CONCISE REPORT
The Coexistence of Acute Myeloblastic Leukemia and Diffuse Histiocytic Lymphoma in the Same Patient as Demonstrated by Multiparameter Analysis

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Measurement of cellular DNA content by flow cytometry demonstrated presence of two distinct aneuploid neoplasms in a patient who developed acute myeloblastic leukemia (AML) 4 mo after diagnosis of a diffuse histiocytic lymphoma (DHL). A lymph node aspirate contained peroxidase-negative, “null,” hyperdiploid (2.6C) DHL cells, while the bone marrow (BM) contained 84% primitive peroxidase-positive tetraploid AML cells (4.0C). Minor populations of hyperdiploid DHL and normal diploid cells could be detected by flow-cytometry in the BM, and all three populations were also seen in the peripheral blood.

SECONDARY ACUTE nonlymphoblastic leukemias (ANLL) are being reported with increasing frequency in patients with non-Hodgkin lymphomas (NHL) who have been treated with intensive combination chemotherapy and radiation therapy. Such secondary leukemias may be difficult to distinguish from leukemic progression of the lymphoma in a given patient. The patient in this study developed a tetraploid acute myeloblastic leukemia (AML) as well as a hyperdiploid diffuse histiocytic lymphoma (DHL) as demonstrated by flow-cytometry, karyotyping, cytochemical staining, and cell marker studies. These studies clarified the distinct phenotypes and genotypes of these two malignancies.

CASE REPORT

The patient, a 41-yr-old Italian man, was admitted to Memorial Hospital in March 1978 with a 6-wk history of painful cervical adenopathy, fever, and night sweats. Prior to admission, a right supraclavicular node biopsy had revealed malignant lymphoma, and the patient received a single course of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), vinblastine, procarbazine, and prednisone. Admission physical examination revealed bilateral cervical, supraclavicular, axillary, and inguinal lymph node enlargement. Diffuse histiocytic lymphoma (DHL) was diagnosed from a left cervical node biopsy. Initial hemoglobin (Hb) was 14.1 g/dl, platelet count 60,000/cu mm, white blood cell count
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(WBC) 4100/cu mm, with a normal differential count. Initial serum BUN, SGOT, LDH, and alkaline phosphatase values were normal. Mediastinal widening was noted on chest x-ray, and lymphangiogram demonstrated enlarged paraortic and pelvic lymph nodes. Bone marrow aspiration was normocellular, with 12.5% large primitive cells. He returned to Italy where he was treated with 2 cycles of cyclophosphamide, vincristine, and prednisone, 1 cycle of adriamycin, bleomycin, and prednisone, and finally 1 cycle of all 5 drugs together with progression of his disease. He returned to the U.S. in June 1978 and received palliative radiation therapy (tumor doses): 2600 rad were delivered to bilateral cervical, 2100 rad to mediastinal, and 1104 rad to paraortic and splenic portals.

He was admitted again in July 1978 with fever, abdominal pain, and pancytopenia. Temperature of 38°C, mild oral mucositis, tonsilar enlargement, progressive inguinal node enlargement, and mild abdominal tenderness were found on physical examination. Hemoglobin was 12.1 g/dl, platelet count 72,000/cu mm, and WBC 1900/cu mm with a normal differential count. Bone marrow aspiration was hypercellular with 66% blasts. Bleomycin, 19 mg/24 hr by continuous intravenous infusion, was administered for 3 days with no signs of improvement. Bone marrow aspiration was repeated, and a left inguinal lymph node aspiration was performed. At this time, the WBC was 700/cu mm with 88% neutrophils and 12% primitive cells. The bone marrow aspiration was hypercellular with 84% peroxidase and Sudan-black-positive blasts. The diagnosis of acute myeloblastic leukemia (AML) concomitant with progressive DHL was made. The patient refused further therapy and returned to Italy where he died 2 wk later.

MATERIALS AND METHODS

Cell Preparations

Fresh bone marrow (BM) smears were stained with tetrachrome, Sudan black, and peroxidase. Approximately 0.5–1.0 ml of aliquots of bone marrow and lymph node (LN) cells were obtained for further studies. Bone marrow and lymph node aspirates and peripheral blood (PB) were diluted with McCoy’s 5A medium containing 20% fetal calf serum (FCS) layered on Ficoll-diatrizoate cushions and spun at 100 g for 20 min. Interphase cell layers were removed, washed twice, and resuspended in medium. Slides were prepared with a cytocentrifuge and stained. Cell viability was tested by trypan blue exclusion and flow-cytometry. Assay for terminal deoxynucleotidyl transferase (TdT) was performed after DEAE-Sephadex chromatography of cell homogenate, as described previously. Bone marrow and lymph node mononuclear cells were studied for the presence of multiple surface markers. Bone marrow cells were cultured for 24 hr in tissue culture medium RPMI 1640 supplemented with fetal calf serum and penicillin-streptomycin. Culture, harvest, and quinacrine dihydrochloride staining of chromosome preparations were performed following conventional methods.

