T-Cell Prolymphocytic Leukemia with a Suppressor Phenotype

ANTONIO T. PLANAS, M.D., KENNETH W. ZAMKOFF, M.D., BERNARD J. POIESZ, M.D., ANTHONY S. KUREC, B.S., and FREDERICK R. DAVEY, M.D.

Departments of Pathology and Medicine, Veterans Administration Medical Center, and Departments of Pathology and Medicine, SUNY Upstate Medical Center, Syracuse, NY 13210 and Barbara Kopp Research Center, Auburn, NY 13021

ABSTRACT

A diagnosis of prolymphocytic leukemia was made from the blood and bone marrow of a 50 year old man. The neoplastic cells were studied by use of light and electron microscopy. Neoplastic cells were focally positive for acid phosphatase and alpha naphthyl acetate esterase. In addition, neoplastic cells formed rosettes with sheep erythrocytes and reacted with Leu-1 and Leu-2a but not Leu-3a antisera. No terminal deoxynucleotidyl transferase (TdT) activity was noted in these cells. It is concluded that these neoplastic cells were phenotypically mature suppressor T lymphocytes. Furthermore, T and B cell prolymphocytic leukemias were compared according to clinico-pathological, cytochemical, ultrastructural, and immunological findings derived from our review of the current literature.

Introduction

Prolymphocytic leukemia, (PLL) originally described by Galton and co-workers,10 is an uncommon form of chronic lymphoid leukemia. Prolymphocytic leukemia is characterized by the presence of marked lymphocytosis (usually greater than 100,000 per μl), massive splenomegaly, moderate hepatomegaly, and minimal lymphadenopathy. The prolymphocytes represent the majority of the cells in the peripheral blood and bone marrow.2,6,10 These cells possess a large vesicular nucleus, a prominent nucleolus and a moderate amount of pale blue cytoplasm. Prolymphocytic leukemia is an aggressive disease in that patients respond poorly to standard treatment for chronic lymphocytic leukemia. In one study,10 the mean survival of patients with PLL was only 11 months.

Lymphocytes form approximately 80 percent of the cases of PLL type as B lymphocytes with intense positivity for surface immunoglobulins (Slg).8 Most cases of the less common T cell variant of PLL have been phenotyped as T helper
The purpose of this report is to describe our experience in regard to a case of PLL in which the neoplastic cells possessed the phenotypic characteristics of suppressor T cells.

Case Report

A 50 year old white male entered the hospital with malaise, pleuritic chest pain, hemoptysis and 45 kilogram weight loss over the preceding 15 months. His initial physical examination revealed a massively enlarged liver and spleen with only minimal lymphadenopathy.

The peripheral white blood cell count was 607,000 per μl with 94 percent prolymphocytes, 4 percent neutrophils, 1 percent bands, and 1 percent monocytes. The hematocrit was 33.5 percent and the platelet count was 40,000 per μl. The bone marrow was hypercellular (>95 percent) and diffusely infiltrated by prolymphocytes. Serum protein electrophoresis and quantitative immunoglobulin determinations for IgG, IgA, and IgM were normal. Other laboratory data were: alkaline phosphatase 296 IU per l (N: 50 to 140); lactic acid dehydrogenase 1,002 IU per l (N: 80 to 200), and uric acid of 9.5 mg per dl (N: 2.6 to 6.1).

