Coincidence of B-Cell Chronic Lymphocytic Leukemia and Cutaneous T-Cell Lymphoma (Mycosis Fungoides): Immunologic Characterization by Monoclonal Antibodies

By Khalil Sheibani, Stephen J. Forman, Carl D. Winberg, and Henry Rappaport

Although rare cases of chronic lymphocytic leukemia (CLL) of the T-cell type have been reported, CLL is more commonly found to be a neoplastic lymphoproliferative disease of B-cell origin. In this article, we describe a patient with long-standing CLL that was immunologically shown to be of the B-cell type, who, during the course of his disease, developed cutaneous T-cell lymphoma (CTCL), which was shown to be of the helper/inducer subtype. The neoplastic lymphoid cells in the skin infiltrate differed morphologically and immunologically from those in the peripheral blood. The occurrence of CTCL during this patient's clinical course represents a second neoplasm arising from a different cell line, rather than a tissue manifestation of the patient's CLL. To our knowledge, this is the first report in which the occurrence of CTCL is documented in a patient with immunologically known B-cell CLL. In addition to establishing the presence of B-cell CLL and CTCL of the helper/inducer T-cell type in the same patient, this case report demonstrates the usefulness and necessity of evaluating lymphoproliferative disorders by means of a multidisciplinary approach.

CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) and cutaneous T-cell lymphoma (CTCL) are lymphoproliferative diseases that usually affect the same general age group; thus, it is reasonable to expect that occasional patients may develop both diseases. However, since patients with CLL rarely develop leukemia cutis, proof of the coexistence of CLL with another lymphoproliferative disease of a different immunologic phenotype requires immunologic studies. Such studies become particularly important when the histologic sections are suboptimal or when the cutaneous infiltrate does not clearly fulfill the criteria for either leukemia cutis or CTCL. We now present a patient with CLL who, during the course of his illness, developed multiple cutaneous lesions that were clinically suggestive of leukemia cutis. The morphologic identification of these lesions as CTCL presented a diagnostic problem that was resolved by immunologic studies.

CASE REPORT

This patient is a 69-yr-old white man who was first diagnosed as having CLL in March 1977, when he presented complaining of fatigue and was found to have an absolute lymphocytosis. No treatment was instituted at the time, and the patient was staged as Rai stage 0. In August 1979, his leukocyte count rose to 90,000, and he was treated with steroids and chlorambucil (4 mg/day). This led to a reduction in his white count, and his disease was controlled by intermittent therapy. In August of 1980, the patient developed a skin rash, which initially involved his face and subsequently extended to his neck, shoulders, back, chest, and torso. Chlorambucil therapy was discontinued because it was clinically thought that the rash was a possible allergic reaction to the drug. Skin biopsies from multiple sites were obtained and the histologic findings were interpreted by various observers as either an inflammatory reaction or an atypical lymphoid infiltrate consistent with involvement by CLL. Immunologic studies done on an additional skin biopsy failed to reveal a monoclonal B-cell lymphoid population, and the findings were considered to be consistent with an inflammatory process.

In September 1981, his white count began to rise, and chemotherapy was re instituted with Velban. There was no improvement in his skin rash, and in January 1982, he was started on oral cyclophosphamide (150 mg/day). Although there was improvement in his hematologic status, the skin rash showed little response to this therapy. The patient was then referred to the City of Hope in August 1982.

Upon admission, the patient was found to have a predominantly papular skin rash involving his head, face, shoulders, back, and trunk with scattered lesions present on his arms and legs (Fig. 1). He was not found to have hepatosplenomegaly or significant lymphadenopathy. The patient had a white cell count of 17,000, with 94% lymphocytes, a Hb of 12.8 g/dl, platelets of 120 x 10^9/liter, and a total protein of 6 g/dl. As part of the clinical evaluation, multiple skin biopsies and a peripheral blood sample were obtained for morphological, immunologic, and cytogenetic studies, and based on the results of these studies, the patient was diagnosed as having cutaneous T-cell lymphoma coexistent with B-cell chronic lymphocytic leukemia. We were unable to obtain a sufficient number of cells from the skin biopsies for karyotype analysis. Cytogenetic studies of peripheral blood lymphocytes showed no significant karyotypic abnormality.