A recently developed method to measure DNA and RNA content simultaneously in unfixed cells using acridine orange (AO) was employed to study BM, LN, and PB cells after separation on a Ficoll-diatrizoate gradient. A computer-interfaced cytofluorograph, model FC200 (Ortho Instruments, Westwood, Mass.) was used to measure 5000 cells in each sample. Analysis of the DNA and RNA distributions and construction of histograms in three-dimensional plots were performed by a Nova 1220 minicomputer and displayed on a Tektronix graphical terminal.

In order to calibrate the RNA distribution, the mean value of lymphocytes obtained from normal donors with G0,1DNA content was used as a reference, and relative RNA values (RNA index—RI) were expressed as follows:

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RI = \frac{\text{Mean G}_{0,1} \text{RNA of sample}}{\text{Mean G}_{0,1} \text{RNA of control lymphocytes}} \times 10
\]

Cells with G0,1DNA content were considered exclusively and chosen for RNA evaluation and comparison because of the increase of RNA content per cell during progression through the cell cycle. The mean RNA value of the total population would reflect proliferation rather than a property of the specific cell type.

RESULTS

The July 1978 BM smear contained strongly Sudan black and peroxidase-positive blasts, while the primitive cells seen in the LN aspirate were Sudan black and peroxidase negative (Table 1). This suggested that myeloblasts were present in
the BM but not in the LN. Cytochemical staining was not performed in fresh PB smears because of the low WBC (700/cu mm) and low percentage of primitive mononuclear cells (12%). After Ficoll-diatrizoate separation of mononuclear cells, 99% of BM and 93% of LN cells were viable as determined by trypan blue exclusion. By DNA–RNA flow-cytometric criteria, 97% of BM, 98% of PB, and 95% of LN cells were viable. Both BM and LN cells were “null” by surface marker analysis. Terminal deoxynucleotidyl transferase (TdT) activity was significantly elevated above normal in the BM sample.

The presence of two aneuploid stem lines in the bone marrow was demonstrated by karyotypic analysis. Fourteen cells in mitosis were available for study. One cell was hyperdiploid with 55 chromosomes. A second cell, which was unsuitable for complete analysis because of poor technical quality, had approximately 53 chromosomes. Thirteen additional cells were hypotetraploid, with a range of 83–85 chromosomes. Duplication of the Y chromosome was identified in each metaphase by quinacrine fluorescence. Quinacrine banding was not of sufficient technical quality to detect specific chromosomal abnormalities.

Flow-cytometry (Fig. 1) demonstrated a single population of G1/G0 cells in the LN with a 2.6C DNA content, while 3 populations of G1/G0 cells with 2.0C, 2.6C, and 4.0C DNA contents were found in the BM and PB. The RNA index of the predominant Sudan black and peroxidase-positive tetraploid line in the BM was 31.4, while that of the hyperdiploid Sudan black and peroxidase-negative LN cells was 15.7.

**DISCUSSION**

The genotypic and phenotypic features of two malignancies were identified by cytochemical staining, cell marker studies, flow-cytometry, and karyotyping. The LN contained a “null” cell, hyperdiploid (2.6C) DHL. Strongly positive staining with Sudan black and for peroxidase in a majority of cells enabled the diagnosis of

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**Table 1. Laboratory Studies**

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<tr>
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<th>LN</th>
<th>BM</th>
<th>PB</th>
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<tbody>
<tr>
<td>Peroxidase and sudan black</td>
<td>-</td>
<td>+</td>
<td>nd</td>
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<tr>
<td>Surface markers</td>
<td>&quot;null&quot;</td>
<td>&quot;null&quot;</td>
<td>nd</td>
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<tr>
<td>TdT Activity</td>
<td>nd</td>
<td>+ (0.91)*</td>
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(U/10⁶ cells)

<table>
<thead>
<tr>
<th>Flow cytometry (DNA content)</th>
<th>2.0C</th>
<th>2.6C</th>
<th>4.0C</th>
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<tr>
<td>DNA index</td>
<td>0%</td>
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<tr>
<td>RNA index</td>
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<td>31.4</td>
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<tr>
<td>Karyotype (Q banding)</td>
<td>83–85 chr., 2Y</td>
<td>83–85 chr., 2Y</td>
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LN = Lymph Node
BM = Bone Marrow
PB = Peripheral Blood
* TdT activity in normal BM <0.1 U/10⁶ cells.
chr. = chromosomes
TdT = terminal deoxynucleotidyl transferase.
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Fig. 1. Computer-drawn DNA–RNA histograms of bone marrow, peripheral blood, and lymph node cells; 5000 cells were measured for each histogram in a flow-cytometer after staining with acridine orange (see Materials and Methods). X-axis: DNA; Y-axis: RNA; Z-axis: cell number. (A) Diploid (2.0C), hyperdiploid (2.6C), and tetraploid (4.0C) cells are found in the bone marrow. The predominant population, consisting of leukemic cells, displays high RNA content and progresses throughout the cell cycle from tetraploid G
 to S and to octoploid G
 DNA values. (B) Diploid (2.0C), hyperdiploid (2.6C), and tetraploid (4.0C) cells are present in the peripheral blood. Almost no proliferation of tetraploid cells can be measured. (C) The predominant lymph node population displays hyperdiploid DNA stem-line (2.6C) representing lymphoma cells. Some cells of this population are found in G
, probably due to previous treatment with bleomycin. Very few diploid cells (2.0C) are also present. The relative frequency of normal diploid cells, hyperdiploid lymphoma cells, and tetraploid leukemia cells is shown in Table 1.

