Large Cell Lymphoma Complicating Acute Lymphoblastic Leukemia

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Non-Hodgkin’s lymphoma (NHL) is a very rare complication of acute lymphoblastic leukemia (ALL). We present the pathologic, clinical, immunologic, and ultrastructural features of the third reported example of NHL following successfully treated ALL. This white girl developed ALL with predominantly L1 cells at 3.5 yr of age. The lymphoblasts were terminal deoxynucleotidyl transferase (TdT) positive and were non-B, non-T cells. She achieved a complete remission with standard induction therapy and has remained in continuous complete remission. Four and one-third years after the onset of ALL, she developed multifocal, pleomorphic large cell lymphoma of the small bowel, which resulted in episodes of intussusception and obstruction. These pleomorphic and frequently multifocal lymphoma cells lacked TdT, common ALL antigen, and all tested markers of B cell, T cell, and histiocyte differentiation. Following three small bowel resections, systemic multiagent chemotherapy, and abdominal irradiation, she is currently free of disease.

SECOND MALIGNANCIES occurring in patients with acute lymphoblastic leukemia (ALL) are rare, with only 43 cases reported.1-4 The most frequent second malignancies are “histiocytic medullary reticulosis,”1 11 acute non-Hodgkin lymphoma,1 12 19 Hodgkin’s disease,20 24 chronic myelogenous leukemia,15 25 27 and various solid tumors.28-41 Only two cases of non-Hodgkin’s lymphoma complicating ALL have been previously reported,30 41 and, in both of these, a large cell lymphoma developed in an extranodal site. We report an additional case of extranodal NHL occurring after successful therapy for ALL. Four and one-third years after the onset of ALL, our patient developed a multifocal, pleomorphic large cell lymphoma of the small bowel, resulting in episodes of intussusception and bowel obstruction. The clinical, morphological, and immunologic features of both the initial ALL and the subsequent NHL are presented. Possible explanations for this phenomenon are suggested.

CASE REPORT

In December 1978, this 3.5-yr-old white girl was evaluated because of pallor, easy bruisingability, and fever of 3-wk duration. Physical examination revealed cervical, inguinal, and axillary lymphadenopathy (maximum node size was 1.5 x 1.5 cm); hepatomegaly and splenomegaly were present. The white blood cell count was 33,100/cu mm with 70% blasts, hemoglobin was 6.7 g/dl, and platelet count was 36,000/cu mm. The bone marrow showed 80%-90% cellularity with 89% lymphoblasts, and most lymphoblasts had typical L1 morphology (Fig. 1A). The periodic acid-Schiff (PAS) and peroxidase stains were negative. Immunologic studies of the bone marrow lymphoblast concentrates showed 4% surface immunoglobulin-bearing cells, 5% cells with complement receptors, and 3% cells displaying spontaneous rosettes with sheep erythrocytes. The cells exhibited high terminal deoxynucleotidyl transferase (TdT) activity. A diagnosis of non-B, non-T acute lymphoblastic leukemia (ALL) was made.

Other laboratory values included serum IgG 626 mg/dl, IgM 186 mg/dl, IgA 20 mg/dl, total protein 7.0 g/dl, albumin 4.3 g/dl, calcium 10 mg/dl, inorganic phosphate 4.4 mg/dl, blood urea nitrogen (BUN) 18 mg/dl, uric acid 9.5 mg/dl, alkaline phosphatase 145 mU/ml, SGOT 26 IU/liter, sodium 145 meq/dl, potassium 4.0 meq/dl, chloride 105 meq/liter, CO2 22, creatinine 0.7 mg/dl, and lactate dehydrogenase (LDH) 815 IU/liter. The cerebral spinal fluid had a 0 cell count, and no blasts were seen on cytospin smears.

The patient was assigned to Children’s Cancer Study Group Protocol no. 162 for treatment of patients considered to be of average risk. Induction was with prednisone, vincristine, and l-asparaginase. Prophylactic central nervous system therapy consisted of cranial irradiation and intrathecal methotrexate. A complete remission was achieved, and maintenance was begun with 6-mercaptopurine, oral methotrexate, vincristine, and l-asparaginase. The patient remained in continuous and complete remission. Maintenance chemotherapy was discontinued approximately 3 yr later (November 1981).

In April 1983, 4 yr and 4 mo after the diagnosis of ALL (1.5 yr after chemotherapy was discontinued) the patient developed abdominal pain following a viral illness. At this time, hemoglobin was 7.8 g/dl with a low mean corpuscular volume (MCV), and iron deficiency anemia was suspected. Two weeks later, she developed intussusception requiring emergency surgery. The “lead point” of the intussusception was a transmural terminal ileal mass measuring 3.5 x 3.5 x 4.5 cm. Several regional lymph nodes were enlarged.