The patient received a trial of electron beam radiation to the mid-back to assess the potential response of his disease. A 10 x 10 cm single posterior field was then treated with an unopposed PA 4-MEV electron beam (6-MEV electrons degraded to 4-MEV). He received 200-rad fractions to a total dose of 2,000 rads. At the completion of radiation therapy, there was no erythema and no changes in the multiple skin nodules within the irradiated field. Since there was no response to electron beam therapy within the...
field, additional, more extensive radiation therapy was not instituted.

During this time, the patient's chronic lymphocytic leukemia remained stable and was well controlled on cyclophosphamide (50 mg/day). He currently receives no therapy for his cutaneous T-cell lymphoma except for medication to control pruritis.

MATERIALS AND METHODS

Cell Preparation and Surface Marker Studies on Cells in Suspension

The separation of mononuclear cells from peripheral blood and routine immunologic studies were carried out as previously described from our laboratory. Lymphocytes were separated from 14 ml of heparinized blood by Ficoll-Hypaque density gradient centrifugation. Spontaneous sheep erythrocyte rosette (SER) formation was done using neuraminidase-treated fresh sheep erythrocytes in Hanks' balanced salt solution (HBSS) and 20% fetal calf serum. Fluorescein-conjugated solid immunoabsorbed F(ab')2 goat anti-human IgG, IgM, IgA, and IgD, κ and λ light chains (Kallestad Laboratories, Chaska, MN) were used for evaluation of the presence or absence of surface immunoglobulin (SIg). Cells were counted under a Zeiss Ortholux fluorescence microscope equipped with epifluorescence and a K-cube for fluorescein excitation. The results were interpreted according to established criteria for monoclonality. The intensity of immunofluorescence was evaluated as faint (+), moderately intense (+ + ), or markedly intense (+ + +).

Monoclonal Antibodies

In addition to the conventional SER and SIg assays, we used a panel of monoclonal antibodies with specific immunoreactivities, as summarized in Table 1. The monoclonal antibodies included: anti-B1 (Coulter Immunology, Hialeah, FL) anti-Leu-1, anti-Leu-2, and anti-Leu-5 (Becton Dickinson Laboratories, Sunnyvale, CA); OK1a and OKT6 (Ortho Pharmaceuticals, Raritan, NJ); and BA-1 and BA-2 (kindly provided by Dr. Tucker LeBien, Department of Pathology, University of Minnesota).

Tissue Preparation and Immunohistochemical Studies on Frozen Sections

Portions of the punch biopsy specimens of the skin were fixed in buffered formalin and stained with hematoxylin and eosin (H & E) for routine histologic examination. Sections for cryostat fresh-frozen immunohistochemistry studies were frozen in isopentane precooled at −120°F in liquid nitrogen. Three sections from each tissue fragment were cut at 6-μm thickness and placed on a single slide. A modification of the Avidin-Biotin-Complex (ABC) technique was used. Following a 5-min fixation of the air-dried frozen sections in acetone, the sections were washed briefly in modified phosphate-buffered saline (PBS) solution. Primary antibodies were placed on two of the three tissue sections at dilutions of 1:50 and allowed to incubate for 30 min. Antibody was not added to the third section, which served as a control for endogenous peroxidase activity. After removal of excessive primary antibody by washing, sections were overlaid with biotinylated, affinity-purified anti-mouse secondary antibody (Vector Laboratories, Inc., Burlingame, CA) at a dilution of 1:100 for 15 min. Subsequently, a preformed complex of Avidin and biotinylated horseradish peroxidase (Vector Laboratories) at 1:80 dilution was applied for 15 min. After removal of excess reagent from the tissue surface with the isotonic buffer system, the substrate