A chest roentgenogram demonstrated an infiltrate in the right upper lung field and a collection of fluid in the right pleural space. Sputum cultures revealed *Haemophilus influenzae* and *Streptococcus pneumoniae*. The patient's pneumonitis subsequently resolved following treatment with ampicillin. The fluid from a right thoracentesis showed numerous prolymphocytes. The patient's PLL was treated with L-asparaginase (35,500 IU per day for 10 days) which produced only a partial lowering of the white cell count. Two months later, the patient developed a periportal edema and pectechial skin rash which was extremely pruritic. The white blood cell count was 1,030,000 per μl and the platelet count was 43,000 per μl. Enlarged lymph nodes were noted bilaterally in the anterior and posterior cervical areas as well as in the axillae. A skin biopsy of a pruritic lesion revealed a leukemic infiltrate in the reticular dermis. There was no involvement of the papillary dermis or epidermis. The patient was treated with vincristine, cyclophosphamide, and prednisone. This therapy produced a reduction in the patient's white blood cell count to 473,000 per μl. However, it had very little effect on the patient's massively enlarged spleen. Because of painful splenomegaly and persistant thrombocytopenia, the patient underwent a splenectomy. He was eventually discharged from the hospital with a white blood cell count of 260,000 per μl. One year after the diagnosis, the patient remains in good condition with no significant complaints. He is presently on chemotherapy with daily oral cyclophosphamide. His last white blood count was 81,000 per μl and platelet count was 100,000 per μl.

**Materials and Methods**

Differential counts of the bone marrow (500 cells) and peripheral blood (200 cells) were performed on Wright-Giemsa stained films. In addition, the films of the peripheral blood and bone marrow were stained for acid phosphatase and for alpha-naphthyl acetate esterase according to standard procedures. Paraffin blocks of the bone marrow biopsy and sections of the spleen, liver, and pancreas were stained with hematoxylin-eosin and examined with a light microscope. Sections from 2.5 percent phosphate-buffered isotonic gluteraldehyde-fixed tissue were cut and then post fixed on phosphate-buffered osmium tetroxide, embedded in Araldite, cut with an LK6 ultra microtome, stained with uranyl acetate, and examined with a Philips EM-300 electron microscope.

Ten milliliters of peripheral blood were obtained in preservative free heparin. Mononuclear cells were separated from other blood elements by centrifugation.* In these preparations, over 90 percent of the mononuclear cells were neoplastic cells as judged by their morphology. Neoplastic cells were evaluated for their ability to form nonimmune rosettes with sheep erythrocytes (E-rosettes) by the previously described method of Jondal. Surface membrane immunoglobulin (Slg) were studied by a direct immunofluorescent technique using fluorescein conjugated polyspecific anti-human serum as well as monospecific anti-human serum (Fab')2 directed against gamma and mu heavy chains, and kappa and lambda light chains. Neoplastic cells were examined for the presence of "Ia-like" antigen by indirect immunofluorescence. To detect Fc receptors the malignant cells were incubated first with aggregated human

---

* Lymphocyte Separation Medium, Litton Bio- netics, Kensington, MD.
gammaglobulin and subsequently with fluorescein labeled anti-human IgG.\textsuperscript{9} The neoplastic cells were evaluated for their ability to form rosettes with mouse erythrocytes (M-rosettes).\textsuperscript{16} The presence of terminal deoxynucleotidyl transferase (TdT) was determined by indirect immunofluorescence using fluorescein labeled goat anti-rabbit IgG after previous incubation of the cells with rabbit anti-calf TdT serum.\textsuperscript{18} The neoplastic cells were also tested for reactivity of monoclonal mouse antibodies against human T-cells (Leu 1, pan-T cell antigen; Leu 2a, suppressor T-cell antigen; Leu 3a, helper T-cell antigen) using a direct immunofluorescence technique.

Results

With the use of a light microscope, the prolymphocytes were observed to be medium sized cells with a moderate amount of pale blue agranular cytoplasm. In the majority of cells, the nuclear contour was round. However, in a minority of cells, the nuclear membrane was folded or convoluted. Occasionally, a bizarre shaped nucleus was noted. A prominent, single nucleolus was present in almost every cell (figure 1). Over 90 percent of the neoplastic cells were focally positive for acid phosphatase and for alpha-naphthyl acetate esterase (figure 2). The bone marrow biopsy was diffusely infiltrated by prolymphocytes. There was a decrease in all other bone marrow elements.

