin, Adriamycin, PD, and splenic irradiation (800 rad). When he came to us again under treatment with chlorambucil, his spleen and liver remained enlarged, Hb and platelets levels were in the normal range, and WBC count was 62,000/μL, with 92% mature-appearing large nongranular lymphocytes (LNGLs); these had rather abundant cytoplasm without granules and irregular nuclei with margination of...
heterochromatin and prominent nucleoli (Fig 1). These features are similar to those previously reported as characteristic of prolymphocytic leukemia (PLL) cells. However, this designation, originally referred to as B-CLL, has been subsequently applied to T-CLL, where immunologic data have shown that “prolymphocytes” are indeed mature T lymphocytes with nucleoli and, usually, a helper phenotype. These cases are therefore now included in Thp-CLL. The patient’s bone marrow was infiltrated with 84% LNLGls. Cytocentriar evaluation showed positivity of peripheral blood mononuclear cells (PBMCs) for dyepeptidyl amino-peptidase IV (DAP IV). At that time, serum IgG was 1,567 mg/dL; IgA, 470; and IgM, 263. Calcaemia was 4.8 meq/L, and signs of increased bone turnover were not present. Serologic tests (September 1982) showed past but not present infections by herpes simplex virus, cytomegalovirus, and Epstein-Barr virus. The search by radioimmunoassay for serum antibodies against HTLV-I was conducted by Dr R.C. Gallo (Bethesda) and found to be negative. Cytogenetic analysis showed a chromosome number ranging between 44 and 47, with multiple numerical and structural clonal anomalies including a break point in 14q12. The patient was then treated with chlorambucil and PD at higher doses; his WBC count dropped to 32,000/μL with slight improvement of organomegaly. Later, severe thrombocytopenia developed and the patient died of cerebral bleeding in February 1983. Autopsy was refused by the next of kin.

Surface Markers

PBMCs were isolated through a Ficoll-Isopaque (Fl) gradient, and sheep rosette-forming cells (SRFC), surface membrane Ig (SmIg) positive cells, and cells bearing receptors for the Fe portion of IgG (FclgG R) were evaluated as reported. In addition, PBMCs were tested with a panel of 13 monoclonal antibodies in an indirect immunofluorescence test. The panel included commercially available reagents of the OK and the Leu series. The patient’s bone marrow was infiltrated with 84% LNLGls. Cytocentriar evaluation showed positivity of peripheral blood mononuclear cells (PBMCs) for dyepeptidyl amino-peptidase IV (DAP IV). At that time, serum IgG was 1,567 mg/dL; IgA, 470; and IgM, 263. Calcaemia was 4.8 meq/L, and signs of increased bone turnover were not present. Serologic tests (September 1982) showed past but not present infections by herpes simplex virus, cytomegalovirus, and Epstein-Barr virus. The search by radioimmunoassay for serum antibodies against HTLV-I was conducted by Dr R.C. Gallo (Bethesda) and found to be negative. Cytogenetic analysis showed a chromosome number ranging between 44 and 47, with multiple numerical and structural clonal anomalies including a break point in 14q12. The patient was then treated with chlorambucil and PD at higher doses; his WBC count dropped to 32,000/μL with slight improvement of organomegaly. Later, severe thrombocytopenia developed and the patient died of cerebral bleeding in February 1983. Autopsy was refused by the next of kin.

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Functional Activities

Response to phytohemagglutinin. The response to phytohemagglutinin (PHA) was evaluated by thymidine incorporation at day 3 in response to optimal doses of purified PHA (P-PHA) in triplicate cultures, as previously reported.

Response to IL-2. Direct response of PBMCs from the patient to IL-2 was evaluated in micro and macro cultures using two IL-2-containing media (IL-2 CM). One was the supernatant obtained from a gibbon cell line (MLA-144), which releases IL-2 in the supernatant and is completely PHA free. The other IL-2 CM (Sup. 4) was obtained from normal lymphocytes obtained from thoracic duct drainage of patients before kidney transplantation. Lymphocytes were stimulated with P-PHA for 90 minutes, washed twice, resuspended in PHA-free medium with irradiated cells from a B-lymphoblastoid cell line, and incubated for 65 hours. Sup. 4 had no detectable PHA (less than 0.0005 μg/mL). In titration experiments with purified IL-2, which was kindly provided by Dr S. Venuta (Universita di Naples, Italy), Sup. 4 gave results comparable to those obtained with a preparation of IL-2 containing approximately 32 U/mL.