<table>
<thead>
<tr>
<th>Antibody Clone</th>
<th>Specific Reactivity</th>
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<tr>
<td>Anti-Leu-1</td>
<td>T cells, thymocytes</td>
</tr>
<tr>
<td>Anti-Leu-5</td>
<td>E-rosette receptor-associated T lymphocytes</td>
</tr>
<tr>
<td>Anti-Leu-3</td>
<td>Inducer/helper T-lymphocyte subclass</td>
</tr>
<tr>
<td>Anti-Leu-2</td>
<td>Suppressor/cytotoxic T-lymphocyte subclass</td>
</tr>
<tr>
<td>OK1a</td>
<td>B lymphocytes, some monocytes, and activated T cells</td>
</tr>
<tr>
<td>OKT6</td>
<td>Human 'common' thymocytes, Langerhans cells, some dendritic reticulum cells</td>
</tr>
<tr>
<td>Anti-B1</td>
<td>All B cells of peripheral blood and lymphoid organs</td>
</tr>
<tr>
<td>BA-1</td>
<td>Peripheral blood B cells, pre-B lymphocytes, CLL cells, most non-Hodgkin's B-cell lymphomas, most non-T, non-B ALL</td>
</tr>
<tr>
<td>BA-2</td>
<td>Cells in early stage of B-cell differentiation</td>
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color-reaction product was developed using 3-amino-9-ethylcarbazole (AEC), according to the method previously described.5 One additional section from each biopsy specimen was H & E stained to serve as a representative frozen tissue section. Sections from normal spleen and reactive lymph nodes were selected and studied in similar fashion as the positive controls. Paraffin-embedded tissue was not used in order to avoid spurious results introduced by fixation and alcohol dehydration.6

Electron Microscopy

Cells were fixed for 2 hr in 2% paraformaldehyde + 2.5% glutaraldehyde solution, postfixed in 1% osmium tetroxide, dehydrated in ascending series of ethanol followed by propylene oxide, and then embedded in epoxy resin. Ultrathin sections were cut, mounted on copper grids, stained with 1% uranyl acetate followed by 1% lead citrate, and examined in a Philips 301 electron microscope.

RESULTS

Characterization of Leukemic Cells in the Peripheral Blood

Review of the peripheral blood smears revealed numerous small, mature lymphocytes, which were consistent with CLL (Fig. 2). No cells having diagnostic features of Sézary cells were identified.

The results of immunologic studies done on cells in suspension that had been prepared from the patient’s peripheral blood are summarized in Table 2. The majority of the patient’s leukemic cells constituted a monoclonal B-cell population expressing lambda-light chains and IgM and IgD heavy chains. The cells exhibited a faint immunofluorescence intensity characteristic of CLL.7 The number of cells typed as having IgM and IgD heavy chains and lambda-light chains was similar to the number positive for the monoclonal antibodies reactive with human B cells (OK1a, anti-B-1, and BA-1).8-10 Less than 20% of the lymphocytes reacted with anti-Leu-5 antibody or formed SER with sheep erythrocytes.

Characterization of the Cutaneous Cellular Infiltrate

The H & E stained sections showed a diffuse infiltration of the dermis and subcutaneous tissue by a pleomorphic lymphoid infiltrate (Fig. 3). Many of the neoplastic lymphocytes had hyperchromatic nuclei and inconspicuous nucleoli; occasional atypical cells with deeply indented nuclei were present. No cells with cerebriform nuclei were evident, however. Marked exocytosis, characterized by patchy infiltration of the epidermis by atypical lymphocytes, was noted, but typical Pautrier microabscesses were not identified.

Transmission electron microscopy revealed atypical lymphocytes with enlarged hyperchromatic nuclei and occasional indentations of the nuclear membranes. No
lymphocytes with the hyperconvoluted nuclear membranes characteristic of CTCL were identified.

The immunologic findings derived from the frozen sections of the skin biopsies are shown in Table 3. The majority of cells constituting the cutaneous cellular infiltrates showed intensely positive surface membrane staining with anti-Leu-1 and anti-Leu-5, the monoclonal antibodies that react with peripheral T cells and with the antigen associated with the E-rosette receptor, respectively, thus indicating the T-cell nature of the cellular infiltrate. The cells also showed an intense positive reaction with the anti-Leu-3 monoclonal antibody, which is specific for helper/inducer T cells; the quantity and pattern of positivity were identical to that of the Leu-1- and Leu-5-positive cells. No positive reactions were observed when serial cryostat-frozen sections were stained with the monoclonal antibodies that are specific for B-cell surface membrane associated antigens (anti-B1 and BA-1). The immunologic phenotype of the cutaneous infiltrate (Leu-1+, Leu-5+, Leu-3+) was identical to the immunologic characterization of the majority of previously reported cases of CTCL.11-14

DISCUSSION

Although a number of cases of chronic lymphocytic leukemia of the T-cell type have been reported,15 CLL is most commonly found to be a lymphoproliferative disease having the immunologic characteristics of B lymphocytes. Our patient had a long history of documented CLL, which was demonstrated immunologically to be of B-cell type. Subsequently, he developed skin lesions with morphological features suggestive of CTCL. The possibility that these skin lesions represented CTCL was not recognized until the patient was admitted to the City of Hope. The immunologic studies done on frozen tissue obtained by biopsy clearly established the T-cell nature of the cutaneous neoplasm and strongly supported the morphological interpretation of CTCL.