AML to be made in the BM. The majority of the BM cells were tetraploid (4.0C). Because of the low WBC and the minority of mononuclear cells in the PB smears, cytochemical characterization of cells was not possible. In the BM, only the majority population of AML cells were identifiable by peroxidase and Sudan black staining. Flow-cytometry of separated mononuclear cell fractions enabled identifi-
cation of diploid (2.0C) normal cells and hyperdiploid (2.6C) lymphoma cells to be made in BM and PB. Karyotypic analysis of BM cells confirmed the presence of two aneuploid stem lines. Thirteen metaphases contained 83–85 chromosomes with 2Y chromosomes. Although flow-cytometry demonstrated that the DNA content of the BM cells was twice that of normal diploid cells, chromosome number was not doubled, suggesting that some chromosomes may have had a greater than normal DNA content. Aneuploidy was also demonstrated in human leukemias and lymphomas with flow-cytometry and karyotyping by Barlogie et al. Only 2 of 88 cases of acute leukemia were found to be tetraploid by these investigators. Use of acridine orange staining in the present case enabled measurements of RNA as well as DNA content of cells to be made by flow-cytometry. The RNA index of the tetraploid BM cells (31.4) was approximately twice that of the hyperdiploid LN cells (15.7). This RNA index may not simply reflect the tetraploid DNA content, since we have seen RNA indices in the range of 15 in diploid, hyperdiploid, and some tetraploid lymphoid malignancies (M. Andreeff, unpublished). We have also found a high RNA index in other cases of nonlymphoblastic leukemia.

No surface markers were detectable on either LN or BM cells. Although a majority of cases of so-called diffuse “histiocytic” lymphomas are actually B cell by surface marker analysis, a minority have been reported to be “null.” Bone marrow cellular TdT was elevated into the range seen in the acute lymphoblastic leukemia, lymphoblastic crisis of chronic myelocytic leukemia, lymphoblastic lymphoma, and a minority of cases of DHL. We believe that the TdT elevation in the bone marrow was due to the presence of DHL cells rather than to the AML, although no further cell separation studies were performed in this patient. We have previously reported a similar finding in a patient with dual population TdT-positive acute lymphoblastic leukemia and TdT-negative acute monocytic leukemia, where both cell populations could be separated by adherence procedures.

An alternative interpretation of these data is that the tetraploid AML represented a clonal evolution of the hyperdiploid lymphoma. The presence of one or more common marker chromosomes in both cell lines would have proven this interpretation to be correct. Unfortunately, the quality of the quinacrine banding was not sufficient to identify marker chromosomes in the present patient. The case for two separate primary malignancies rests on two points. First, although we have seen simultaneous occurrence of acute lymphoblastic and nonlymphoblastic leukemias, we are unaware of any case of malignant lymphoma evolving into an ANLL. Strongly positive peroxidase and Sudan black staining in the majority of bone marrow blasts established the diagnosis of AML and such cells were not found in the lymph node. Secondly, although it is formally possible that the hyperdiploid line could have evolved into the hypotetraploid line, the number of somatic events necessary to bring the hyperdiploid to a hypotetraploid chromosome number make this extremely unlikely. The diagnosis of malignant lymphoma antedated that of AML, so it is doubtful that the hyperdiploid lymphoma line evolved through chromosome loss from the hypotetraploid AML line.

The occurrence of ANLL has been reported in patients with NHL treated extensively with chemotherapy and/or radiation therapy. The majority of these cases probably represent secondary malignancies induced by the chemotherapeutic agents and/or the radiation therapy. In our patient, only 5 mo elapsed between the
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Diagnosis of DHL and AML, which is less than the 29–132 mo reported by Rowley et al. for 10 patients with ANLL after treatment for NHL. Since flow-cytometry was not performed on BM and PB cells initially, it is impossible to exclude the possibility that the AML was present when the DHL was first diagnosed. This case illustrates the application of flow-cytometry along with karyotyping, cytochemical staining, and cell marker studies to elucidate dual malignant cell populations with distinct phenotypic features in a single patient. This comprehensive analysis should be of value in recognizing those patients with more than one malignant cell lineage that might require different therapeutic approaches than those currently used for patients with leukemic progression of an underlying lymphoma.

REFERENCES

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