IL-2 production. IL-2 production was tested on supernatants of PBMCs from patient S.B. or from normal donors (106 cells per milliliter), incubated for 48 hours in RPMI 1640 with 1% of fetal calf serum (FCS) and P-PHA (0.5 μg/mL). Dilutions of the supernatants obtained from the patient and from normal donors were tested for induction of thymidine incorporation of normal T lymphocytes cultured for 14 days with IL-2 CM (cultured T cells [CTC]) at 48 hours, as previously described.

Modulation of Tac antigen. Modulation of Tac antigen was performed by Tsudo et al. Tac-positive PBMCs from patient S.B. were incubated in RPMI 1640 with 5% FCS and antibiotics for 48 hours in the continuous presence of appropriate amounts (10 μL of ascites diluted 1:100/106 cells) of anti-Tac monoclonal antibody. Cells were then washed three times, and reactivity with anti-Tac was assessed by indirect immunofluorescence. A control, PHA-stimulated culture of PBMCs obtained from a normal donor was also set up. In this case, after 48 hours of culture with P-PHA, cells were washed, tested for Tac expression, and reincubated for an additional 48 hours of culture in PHA-free medium in the continuous presence of anti-Tac, and then tested again for Tac expression.

Test for helper and suppressor activities on B cell differentiation (BCD). This test was performed as described, with a few modifications. Briefly, for testing the suppressor activity, 10% to 25% PBMCs from the patient were added to PBMCs obtained from a normal donor and cultured for seven days in the presence of pokeweed mitogen (PWM), in triplicate experiments. Suppression in the BCD was then evaluated by comparing the number of plasma cells (as observed microscopically after staining the cytospin preparations with fluorescent anti-Ig antibodies) per 10,000 viable cells observed in the cultures, with the number of plasma cells present in the control cultures (normal PBMCs and PWM). Evaluation of helper activity was also assessed on PWM-stimulated cultures, but the patient’s PBMCs were added to normal OKT3-depleted cells. Depletion was obtained by incubating normal PBMCs with appropriate amounts of OKT3 and complement, washing, and passing the cells on a F1 gradient to remove dead cells. Efficiency of separation was then evaluated by the SRFC test. Control cultures were normal unseparated PBMCs and PWM, and normal OKT3-
depleted cells cocultured with normal B cell-depleted lymphocytes (depletion was obtained by incubating the cells with Coulter Clone B1, CCB1, monoclonal antibody, and complement) and PWM. Plasma cells were evaluated on day 7 on cytocentrifuge smears stained with anti-Ig fluorescent antibody.

Suppression was also evaluated on lectin-induced response (LIR) of normal PBMCs as previously reported. 26

Survey of leukemic cells using cloned DNA sequences of HTLV. This survey was performed as previously reported in detail. 27 Briefly, DNA was extracted from leukemic cells by the proteinase K SDS method. Thirty micrograms were digested with PstI and run on agarose gels, transferred on nitrocellulose according to Southern, 21 and hybridized in 50% formamide, 3x SSC 5 x Denhart, 0.5% SDS, 50 mmol/L TRIS HCl, pH 7.4, 300 µg/mL tRNA 10% dextran sulphate overnight at 37 °C with HTLV I SstI-HindII and HTLV II EcoRI SstI 28 cloned DNA nicktranslated probes. Washed under stringent conditions (1 x SSC at 60 °C) and autoradiographed with Kodak XAR film at –70 °C.