Following morphological, cytochemical, immunologic, and ultrastructural evaluation, a diagnosis of malignant lymphoma, diffuse, large cell, immunoblastic, involving small bowel and adjacent lymph nodes, was made. Bone marrow aspirate and biopsy specimens were normal. The cerebrospinal fluid contained 10 cells/cu mm, which were mature lymphocytes and histiocytes.

After surgery, a metastatic work-up was negative, except for a left upper quadrant mass revealed by CT scan. A second laparotomy revealed a 10 x 3 x 2 cm circumferential mass in the proximal jejunum and a 5 x 4 x 4 cm circumferential ileal mass at the ligament of Treitz, with enlarged local lymph nodes. A separate 2 x 2 cm mass was identified at the site of the earlier bowel resection. These three masses were resected and were histologically identical to the original pleomorphic large cell lymphoma. The spleen and liver were grossly unremarkable; liver biopsies showed no evidence of lymphoma or leukemia.

After surgery, the patient received cyclophosphamide, methotrex-

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transferase (TdT) activity assays were performed according to previously published techniques.**45** Cerebrospinal fluid (CSF) cell counts were performed in counting chambers: Wright's stain, peroxidase, and nonspecific esterase (a-naphthol butyrate substrate). Fresh frozen tumor tissue was evaluated by immunofluorescence using fluorescein-conjugated antisera to IgM, G, A, D, E, κ, and λ (Kallestad, Inc.), and an indirect immunofluorescence procedure was used to evaluate the presence of TdT (Bethesda Research Labs, Bethesda, MD). In addition, frozen tumor tissue was evaluated by immunofluorescence techniques using monoclonal antibodies T101, OKT4, OKT8, OKT6, BA1, OKM1, T29/33, and J5(CALLA).**46,47** Immunoperoxidase staining using the peroxidase-antiperoxidase (PAP) technique was performed on formalin-fixed, paraffin-embedded tissue using antisera to IgM, A, D, G, E, κ, λ, and lysozyme (DAKO, Santa Barbara, CA).

Electron microscopy was performed on glutaraldehyde-fixed tissue using standard techniques. The tissue was sectioned at 640 Å, stained with uranyl acetate and lead citrate, and examined with a Phillips 300 electron microscope.

**RESULTS**

Most of the lymphoblasts from the initial (1978) bone marrow had scant cytoplasm and inconspicuous nuclei characteristic of L1 lymphoblasts (Fig. 1A). The ratio of L1:L2 lymphoblasts was 91:9. The bone marrow biopsy was mildly hypercellular and showed an extensive diffuse infiltrate of leukemic cells exhibiting high mitotic activity. These cells were surface immunoglobulin and E rosette-negative, and possessed high TdT activity (Table 1).

The cytologic features of the subsequent multifocal lymphoma involving the small bowel were dramatically different from the initial lymphoblasts (Fig. 1B). These cells were extremely large (up to 80 μ in diameter) and pleomorphic, with abundant to voluminous, pale to pyroninophilic cytoplasm. Occasional intracytoplasmic erythrocytes were seen. The often eccentrically located nuclei varied from round to irregularly folded and lobulated; nucleioli varied, but many were prominent and centrally located. Numerous multinucleated cells were seen; Reed-Sternberg-like cells were easily identified (Fig. 2). Many neutrophils and eosinophils were admixed with the lymphoma cells. The tumor extended through all layers of the bowel wall in multiple, widely separate sites within the jejunum and ileum. Several adjacent lymph nodes showed either complete nodal effacement or a paracortical infiltrate of tumor cells. On imprint smears, the tumor cells were negative by peroxidase and nonspecific esterase and positive for CD3, CD4, CD5, and CD10. A polyclonal plasma cell infiltrate was also apparent.

**MATERIALS AND METHODS**

All bone marrow aspirate and biopsy sections and special stains were prepared according to standard techniques. The leukemia cells on Wright's stained smears were classified as L1, L2, or L3 according to revised French American British criteria.**42,43** Periodic acid-Schiff (PAS) and peroxidase stains were performed on bone marrow smears. A Ficoll-Hypaque gradient was used to concentrate leukemic cells from the initial (1978) bone marrow specimen, and suspensions of the cells obtained were evaluated by immunofluorescence using antisera directed against IgM, G, A, D, E, κ, and λ. (Kallestad, Inc., Austin, TX). Spontaneous sheep erythrocyte rosetting of these cells (E rosette) was evaluated at 37°C. Terminal deoxynucleotidyl transferase (TdT) activity assays were performed according to previously published techniques.**44,45** Cerebrospinal fluid (CSF) cell counts were performed in counting chambers; a cytopsin smear of CSF was then made and stained with Wright's stain.