Rare cases of mycosis fungoides have been reported to be coexistent with Hodgkin's disease,16,17 hairy cell leukemia,18 and monoclonal gammopathy;19 however, we know of no reported cases of B-CLL and CTCL in the same patient. This case may represent the first such case that has been documented immunologically.

The occurrence of CTCL in a patient with documented CLL may represent (1) the spontaneous development of a second neoplasm unrelated to the CLL or the patient's previous therapy, (2) a chemotherapy-induced lymphoproliferative disease arising from a cell line different from the CLL, or (3) a "modulation" or "switch" in the immunologic phenotype of a neoplastic

Table 3. Immunologic Characterization of the Cutaneous Neoplasm by Frozen Section Studies on the Skin

<table>
<thead>
<tr>
<th>Antibody</th>
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<tbody>
<tr>
<td>Anti-Leu-1</td>
<td>+</td>
</tr>
<tr>
<td>Anti-Leu-5</td>
<td>+</td>
</tr>
<tr>
<td>Anti-Leu-3</td>
<td>+</td>
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<tr>
<td>Anti-Leu-2</td>
<td>–</td>
</tr>
<tr>
<td>BA-1</td>
<td>–</td>
</tr>
<tr>
<td>BA-2</td>
<td>–</td>
</tr>
<tr>
<td>Anti-B1</td>
<td>–</td>
</tr>
<tr>
<td>Anti-IgM</td>
<td>–</td>
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<td>Anti-IgD</td>
<td>–</td>
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<tr>
<td>Anti-xLC</td>
<td>–</td>
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<tr>
<td>Anti-ALC</td>
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+ = Reactive, – = nonreactive.
cellular line derived from the patient’s original CLL. In the latter case, the switch in phenotype may be spontaneous, or related to the previous chemotherapy. Based on both the clinical features and the morphological and immunologic findings, we propose that the neoplastic cutaneous lymphoid infiltrate in this patient is a second neoplasm originating from a different cell line, rather than representing evolution of a T-cell lymphoma from CLL of the B-cell type. The development of a second malignancy in patients receiving chemotherapeutic agents has been well established and is presumed to be related to the oncogenic potential of these agents. The duration between the initiation of chemotherapy and the development of a second neoplasm is usually longer, however, than that observed in our case, in which the interval between the onset of chemotherapy for the CLL and the development of the skin rash was less than a year.

Although lymphoid cells with surface marker characteristics of both B and T cells have recently been found in a patient with B-CLL, simultaneous expression of B- and T-cell surface membrane characteristics on the same lymphoid population was not encountered in this case. In addition, the immunologic characteristics of the lymphoid population in the peripheral blood were typical for B-cell chronic lymphocytic leukemia, whereas the immunophenotype of the skin lymphoid infiltrate was the one most commonly encountered in CTCL. These findings provide further evidence for two separate lymphoproliferative disorders.

This case demonstrates the value and necessity of a multidisciplinary approach to the diagnosis and study of lymphoproliferative disorders. The availability of recently developed immunologic techniques for the study of frozen tissue and cells in suspension makes it possible to define precisely the expression of functional properties on neoplastic mononuclear cells. Immunologic techniques applied to frozen tissue are particularly well suited for the study of skin specimens, since it is frequently difficult to obtain sufficient numbers of cells from skin for cell suspension studies. The results can be further correlated with the morphological, cytochemical, and ultrastructural findings, and with the results of cell suspension studies, so that a specific diagnosis can be established.

REFERENCES

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Coincidence of B-cell chronic lymphocytic leukemia and cutaneous T-cell lymphoma (mycosis fungoides): immunologic characterization by monoclonal antibodies

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