RESULTS

Surface Markers

Surface markers were done on PBMCs from patient S.B. on three bleedings, in September, October, and December 1982, with virtually identical results. Results obtained in October 1982, when functional studies and DNA survey were performed, are reported in Table I. Cells reacted with reagents specific for mature T cells (OKT3, OKT1, OKT11, UCHT1), whereas they were negative with markers expressed by immature or activated cells (OKT6 and OKT10). In addition, cells reacted with OKT4 and 3A1 monoclonal antibodies and were OKT8 negative, thus showing the phenotype of a normal subpopulation of mature lymphocytes among which helper T lymphocytes are included. This finding was further supported by positivity for DAP IV. 17 Unlike all previous cases of Thp-CLL studied, 4 fresh cells from patient S.B. were positive with anti-Tac monoclonal antibody. This result prompted us to perform an extensive characterization of these cells, since a Tac positivity has been reported in HTLV-I–positive ATL cells.

Functional Activities

Leukemic cells had an impaired response in vitro to P-PHA. Mean (± SD) cpm in triplicate cultures with P-PHA were 151.3 ± 45.5 v 68.6 ± 41.3 cpm in control, unstimulated cultures. Stimulation index (SI) was thus 2.2 (our standards give 30 to 200 SI values in normal donors).

As the patient’s cells spontaneously expressed a Tac positivity, we evaluated their direct response to IL-2 CM. However, as shown in Table 2, a weak response was obtained with two different IL-2 CM. These data were confirmed by the inability to grow the patient's cells in macrocultures with IL-2 CM. In these tests we experienced the death of all the cells by ten days without the formation of clusters of cells, despite the use of two highly efficient IL-2 CM with or without the presence of irradiated cells of a B lymphoblastoid cell line.

IL-2 production by leukemic cells was absent (Table 3): A 1:2 dilution of the supernatant obtained after 48 hours of culturing the patient’s cells with P-PHA was tested for its ability to induce thymidine incorporation on normal CTCs after two days of cultivation. Results showed no stimulation as compared with a strong incorporation (mean, 59,321 cpm) obtained with Sup. 4, containing approximately 32 U/mL of IL-2. Supernatants obtained from PBMCs of two normal donors (ND) and cultured for 48 hours with P-PHA showed considerable stimulation, using the same test.

Modulation of Tac antigen on leukemic cells was found to be induced by anti-Tac antibody, as reported in Table 4. Modulation by normal PHA-blasts is also shown.

Despite the helper phenotype of the patient cells, helper as well as suppressor activities on BCD of these cells were virtually undetectable (Fig 2). T cell-depleted normal PBMCs (103) plus B cell-depleted normal PBMCs (103) and PWM gave 15,950 plasma cells per well, whereas the addition of T-CLL cells to normal T-depleted PBMCs and PWM gave, in the same experiment, less than 1,000 plasma cells per well. In addition, suppression by leukemic cells was not

Table 1. Surface Markers of PBMCs Isolated From Patient S.B.

<table>
<thead>
<tr>
<th>Marker</th>
<th>S.B. (%) pos.</th>
<th>Normal Donors (201) (Means ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRFC</td>
<td>95</td>
<td>69.0 ± 7.3</td>
</tr>
<tr>
<td>FcγGR</td>
<td>6</td>
<td>18.0 ± 2.3</td>
</tr>
<tr>
<td>SmIg</td>
<td>1</td>
<td>7.0 ± 1.8</td>
</tr>
<tr>
<td>OKT6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>OKT11</td>
<td>95</td>
<td>69.2 ± 6.3</td>
</tr>
<tr>
<td>UCHT1</td>
<td>95</td>
<td>63.6 ± 6.1</td>
</tr>
<tr>
<td>OKT3</td>
<td>97</td>
<td>63.3 ± 6.0</td>
</tr>
<tr>
<td>OKT4</td>
<td>93</td>
<td>44.8 ± 3.8</td>
</tr>
<tr>
<td>OKT8</td>
<td>6</td>
<td>26.6 ± 4.3</td>
</tr>
<tr>
<td>OKT10</td>
<td>3</td>
<td>7.2 ± 2.0</td>
</tr>
<tr>
<td>OK1a</td>
<td>1</td>
<td>10.1 ± 2.3</td>
</tr>
<tr>
<td>Anti-Tac</td>
<td>88</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Leu7</td>
<td>1</td>
<td>16.1 ± 5.6</td>
</tr>
<tr>
<td>OKM1</td>
<td>3</td>
<td>14.4 ± 4.0</td>
</tr>
<tr>
<td>3A1</td>
<td>3</td>
<td>48.3 ± 4.1</td>
</tr>
<tr>
<td>VIL1A</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are expressed as a percentage of positive cells.