Sections of spleen showed a diffuse infiltration in the red pulp and a focal infiltration in the white pulp. Mitotic figures were exceedingly rare. The liver biopsy demonstrated a dense portal infiltrate of prolymphocytes. The hepatic sinusoids were relatively spared of the leukemic cells. The peripancreatic fibrofatty tissue was infiltrated by prolymphocytes. The skin biopsy showed an infiltrate of leukemic cells in the reticular dermis (figure 3). No infiltrates were observed within the papillary dermis or epidermis.

The ultrastructure of the peripheral blood prolymphocytes showed a moderate amount of cytoplasm exhibiting occasional short cytoplasmic projections. The cytoplasm contained some ribosomes, polyribosomes and occasional short segments of endoplasmic reticulum. Only a few mitochondriae were present and were clustered in one area of the cytoplasm. The Golgi apparatus was not well developed. An occasional prolymphocyte showed membrane-bound, electron-dense granules in the cytoplasm. The nuclear contour was usually round, but a small number of cells exhibited a rather irregular nuclear shape. Marginated heterochromatin with a prominent single nucleolus was present in almost every cell (figure 4). Intracytoplasmic inclusions were not present in any of the cells.

The results of the immunologic marker studies are recorded in table I. The majority of neoplastic cells formed nonimmune rosettes with sheep erythrocytes and reacted with monoclonal Leu-1 and Leu-2a antisera. In contrast, none of the cells reacted with Leu-3a antiserum nor displayed TdT. In addition, only a very small proportion of cells contained B lymphocyte markers. Thus, neoplastic prolymphocytes were T-cells having a suppressor phenotype.

Discussion

Prolymphocytic leukemia is a rare variant of chronic lymphoid leukemia. Approximately 80 percent of the cases of prolymphocytic leukemia are of B cell lineage.\textsuperscript{8} Most of the cases of T cell prolymphocytic leukemia are phenotyped as T
Figure 1. Peripheral blood film from patient with prolymphocytic leukemia. Note the prominent nucleoli and moderately abundant cytoplasm (Wright-Giemsa ×1000).

Figure 2. Peripheral blood film stained for alpha-naphthyl acetate esterase. Note the focal location of reaction product (×1000).
Figure 3. Biopsy of skin. The neoplastic lymphocytes infiltrate the reticular dermis and do not involve the papillary dermis or the epidermis (Hematoxylin-Eosin stain × 450).

Figure 4. Electron micrograph of prolymphocytes from buffy coat of peripheral blood. Note the prominent compact nucleolus, heterochromatin, and cytoplasm containing mitochondriae and polyribosomes.
TABLE I
Summary of Immunologic Assays on Neoplastic Lymphocytes

<table>
<thead>
<tr>
<th>Assay</th>
<th>Percentage of Positive Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>E rosettes</td>
<td>97</td>
</tr>
<tr>
<td>Surface membrane immunoglobulin</td>
<td>1</td>
</tr>
<tr>
<td>&quot;la-like antigen&quot;</td>
<td>2</td>
</tr>
<tr>
<td>Fc receptors</td>
<td>0</td>
</tr>
<tr>
<td>Mouse rosettes</td>
<td>6</td>
</tr>
<tr>
<td>Terminal deoxynucleotidyl transferase</td>
<td>0</td>
</tr>
<tr>
<td>Leu-1 antigen*</td>
<td>92</td>
</tr>
<tr>
<td>Leu-2a antigen*</td>
<td>88</td>
</tr>
<tr>
<td>Leu-3a antigen*</td>
<td>0</td>
</tr>
</tbody>
</table>

*The neoplastic cells were also tested with monoclonal mouse antibodies against T-cells from Ortho Diagnostic, Raritan, NJ with the following results: OKT3, 100 percent of cells positive; OKT4, one percent of cells positive; OKT8, 100 percent of cell positive; and OKT11, 100 percent of cells positive.

helper cells. A case is presented here phenotyped as a T suppressor cell.

