Multimerker Analysis of T-Cell Chronic Lymphocytic Leukemia

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A 68-yr-old male with chronic lymphocytic leukemia (CLL) presented with splenomegaly and skin infiltration but no lymphadenopathy. The peripheral blood WBC count was 300 × 10^9/liter, with 95% small mature-appearing lymphocytes that were E-rosette positive and EAC-rosette negative. Further characterization of the patient’s cells was performed using antisera with known lymphoid subpopulation specificity. Anti-p23,30, which reacts with normal circulating B cells but not with T cells or thymocytes, was unreactive with the patient’s cells. Anti-311, which reacts with both thymocytes and circulating T cells, was reactive with the patient’s cells. Anti-Bk, which reacts only with thymocytes and not with circulating T cells, failed to react with the patient’s cells. The enzyme terminal deoxynucleotidyl transferase, present in thymocytes but absent from circulating T cells, was also absent from the patient’s lymphoid cells. Multimerker analysis therefore showed a mature T-lymphocyte phenotype on this patient’s leukemic cells. Further functional analysis will probably show that such cells represent clonal expansion of a mature T-cell subpopulation, analogous to the B-cell clonality of common-variant CLL.

Chronic lymphocytic leukemia (CLL) has in most cases been clearly demonstrated to represent a malignant proliferation of B lymphocytes, as defined by the presence of normal B-cell markers such as surface IgM and IgD immunoglobulins, complement receptors, and the ability to synthesize immunoglobulin light chains in vitro. Recently, however, there have been reports of rare patients with CLL whose cells have T-cell markers, identified by E-rosette formation, lysis by anti-T-cell sera, and absence of B-cell markers. We report here a case of T-cell CLL with surface markers distinct from thymocytes but identical to circulating normal T cells, suggesting a malignant clonal proliferation of a subclass of mature T cells.

Case Report

H.S., a 68-yr-old male, was referred to the Peter Bent Brigham Hospital in November 1976 because of recently discovered peripheral blood lymphocytosis. Routine blood count, in preparation for a transurethral resection, revealed a hematocrit of 36%, white blood cell count of 300 × 10^9/liter with over 90% of the cells being small adult lymphocytes by Wright-Giemsa morphology, and 40 × 10^9/liter platelets. There was no history of infection, fever, night sweats, weight loss, or increased bruisability or bleeding. The patient had noticed a nonpruritic rash of his chest and back for several days. On physical examination, multiple violaceous...
papules and nodules with some element of hemorrhage were noted over the sternal and lumbar areas. Scattered petechiae were found on the wrists and fingers. No lymphadenopathy was noted, but hepatosplenomegaly was present. A bone marrow biopsy was hypercellular, with 60%, small lymphocytes with inconspicuous nuclei and varying quantities of cytoplasm. A skin biopsy of the lower back revealed prominent infiltrates of small lymphocytes of similar morphology to those seen in the bone marrow about the adnexae and vessels in the dermis. Coombs’ tests, direct and indirect, were negative. Total protein was 6.1 g/dl with an albumin of 3.0 g/dl, IgG of 0.98 g/dl, IgA of 0.21 g/dl, and IgM of 0.086 g/dl.

The patient was treated with weekly intravenous cyclophosphamide and vincristine and daily oral prednisone. His course was characterized by rapid improvement of his hematologic parameters and resolution of his skin lesions and hepatosplenomegaly. Within 1 mo he achieved normal peripheral blood counts.

MATERIALS AND METHODS

Cytologic Studies

In addition to Wright-Giemsa staining of the peripheral blood and bone marrow smears, periodic acid Schiff (PAS) and acid phosphatase (with and without tartrate) staining were performed.15

Cell Surface Markers

Cells were prepared for surface marker studies from heparinized blood, and lymphocytes were purified on Ficoll-Hypaque density sedimentation according to Böyum.16 Three antisera of known specificity were employed.

Antiserum to a glycoprotein antigen complex of 23,000- and 30,000-dalton subunits (p23,30), isolated from a human lymphoblastoid B-cell line and highly specific for all human B cells, was prepared as described previously.17 This antiserum is completely unreactive with human thymocytes, T-cell acute lymphocytic leukemia (ALL), T-cell lines, and normal peripheral blood T cells but is reactive with B cells and B-cell CLL.18

Antiserum to a population of acute T-cell lymphoblastic leukemic cells (anti-BK) were raised by injecting rabbits intravenously with 3 x 10⁶ lymphoblasts from patient B.K., as previously described.18 This antiserum reacts with thymocytes, T-cell leukemic cell lines, and T-cell ALL but is unreactive with peripheral T cells. Fifty patients with ALL have been studied with this reagent and only 10 patients’ cells reacted, all of whom were E-rosette positive. The non-reactive patients were E-rosette negative. Similarly, 20 patients with acute myelogenous leukemia (AML) and 10 patients with CLL were negative (Schlossman SF: unpublished observations).

