LYMPHOPROLIFERATIVE DISORDERS involving post-thymic lymphocytes constitute a heterogeneous group of neoplastic and nonneoplastic conditions. Several of these conditions feature chronic lymphocytosis: T cell chronic lymphocytic leukemia (T-CLL); T cell prolymphocytic leukemia (T-PLL); adult T cell leukemia/lymphoma (ATL); cutaneous T cell lymphomas of the mycosis fungoides/Sézary syndrome type (CTCL); T cell "lymphosarcoma" cell leukemia (T-LSCL); other "peripheral" T lymphocyte types; rare examples of T cell hairy cell leukemia (T-HCL); and chronic T cell lymphocytosis.

In vitro immunologic studies in some of these cases often demonstrated residual functional activity within the expanded population of lymphocytes. Correlation of cell function with cell phenotype have further defined the lymphoproliferative disorders in terms of their putative normal counterparts within the immune system and contributed to the recognition of distinctive clinicopathologic entities.

We report here a case of a T cell lymphoproliferative disorder that we classify as a T cell chronic lymphocytic leukemia, ie, the "knobby type" of Lukes and Meyer. The leukemic cells express a composite "helper" and "suppressor" cell phenotype and show evidence of in vitro helper and suppressor activity.

**CLINICAL HISTORY**

The patient is a 55-year-old white man who had a two-week history of intermittent pain in the left upper quadrant. Physical examination disclosed an entirely normal skin, nonenlarged axillary and inguinal lymph nodes, an enlarged liver palpable 4 cm below the right costal margin, and an enlarged spleen palpable 10 cm below the left costal margin.

Laboratory studies reported the following peripheral blood values: a hemoglobin of 10.7 g/dL; a leukocyte count of 195 x 10^9/L with 5% segmented neutrophils, 1% bands, 93% lymphocytes, and 1% monocytes; a platelet count of 40 x 10^9/L. Liver function tests was normal, which is consistent with the peripheral T cell phenotype. The patient underwent splenectomy, and the spleen showed very low levels of T3 and T4 by immunoperoxidase and undetectable levels by immunofluorescence. The morphology of the splenic infiltrate was not significantly different from that in the initial bone marrow. Human T cell leukemia virus (HTLV) antigen and antibody tests were negative. The cells in this leukemia apparently are derived from a transitional stage of maturation between the cortical and medullary thymocyte. A small subset of lymphocytes of identical phenotype to this leukemia has been identified in normal individuals.

This report discusses a case of T cell chronic lymphocytic leukemia (T-CLL) in an elderly white man whose lymphocytes expressed a post-thymic phenotype except for the coexpression of T4 and T8 on 80% to 95% of the cells. Because of the uncommon phenotype, in vitro functional assays were performed that showed decreased mitogenic responses but normal helper activity for B cell immunoglobulin secretion and normal suppressor activity of lectin-induced mitogenesis. Morphologic evaluation by both light and electron microscopy and cytochemical staining were consistent with the "knobby" type T-CLL. Adenosine deaminase and terminal deoxynucleotidyl transferase (TdT) levels were low, but the acetylcholinesterase level produced abnormal values: alkaline phosphatase, 104 IU/L (reference range, 5 to 59); lactic dehydrogenase, 106 IU/L (34 to 110); and aspartate aminotransferase, 42 IU/L (5 to 30). Serum calcium, electrolytes, urea nitrogen, uric acid, glucose, and protein electrophoresis levels were within normal limits.

The clinical impression was chronic lymphocytic leukemia, and the patient was treated with chlorambucil (0.1 mg/kg/d) and allopurinol (300 mg/d). Prednisone (50 mg/d) was added after one month but did not produce improvement.

The patient required packed RBC transfusion and leukopheresis. After three months of treatment, lymphocyte phenotyping on the bone marrow aspirate and peripheral blood was interpreted as demonstrating a T cell chronic lymphocytic leukemia. Therapy was changed to pulses of cyclophosphamide, 1,000 mg intravenous (IV) push, and vincristine, 1 mg IV push, with daily prednisone, 50 mg, and procarbazine, 100 mg. Two months later the leukocyte count was still 170 x 10^9/L, and 1,000 rad was given to the spleen over four weeks. The patient was hospitalized with sepsis and pancytopenia, and a spleenectomy was performed.