The distinction between B cell and T cell prolymphocytic leukemia can not be made using clinical data alone. B cell and T cell PLL most commonly affect individuals over 60 years of age. Approximately twice as many males develop this disease as females. In both B and T cell PLL, patients present with massive splenomegaly, moderate hepatomegaly and modest lymphadenopathy. Skin involvement may be more common in the T cell than the B cell variant of PLL. The skin infiltrate seen with T cell PLL is present within the deep dermal tissue as opposed to the lymphoid infiltrates of Sézary syndrome or Mycosis fungoides in which the lymphoid infiltrates are usually found in the papillary dermis and epidermis.

B cell and T cell PLL cannot be distinguished from each other on the basis of the morphology of the prolymphocytes. In both variants, the neoplastic cells are medium size lymphocytes with moderate amount of deep blue agranular cytoplasm. Prominent nucleoli are observed in the lymphoid cells from both cases of B cell and T cell PLL. No consistent ultrastructural characteristic differentiates B cell PLL from T cell PLL.

The use of cytochemical stains may be a reliable method in differentiating B cell from T cell PLL. Previously reported cells from patients with T cell PLL characteristically showed strong localized activity with alpha-naphthyl acetate esterase and with acid phosphatase. In contrast, B cell PLL contains none or only faint amounts of these hydrolases.

Several studies have indicated that circulating T lymphocytes from normal individuals are rich in acid phosphatase and alpha-naphthyl acetate esterase. In addition, these hydrolases are characteristically displayed as localized granules when studied with cytochemical techniques. However, not all T lymphocytes stain positively for these enzymes. Grosi and co-workers indicated that T cells with Fc receptors for IgM, but not T cells with Fc receptor for IgG, would possess a localized granular reaction for alpha-naphthyl acetate esterase. Bevan and co-workers found a similar distribution of acid phosphatase reactivity among subsets of T cells. When acid phosphatase and alpha-naphthyl acetate esterase activity were examined on T cell subpopulations defined by monoclonal antibodies, Leu 3a+ cells displayed a higher percentage of focal positivity than did Leu 2a+ cells but the overlapping populations were great and the difference in cytochemical reactivity was not considered to be clinically useful. Furthermore, the presence of acid phosphatase and alpha naphthyl acetate esterase reactions did not correlate with the membrane phenotypes as defined by monoclonal antibodies in 11 cases of T cell PLL. In the current case, focal acid phosphatase and alpha-naphthyl acetate esterase reactions occurred in neoplastic cell phenotyped as
T suppressor cells. In addition, it is our experience that in some cases the membrane phenotype does not correspond to the functional activity of the cells. (These findings will be reported in a forthcoming paper.)

B cell and T cell PLL can best be differentiated from each other by the use of immunologic lymphocyte markers. In the current case, the T cell nature of the neoplastic cells was determined by their ability to form nonimmune rosettes with sheep erythrocytes and reactivity with Leu 1 antiserum. The suppressor phenotype was determined by the positive reaction with Leu 2a antiserum and negative reaction with Leu 3a antiserum. Since TdT activity is found in immature T and B cells, the lack of TdT activity in the neoplastic cells from the current case is consistent with the hypothesis that these prolymphocytes are derived from mature suppressor T cells.

There is insufficient information in the available published cases regarding the clinical course and response to therapy between cases of B cell and T cell PLL. Both variants of PLL, however, appear to respond less well to therapy and have a shorter survival than cases of chronic lymphocytic leukemia.¹²,¹⁰ However, as therapies become increasingly directed at specific lymphocyte subpopulations, determining the B or T cell nature of the cells in PLL [may] be increasingly clinically important.

Acknowledgments

Thanks are extended to Ms. Maureen Barcza for her invaluable technical assistance and to Ms. Sarah Fuoco for secretarial assistance.

References