Another antiserum, designated as anti-311, was prepared by immunizing rabbits with lympho-

| Table 1. Antisera Characteristics |
|-------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Cell Source             | E Rosettes     | EAC Rosettes   | Anti-p23,30  | Anti-311  | Anti-BK  | TdT  |
| Peripheral blood T cells| +              | -              | -             | +          | -       | -   |
| Peripheral blood B cells| -              | +              | +             | -          | -       | -   |
| Thymocytes              | +              | -              | -             | +          | +       | +   |
| Common-variant CLL (B cell)| -          | +              | +             | -          | -       | -   |
| Patient H.S.            | +              | -              | -             | +          | -       | -   |

+, greater than 90% reactivity, except for EAC rosettes, which were considered positive if greater than 50%; -, less than 2% reactivity. For TdT, the range of enzyme activity was 0.8-2.1 enzyme units/10⁸ thymocytes. Negative samples contained less than 0.002 enzyme units/10⁸ cells.
blasts from a patient (M.D.) with E-rosette-positive ALL. After extensive absorption this antiserum reacts with thymocytes, T-cell leukemic cell lines, T-cell ALL, and normal human peripheral blood T cells but not with normal or leukemic B cells or p23,30-positive cells. The characteristics of these three antisera are listed in Table 1.

Reactivity to the three antisera was studied by indirect immunofluorescence. Analysis and quantitation of fluorescence-staining cells were performed with a Becton-Dickenson fluorescence-activated cell sorter (FACS-I), as described previously. The data obtained were displayed in the form of a histogram, plotting cell number versus intensity of fluorescence.

**Sheep Red Blood Cell Rosetting**

Spontaneous rosette formations with sheep erythrocytes (E rosettes) and with sheep erythrocytes-antibody-complement (EAC) were performed according to methods previously described.

**Terminal Deoxynucleotidyl Transferase (TdT)**

TdT was assayed by Dr. Ronald McCaffrey as described previously.

**RESULTS**

The peripheral blood and bone marrow lymphocytes demonstrated no perinuclear PAS-positive granulations. Virtually all of the lymphocytes were positive for acid phosphatase, but none was tartrate resistant. TdT was absent from the peripheral blood lymphocytes. The binding of the three different antisera by the patient’s lymphocytes was studied with fluorescent analysis, utilizing the FACS-I. The cells were nonreactive with anti-HTL and anti-p23,30 but strongly reactive with anti-311. Rosette analysis showed greater than 90\(^\circ\), of the cells to be E-rosette positive and EAC-rosette negative. A summary of the reactivity of the various markers with the patient’s lymphocytes and other subsets of lymphocytes is shown in Table 1.

**DISCUSSION**

CLL of T-cell origin is an unusual, but distinct, disease entity. Clinically, these patients have been characterized by splenomegaly without lymphadenopathy, neutropenia, and occasionally skin infiltration. The diagnosis of T-cell CLL in this case was initially suggested because of the clinical presentation and was confirmed by acid phosphatase-positive lymphocytes and E-positive and EAC-negative rosettes. E-rosette positivity, however, has been present in all stages of differentiation of T-cells.

In order to characterize the lymphocytes further, cell sorter analysis was performed using anti-p23,30, a normal B-cell antiserum, and two different T-cell antisera, anti-Bk and anti-311. In addition, TdT, a unique DNA polymerizing enzyme present in thymocytes but not in circulating T cells, was assayed. The cells in this case were negative for anti-p23,30, TdT, and anti-BK but reacted positively with anti-311. Thus this patient’s leukemic T cells appeared by multimarker analysis to be similar to normal peripheral T cells, perhaps representing a clonal expansion of a subclass of normal T cells (which might be identifiable by functional assays) analogous to common-variant CLL, which is characterized by a clone of B-cell subtypes. Attempts should be made in future cases of T-cell CLL to determine if in fact such clonal expansion of T-cell subsets does occur.
REFERENCES

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