The patient subsequently improved, and chemotherapy was discontinued. Bone marrow biopsy, however, still demonstrated extensive infiltration by lymphocytes, but the patient is able, at this time (six months after splenectomy, one year after diagnosis), to maintain a normal hemoglobin and neutrophil count, with platelets in the range of 80 to 160 x 10^9/L and a lymphocytosis of 30 to 40 x 10^9/L.

**MATERIALS AND METHODS**

**Cell Isolation and Preservation**

The mononuclear cell fraction was obtained by Ficoll-Hypaque gradient centrifugation (Pharmacia Fine Chemicals Inc, Piscata...
Peripheral blood smears stained with Wright’s stain showed lymphocytosis, aniso- and poikilocytosis, and thrombocytopenia. The leukemic cells (Fig 1A) were slightly larger than erythrocytes and were pleomorphic, with indented or lobulated nuclei, relatively coarse chromatin, indistinct nucleoli, and moderately scant, agranular cytoplasm. The bone marrow and spleen showed a diffuse infiltrate of lymphoid cells, slightly larger than normal mature lymphocytes. These cells appeared fairly uniform in size and exhibited an irregular nuclear contour, densely staining chromatin, rare nucleoli, and little cytoplasm. Transmission electron microscopy (Fig 1 B and C) demonstrated the pleomorphic cell population. The leukemic cells had moderate amounts of cytoplasm, and some cells possessed microvilli. Many of these cells showed a prominent Golgi apparatus and numerous mitochondria, as well as occasional heterolysosomes. There were many free ribosomes and a small amount of rough endoplasmic reticulum.

Fig 1. (A) Morphology of leukemic cells from bone marrow aspirate (Wright’s stain, original magnification × 1600), (B) transmission electron micrograph of lymphocyte pellet (original magnification × 9,900), (C) nuclear and cytoplasmic features (original magnification ×40,000). The cells were fixed in Karnovsky’s solution and post-fixed with osmium tetroxide. Thin sections were stained with uranyl acetate and lead citrate.

RESULTS

Peripheral blood smears stained with Wright’s stain showed lymphocytosis, aniso- and poikilocytosis, and thrombocytopenia. The leukemic cells (Fig 1A) were slightly larger than erythrocytes and were pleomorphic, with indented or lobulated nuclei, relatively coarse chromatin, indistinct nucleoli, and moderately scant, agranular cytoplasm. The bone marrow and spleen showed a diffuse infiltrate of lymphoid cells, slightly larger than normal mature lymphocytes. These cells appeared fairly uniform in size and exhibited an irregular nuclear contour, densely staining chromatin, rare nucleoli, and little cytoplasm. Transmission electron microscopy (Fig 1 B and C) demonstrated the pleomorphic cell population. The leukemic cells had moderate amounts of cytoplasm, and some cells possessed microvilli. Many of these cells showed a prominent Golgi apparatus and numerous mitochondria, as well as occasional heterolysosomes. There were many free ribosomes and a small amount of rough endoplasmic reticulum.
Adenosine deaminase activity was low, which is consistent with the low TdT activity of the cells; acetylcholinesterase activities of the patient's erythrocytes and lymphocytes were normal (Table 3), which is consistent with the T cell phenotype.

Peripheral blood counts and immunoglobulin levels over the ten-month follow-up are shown in Table 1A. Peripheral blood and bone marrow lymphocytes showed T cells lacking thymic-stage differentiation or activation antigens but coexpressing T4 and T8 on 70% to 80% of the cells (Table 4). Thus, their phenotype is Leu-1', ER', T11', T4', T8'. Both Fcα and Fcγ receptors were present at levels of 11% and 45%, respectively. A notable feature was the absence of detectable T3 or T4 on the spleen cells by immunofluorescence of cell suspensions, although these antigens were present as shown by a weak reactivity in immunoperoxidase reaction on frozen sections of spleen. The spleen also showed small scattered clusters of cells demonstrating T9, T10, Ia, and M1.

Serum immunoglobulin was low to normal (Table 1). In vitro functional studies showed a reduced mitogenic response to PHA and con-A (Tables 5, 6, 7); however, the leukemic cells gave normal results for con-A–stimulated suppression and helper cell activity.