Table 2. Direct Response of Leukemic Cells to IL-2

<table>
<thead>
<tr>
<th>S.B.</th>
<th>68 ± 41</th>
<th>222 ± 87</th>
<th>124 ± 47</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLA 244</td>
<td>8,120 ± 2,076</td>
<td>43,399 ± 839</td>
<td>43,274 ± 4,185</td>
</tr>
</tbody>
</table>

Fresh PBMCs from patient S.B. and cells from a normal donor cultured for 14 days with IL-2 CM (CTC) were incubated for 48 hours in different culture conditions. Results are means ± SD of three experiments.
Table 3. IL-2 Production by Leukemic Cells

<table>
<thead>
<tr>
<th>nCTC and:</th>
<th>Mean cpm ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium alone</td>
<td>224 ± 32</td>
</tr>
<tr>
<td>T-CLL Sup.</td>
<td>283 ± 62</td>
</tr>
<tr>
<td>ND 1 Sup.</td>
<td>25,730 ± 3,520</td>
</tr>
<tr>
<td>ND 2 Sup.</td>
<td>49,125 ± 5,218</td>
</tr>
<tr>
<td>Sup.4</td>
<td>59,321 ± 3,736</td>
</tr>
</tbody>
</table>

Normal T lymphocytes were cultured for 14 days with IL-2 CM, washed, and then incubated for 48 hours with (a) medium alone, (b) Sup. 4, or (c) 1:2 dilutions of supernatants obtained by incubating PBMCs from patient S.B. or from two normal donors (ND) with P-PHA for 48 hours. Results are the means ± SD cpm (thymidine incorporation) of three experiments. nCTC, normal cultured cells.

demonstrable. Indeed, 25% or 10% T-CLL cells had no suppressor effect on combined normal B and T cells. Finally, 10% T-CLL added to normal PBMCs and PHA had no suppressive effect on PHA-induced thymidine incorporation, thus showing a lack of suppression in LIR (Fig 2).

Survey of Leukemic Cells DNA for HTLV Sequences

DNA survey of leukemic cells was negative for proviral HTLV-I (Fig 3). Identical results were obtained with HTLV-II probe (data not shown), indicating that, different from ATL cells, HTLV infection had not occurred in leukemic cells from our patient.

DISCUSSION

We report a patient with Thp-CLL whose cells were positive for the anti-Tac monoclonal antibody, and thus expressed the phenotype of ATL (OKT3⁺, OKT4⁺, OKT8⁻, Tac⁻)⁴⁰ rather than of Thp-CLL cells (OKT3⁺, OKT4⁺, OKT8⁻, Tac⁻).⁴ Despite this phenotype, the patient had Thp-CLL, since his cells, unlike ATL cells,⁴ lacked HTLV-I and -II related sequences in their DNA. Thus, the expression of Tac antigen is not restricted to leukemic cells from patients with HTLV-I⁺ ATL.

Outside of endemic areas for ATL, the differential diagnosis between Thp-CLL and ATL may be trouble-
some. Although these diseases are considered two distinct clinicopathologic entities, they are both T-CLL of adults sustained by the expansion of a subpopulation of mature Thp-lymphocytes, with frequent splenomegaly, skin lesions, chromosome abnormalities, and, usually, an aggressive clinical course.