The bowel masses were sectioned at 4 μ and stained with hematoxylin and eosin, PAS, methyl green pyronine, and chloroacetate esterase. Imprint smears of the bowel masses were stained with Wright's stain, peroxidase, and nonspecific esterase (α-naphthol butyrate substrate). Fresh frozen tumor tissue was evaluated by immunofluorescence using fluorescein-conjugated antisera to IgM, G, A, D, E, κ, and λ (Kallestad, Inc.), and an indirect immunofluorescence procedure was used to evaluate the presence of TdT (Bethesda Research Labs, Bethesda, MD). In addition, frozen tumor tissue was evaluated by immunofluorescence techniques using monoclonal antibodies T101, OKT4, OKT8, OKT6, BA1, OKM1, T29/33, and J5(CALLA).**46,47** Immunoperoxidase staining using the peroxidase-antiperoxidase (PAP) technique was performed on formalin-fixed, paraffin-embedded tissue using antisera to IgM, A, D, G, E, κ, λ, and lysozyme (DAKO, Santa Barbara, CA).

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specific esterase stains. Likewise, the chloroacetate esterase stain on tissue sections was negative. The tumor cells lacked B cell, T cell, or true histiocytic features (negative surface immunoglobulin, cytoplasmic immunoglobulin, BA1, T101, OKT4, OKT8, OKT6, and OKM1). The tumor cells were also negative for TdT and common ALL antigen (cALLA, J5) (Table 1). By immunoperoxidase staining, the cells failed to show evidence of B cell or true histiocytic differentiation. The monoclonal antibody T29/33 was strongly reactive in tumor cells, indicating their hematopoietic-lymphoreticular origin.52

By electron microscopy, the cells had abundant cytoplasm that contained moderate amounts of rough endoplasmic reticulum and occasional lysosomes and mitochondria. Some microvilli were present. No junctions of any type were identified. No convincing evidence of differentiation toward histiocytes or plasma cells was demonstrated.

DISCUSSION

The development of a large cell lymphoma after successful therapy for ALL is a rare occurrence; only two reports of this phenomenon are found in the literature.30,41 Both these reports describe large cell lymphomas that arose in extranodal sites (brain and lung) from 1 to 15 yr after the diagnosis of ALL. Similarly, our patient developed a multifocal, pleomorphic large cell lymphoma of the small bowel 4.33 yr after the onset of ALL. In our case, the lymphoblasts of the original ALL did not resemble the bizarre pleomorphic lymphoma cells of the second malignancy (Fig. 1, A and B). These pronounced morphological differences are insufficient evidence to suggest that these two tumors are unrelated, as there are several hematologic malignancies, notably chronic lymphocytic leukemia and multiple myeloma, in which morphological transformations of well-differentiated cells to anaplastic cells are well described.54-58 However, the immunologic features of our patient's two malignancies also appear to be different. The original ALL cells lacked B and T cell features and were TdT positive. The cells of the subsequent pleomorphic large cell lymphoma lacked TdT and cALLA and typed as null cells. The only positive immunologic result on the NHL was obtained with the monoclonal antibody T29/33, a marker for cells of hematopoietic origin. Because the lymphoma cells lacked TdT, this NHL cannot represent a straightforward extramedullary relapse of the original ALL. However, it is still possible that these two tumors are related. Perhaps the ALL cells were somehow induced to differentiate into the lymphoma cells. This could have been spontaneous or influenced by the drug therapy. Certainly, there are several reports of drug-induced differentiation in vitro of ALL and in vitro and in vivo of ANLL cells.60-64 One could also suggest that the therapy for the ALL, either by direct drug interactions or by immunosuppression, somehow caused this NHL. NHL following therapy for Hodgkin's disease or other malignancies is well documented,65-67 but the low incidence of subsequent NHL in ALL patients makes this explanation less likely.

It is also possible that patients with ALL have an increased propensity for subsequent unrelated tumors, although, to date, the reported frequency of this complication is low. In our literature review, we identified only 43 ALL patients who developed second malignancies.1,41 However, the actual number of second malignancies may be somewhat less, as recent reports suggest that the histiocytic medullary reticulosis-like disorders described in ALL patients may be secondary to viral infections.68-72 Thus, some of the 11 ALL patients reported as developing histiocytic medullary reticulosis may actually have had virus-associated hemophagocytic syndrome.54,68,72 The other second malignancies reported in ALL are ANLL, Hodgkin's disease, CML, and a variety of solid tumors, including the two cases of NHL.1,12,41

We report the third case of NHL complicating ALL. In our case, the NHL was a pleomorphic large cell lymphoma with immunoblastic features that lacked all tested markers for B cell, T cell, and histiocyte differentiation, as well as markers for ALL cells, such as TdT and cALLA. The lymphoma involved multiple discrete foci of the small bowel and
resulted in episodes of intussusception and bowel obstruction. The initial surgical resection was unsuccessful and the lymphoma persisted despite chemotherapy. All grossly apparent lymphoma was resected during the third laparotomy. The patient is currently alive without evidence of lymphoma 5 mo after the diagnosis of NHL (4 yr and 9 mo after the diagnosis of ALL). She remains in continuous complete remission from ALL.

**NOTE**

At nine months after the diagnosis of NHL, the patient developed massive intraabdominal relapse and ascites. She remains in complete continuous remission from ALL. (Added in proof.)

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