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### Table 1. Sequential Peripheral Blood Counts and Immunoglobulin Levels

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Leucocyte count†</td>
<td>207-257</td>
<td>318</td>
<td>162</td>
<td>37.5</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>2-5</td>
<td>2</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>93-94</td>
<td>99</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>1-2</td>
<td>0</td>
<td>4</td>
<td>(1-11)</td>
</tr>
<tr>
<td>Immature cells (%)</td>
<td>1-2</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Platelets†</td>
<td>19-24</td>
<td>24</td>
<td>66</td>
<td>90</td>
</tr>
<tr>
<td>IgG (mg/dL)</td>
<td>579</td>
<td>965</td>
<td></td>
<td>(666-1.647)</td>
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<tr>
<td>IgM (mg/dL)</td>
<td>65</td>
<td>75</td>
<td></td>
<td>(61-309)</td>
</tr>
<tr>
<td>IgA (mg/dL)</td>
<td>43</td>
<td>174</td>
<td></td>
<td>(86-370)</td>
</tr>
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</table>

*Two specimens one week apart.
† x 10⁹/L.

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### Table 2. Cytochemical Reactions of Circulating Lymphocytes

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Percentage of Cells Reacting</th>
<th>Pattern</th>
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<tbody>
<tr>
<td>Acid phosphatase</td>
<td>80</td>
<td>Punctate</td>
</tr>
<tr>
<td>α-Naphthyl acetate esterase</td>
<td>83</td>
<td>Punctate</td>
</tr>
<tr>
<td>α-Naphthyl butyrate esterase</td>
<td>84</td>
<td>Punctate</td>
</tr>
<tr>
<td>β-glucuronidase</td>
<td>96</td>
<td>Punctate</td>
</tr>
<tr>
<td>Periodic acid-Schiff</td>
<td>7</td>
<td>Finely-to-coarsely granular</td>
</tr>
</tbody>
</table>

---

### Table 3. Acetylcholinesterase and Adenosine Deaminase Activities

<table>
<thead>
<tr>
<th>Activity</th>
<th>Patients</th>
<th>Controls</th>
<th>B-CLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase (mol/L/10⁹ cells × 10⁻⁴)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes 25 °C</td>
<td>1.7 ± 0.5*</td>
<td>1.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>37 °C</td>
<td>2.7 ± 0.6</td>
<td>2.4 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Erythrocytes 25 °C</td>
<td>6.5 ± 1.0</td>
<td>5.5 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>37 °C</td>
<td>8.8 ± 1.1</td>
<td>9.1 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Adenosine deaminase (µmol/L/h/mg protein)</td>
<td>1.0 ± 0.2</td>
<td>1.9 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

*Error is expressed as ± SEM.
Table 4. Lymphocyte Phenotyping*  

<table>
<thead>
<tr>
<th>Marker</th>
<th>Peripheral Blood* (% Cells Positive)</th>
<th>Bone Marrow (% Cells Positive)</th>
<th>Spleen Cells (% Cells Positive)</th>
<th>Spleen Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER, 4°</td>
<td>89 ± 1 (3)</td>
<td>83</td>
<td>60</td>
<td>Strongly positive</td>
</tr>
<tr>
<td>ER, 37°</td>
<td>0 (3)</td>
<td></td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>FcRt</td>
<td>11 ± 1 (2)</td>
<td></td>
<td></td>
<td>Clusters of positive cells</td>
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<tr>
<td>FcRt</td>
<td>45 ± 4 (2)</td>
<td></td>
<td></td>
<td>Clusters of positive cells</td>
</tr>
<tr>
<td>Leu-1</td>
<td>96 ± 5 (4)</td>
<td></td>
<td></td>
<td>Strongly positive</td>
</tr>
<tr>
<td>T11</td>
<td>95 ± 6 (4)</td>
<td>86</td>
<td>98</td>
<td>Strongly positive</td>
</tr>
<tr>
<td>T3</td>
<td>90 ± 9 (4)</td>
<td>98</td>
<td>97</td>
<td>Strongly positive</td>
</tr>
<tr>
<td>T4</td>
<td>79 ± 13 (4)</td>
<td>73</td>
<td>0</td>
<td>Weakly positive</td>
</tr>
<tr>
<td>T8</td>
<td>93 ± 8 (4)</td>
<td>89</td>
<td>0</td>
<td>Weakly positive</td>
</tr>
<tr>
<td>T6</td>
<td>0 (1)</td>
<td></td>
<td></td>
<td>Clusters of positive cells</td>
</tr>
<tr>
<td>T9</td>
<td>2 ± 2 (2)</td>
<td></td>
<td></td>
<td>Clusters of positive cells</td>
</tr>
<tr>
<td>T10</td>
<td>1 ± 1 (2)</td>
<td></td>
<td></td>
<td>Clusters of positive cells</td>
</tr>
<tr>
<td>slg</td>
<td>&lt;1 (3)</td>
<td>3</td>
<td>2</td>
<td>Clusters of positive cells</td>
</tr>
<tr>
<td>B1</td>
<td>0 (2)</td>
<td></td>
<td></td>
<td>Clusters of positive cells</td>
</tr>
<tr>
<td>La</td>
<td>&lt;1 (2)</td>
<td></td>
<td></td>
<td>Clusters of positive cells</td>
</tr>
<tr>
<td>Leu-7</td>
<td>&lt;1 (2)</td>
<td></td>
<td></td>
<td>Clusters of positive cells</td>
</tr>
<tr>
<td>M1</td>
<td>&lt;1 (2)</td>
<td></td>
<td></td>
<td>Clusters of positive cells</td>
</tr>
<tr>
<td>CALLA</td>
<td>0 (3)</td>
<td></td>
<td></td>
<td>Clusters of positive cells</td>
</tr>
<tr>
<td>TdT</td>
<td>0 (2)</td>
<td></td>
<td></td>
<td>Clusters of positive cells</td>
</tr>
</tbody>
</table>