Clinical, immunologic, and epidemiologic features have been reported as characteristic of ATL. Nevertheless, none is specific for ATL. Nevertheless, none is specific for ATL nor is present in all cases. ATL patients may have a paraneoplastic syndrome, characterized by increased bone turnover and hypercalcemia, which is peculiar to ATL but is not present in all cases. Other clinical signs (such as skin lesions or splenomegaly) may be present in other T-CLL. Thus, the differential diagnosis remains difficult on a clinical ground. Immunologic tests have shown that ATL cells, in contrast to their mature Thp, may exert in vitro suppressor activity. Again, this property is present only in some ATL cases, whereas in other cases, helper or no activities have been demonstrated. Surface markers may be of help. In most, but not all, cases, ATL cells are OKT10- and the well-documented expression of the Tac antigen on unstimulated ATL cells is considered of importance, since other T-CLL are negative. However, personal data clearly demonstrate that Tac expression may also be present on HTLV-negative Thp-CLL. Cases of ATL have been described mainly in southern Japan and in the West Indies, but also elsewhere, thus precluding the use of a strict epidemiologic criterion for the diagnosis. Recent studies have shown that almost all ATL patients have serum antibodies against HTLV. We have previously pointed out the possible importance of detecting anti-HTLV antibodies in the differential diagnosis of chronic T cell leukemias. Patients with ATL who lacked anti-HTLV serum antibodies but had HTLV-related sequences in their DNA, have been recently reported. We have extended our survey to this test, thus showing that HTLV-negative leukemic T cells may bear the Tac antigen.

It has been proposed that the expression of the Tac antigen may be a marker of cellular infection by HTLV-1. A network of interactions may link HTLV to the IL-2 system. The patient reported here, however, indicates that Tac antigen is not necessarily linked to HTLV infection. In our patient, the presence of the Tac receptor may be the result of an infection of his cells by an unidentified retrovirus that shares little or no homology with HTLV (I or II), or it may merely be the result of in vivo cellular activation by other mechanisms. The possibility cannot be excluded that Tac expression may be a marker of distinct clinical phases of some leukemias or that the patient had been exposed to HTLV previously (hit-and-run type infection). However, this latter hypothesis seems unlikely because anti-HTLV-I serum antibodies were negative in our patient, thus showing a lack of immunologic memory for HTLV. Yet some differences are present in the expression of Tac antigen by ATL cells as compared with those of our patient. Tac+ cells from some ATL patients can directly respond to PHA-free IL-2 CM, thus confirming that the Tac receptor may maintain its biologic function. On the contrary, cells from our patient were unable to produce or respond to IL-2, and this inability could not be reversed by the addition of phorbol myristate acetate to the cultures (data not shown). This event may result from alterations present either in the Tac receptor, or in some of the steps that eventually lead to cellular activation and division. In this light, it may be remarkable that a lack of gamma interferon production was also detected in cells from this (and other) case of Thp-CLL. Another difference was that, unlike ATL cells, cells from our patient modulated the antigen in the presence of anti-Tac antibody, as do normal activated T blasts.

At present no simple criteria are available for a clear-cut distinction between ATL and Thp-CLL. When a patient presents all the characteristic features of ATL, the distinction is rather easy in endemic areas. However, several patients may have clinical and immunologic data that are consistent with both ATL and Thp-CLL, and the two diseases may thus appear as a smooth continuum rather than as two discretely separated entities. In such cases, Tac positivity may be unreliable and the demonstrations of anti-HTLV-I serum antibodies or of DNA integrated HTLV-I sequences may be the only feature that allows the distinction between the diseases. It could be hypothesized that in Thp-CLL, the induction of leukemia is caused by a different mechanism (not involving HTLV) that results in an ATL-like disease, probably because the transformed cell has reached a similar degree of biologic maturation. Albeit the presence of HTLV has been regarded by itself as a marker of a severe prognosis, both HTLV+ ATL and HTLV+ Thp-CLL are usually aggressive lymphoproliferative disorders. Thus, the detection of a Thp on cells from adults with lymphoproliferative disorders of mature T cells, should be regarded as a more general predictor of a possibly severe prognosis.

ACKNOWLEDGMENT

We thank Drs T.A. Waldmann (Bethesda), A.S. Fauci (Bethesda), and W. Knapp (Wien) for their generous gift of monoclonal antibodies; Dr A. Faggioni (Rome) for kindly supplying IL-2 CM supernatants of the MLA-144 cell line; and Dr G. Semenzato (Padova) for his useful comments on the manuscript.
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Tac-positive, HTLV-negative, T helper phenotype chronic lymphocytic leukemia cells

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