*Peripheral blood lymphocytes tested over a ten-month period. The number of determinations is given in parentheses. The mean ± SD is indicated where possible.
†Fc-receptors determined on cryopreserved cells.

DISCUSSION

For much of the decade following its initial description, T cell CLL has been a nosologic entity accompanied by confusion and controversy. Series of cases have been reported with only moderate lymphocytosis and frequently an indolent clinical course, whereas others emphasized the aggressive nature and resistance to therapy of this disease. In addition, a disproportionately high frequency of cases with a very aggressive form of this disease was reported in Japan. Finally, T cell CLL must be distinguished from the usually aggressive subacute T lymphocytic leukemia (PLL) that is clinically indistinguishable from Galton’s PLL of the B cell type, as well as from other T-lymphoproliferative disorders with leukemic manifestations.

Three major forms of T cell CLL have been described: (1) azurophilic T cell CLL, in which the cells have round, regular mature nuclei and azurophilic cytoplasmic granules; (2) "knobby" type T cell CLL of Levine et al., so-called because of the very irregular, indented nuclear configuration; and (3) the adult T cell leukemia/lymphoma (ATL) exemplified by the Japanese cases, which comprise cells characterized by extremely pleomorphic, lobulated, or cloverleaf nuclei with a marked variation in cell size.

Cytochemically, azurophilic cells show a diffuse or focal granular pattern with acid phosphatase but are negative for nonspecific esterase. The "knobby" cell is characterized by dot-like acid phosphatase and a spot-like nonspecific esterase reaction, and ATL is characterized by a granular phosphatase positivity and a few cells showing a spot like nonspecific esterase (NSE) reaction. The phenotype of the "knobby" T cell CLL and ATL cases is T1, T4, while azurophilic T cell CLL is primarily T1, T8.

The validity of this classification scheme for T cell CLL receives strong support from the differences in prognosis and clinical findings between the three forms. Most of the patients with azurophilic T cell CLL are asymptomatic with nonprogressive disease. In contrast, median survival for "knobby" T cell CLL and ATL is 12 months and 4½ months, respectively.

Table 6. Lymphocyte Suppressor Cell Activity

|Suppressor Index (%)|  
|-------------------|---
|Patient’s lymphocytes| 56 ± 6
|Control lymphocytes| 35–70

Table 7. Lymphocyte Helper Cell Activity

|IgG Production In Vitro (μg/mL)|  
|-----------------------------|---
|B cells alone                | <1
|T cells alone                | <1
|Control T cells plus normal B cells| 11
|Patient’s T cells plus normal B cells| 12
Normal T lymphocytes can be identified on the basis of their expression of T4 or T8 in conjunction with other markers such as T11, T1, T3, and T12. Generally, one or the other of the markers T4 or T8 is expressed, although there is strong evidence that the expression of these antigens by post-thymic lymphocytes is neither mutually exclusive nor reciprocal, both in normal individuals or in the presence of disease. Van den Griend et al demonstrated an ER, Leu-1, T3+, T8+, and T4+ subset of high-density lymphocytes comprising of about 2% of the peripheral blood lymphocytes of normal subjects. The cells also lacked T6, T10, and TdT. They were viable but showed no proliferation or immunologic function. A negative subset was also observed for both T4 and T8 in 25% of the lymphocytes of a patient who had received a successful bone marrow transplant. Another group found the T3+, T4+, T8+ phenotype on 1.6% of E rosette fractionated peripheral blood lymphocytes from normal controls and up to 16% of the same fraction from a patient with B cell CLL.

Conversely, there is considerable evidence of a normal population of post-thymic lymphocytes, which are defined by the presence of T11 and T3 and absence of T6 and TdT, but which co-express T4 and T8. Janossy et al detected T4 and T8 on 2.6% of peripheral blood lymphocytes from seven normal subjects and 6.5% of tonsillar lymphocytes stained for both T4 and T8. Tidman et al reported 1% to 2% of peripheral blood lymphocytes normally express both T4 and T8. Berrih et al determined T cell subset from 19 patients with myasthenia gravis and showed that 15% of the patients' lymphocytes had T4 and T8 markers (T6 was negative and T10 occasionally increased). Post-thymectomy, the T4+, T8+ cells rapidly disappeared in most patients. An expanded population of T4+, T8+ lymphocytes in chronic active hepatitis has also been reported.

Neoplastic proliferation of lymphocytes with both markers have been described. One case of T cell lymphosarcoma cell leukemia (T-LSCL), in which the patient had a marked lymphocytosis of cells carrying T1, T4, and T8, but no T6 was classified as a lymphoma because the bone marrow infiltration was only approximately 20% with marked dermal involvement. Two of eight prolymphocytic leukemias coexpressed both T4 and T8, one with T4 on half the cells. One case of a very aggressive T cell CLL was of this phenotype, although functional assays showed negative results.

Sézary syndrome and ATL are generally the T4 or T8 phenotype, but two reports describe both T4 and T8 positivity in Sézary syndrome. Lennert et al showed that of 16 peripheral T cell lymphomas, five had T4+, T8+ populations. A leukemia was also described by Saxon et al in a patient with ataxia telangiectasia with both helper and suppressor cell markers. The cells showed a normal mitogenic response to PHA and had normal helper and suppressor activity. The patient had a t(4q+ translocation, and Fc, and Fc, receptors were detected on 45% and 10% of the cells, respectively.

It is important to compare these other examples with this case report, in which the cells showed decreased mitogenic response but normal B cell helper activity. The different phenotype of the spleen cells, with the low degree of reactivity for T3 and T4 (although weakly positive on immunoperoxidase stained sections), may simply be the result of splenic radiation, although more primitive blastlike cells have also shown the loss of differentiation antigens in T cell lymphomas.

We believe that the phenotype and functional properties of the leukemic cells in our patient are consistent with a stage in thymic development in which the thymocyte has developed T1 and T3, but lost T6 before diverging into separate T4 and T8 populations. This stage is not formally postulated in the model by Reinherz et al, but it seems unreasonable to maintain that continuous biologic processes occur in discrete stages without the existence of transitional forms. Cortical thymic cells bearing T1 and T3 have been isolated and shown to be functionally competent in vitro, and there is evidence that a small subset of T4 and T8 cells exists in normal individuals that may disappear after thymectomy.

The leukemia described here appears to be a neoplastic proliferation of this latter subgroup of cells, and contrary to most other reports of cells possessing both markers, the percentage of cells expressing both T4 and T8 markers was very high (80% to 90%), which is unusual. If the immunologic studies are ignored, then this patient's cells are morphologically and cytochemically the "knobby" type, even though Fc receptors would put them in the azurophilic class. The most striking observation, however, is the relatively benign course this patient now seems to be experiencing, which is more in keeping with the "azurophilic" class of T cell CLL. Finally, it should be added that now (1½ years after diagnosis), the patient's cells have lost most of their T4 reactivity (15% to 20%) and resemble those observed in the spleen six months ago.

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T cell chronic lymphocytic leukemia with lymphocytes of unusual immunologic phenotype and function

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