Acute Leukemia Presenting with Myeloid and Lymphoid Cell Markers*

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ABSTRACT

A case is described of acute leukemia whose neoplastic cells possessed myeloid and lymphoid characteristics. Neoplastic cells possessed cytoplasmic granules containing Sudan black B material and diaminobenzidine myeloperoxidase. In addition, these leukemic cells were positive for terminal deoxynucleotidyl transferase, 1a antigen, and the common acute lymphocytic leukemia antigen. These findings indicate that biphenotypic cell markers may exist in cases of acute leukemia. It is our belief that these results are best explained as either a mixed myeloid-lymphoid leukemia or a stem cell leukemia capable of differentiating into myeloid and lymphoid cells.

Introduction

The appearance of acute myeloid leukemia (AML) as a second malignancy has been reported with increased frequency in patients with ovarian carcinoma, polycythemia vera, Hodgkin's disease, and multiple myeloma.1,12,24,25,30 Occasionally, cases of AML following non-Hodgkin's lymphoma, chronic lymphocytic leukemia, and acute lymphocytic leukemia have occurred.21,22,27 Even more rare are cases of acute leukemia with neoplastic cells exhibiting cytochemical and/or immunologic characteristics of both myeloid and lymphoid cell lines.16

The purpose of this report is to present morphologic, cytochemical, and immunologic studies on the leukemic cells from a patient with acute leukemia. In this case, the leukemic cells exhibited characteristics of both acute myeloid and acute lymphoid leukemia at the time of presentation before any chemotherapy was given to the patient.

Case Report

A 59 year old woman was admitted to the hospital because of marked fatigue, a 20 pound weight loss, and bleeding from the nose and mouth. Her physical examination revealed a left facial nerve palsy, pallor, epistaxis, and gingival bleeding. There was no lymphadenopathy or organomegaly. A roentgeno-
graph of the chest was normal. Pertinent laboratory values were as follows: leukocyte count \(31.6 \times 10^3\) per \(\mu\)l with a differential count of 75 percent blasts, rare promyelocyte and myelocyte, six percent bands, nine percent neutrophils and 10 percent lymphocytes. There were five nucleated red blood cells per 100 leukocytes. The bone marrow aspirate films contained 92 percent blasts and the biopsy was hypercellular. A diagnosis of acute leukemia was made and the patient received induction therapy consisting of daunorubicin, cytosine arabinoside, and vincristine administered intravenously, and prednisone given orally. Leukemic cells were also identified in the spinal fluid samples, and methotrexate was administered intrathecally. Following therapy, the patient developed pancytopenia and the bone marrow aspirate and biopsy were hypocellular. Unfortunately, the patient developed bacterial septicemia and, despite antibiotic therapy, died 16 days after the start of chemotherapy.

**Materials and Methods**

A 500 cell differential count was performed on each bone marrow aspirate previously stained with Wright-Giemsa reagents.

**Cytochemical Stains**

Bone marrow aspirates and peripheral blood air dried smears were stained for the presence of 3,3'-diaminobenzidine (DAB) peroxidase, neutral lipids (Sudan black B), alpha-naphthyl acetate esterase, alpha-naphthyl butyrate esterase, acid phosphatase, and periodic acid-Schiff (PAS) material. The percentage of positive cells was calculated from a 200 cell count.

**Immunologic Studies**

Ten milliliters of peripheral blood were obtained in preservative-free heparin. Mononuclear cells were separated from other blood elements by centrifugation and a gradient composed of Ficoll and diatrizoate salts.* Leukemic cells were evaluated for their ability to form nonimmune rosettes with sheep erythrocytes (E-rosettes) and to contain surface and cytoplasmic immunoglobulins. Leukemic cells were examined for the presence of Ia-like antigen by indirect immunofluorescence. To detect Fc receptors, the malignant cells were first incubated with aggregated human gammaglobulin and subsequently with fluorescein labeled antihuman IgG. 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. The tumor cells were also examined for the presence of human T-cell antigen using a direct immunofluorescence technique.

**Results**

**Histopathology and Cytology**

The bone marrow aspirate and biopsy showed blasts measuring 12 to 20 \(\mu\) in diameter and possessing irregularly shaped nuclei. The nuclear to cytoplasmic ratio was moderately high revealing only a slight amount of light blue cytoplasm. The nuclear chromatin was fine and one to two small nucleoli were present in the majority of leukemic cells (figure 1).

**Cytochemical Studies**

Thirty-nine percent of leukemic cells from the bone marrow aspirate were positive for DAB-peroxidase reaction and 31 percent were positive for Sudan black B (figure 2). Auer rod like structures were observed in both the DAB peroxidase and Sudan black B stained preparations. Ninety percent of the cells were positive for acid phosphatase. Leukemic cells were negative for PAS and alpha-naphthyl acetate esterase (table I).

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* Lymphocyte Separation Medium, Litton BioNetics, Kensington, MD.
Immunologic Marker Studies

Ninety percent of leukemic cells were positive for TdT (figure 3), 84 percent positive for the common ALL antigen and 96 percent positive for Ia-like antigen. Leukemic cells were negative for other immunologic markers (table I).

Discussion

In the current case, leukemic cells were positive for DAB myeloperoxidase, Sudan black B as well as for TdT, common ALL antigen, and Ia antigen. Myeloperoxidase activity is present at all stages of neutrophil development and is
TABLE I
Summary of Cytochemical and Immunologic Marker Studies of Leukemic Cells

<table>
<thead>
<tr>
<th>Assay</th>
<th>Positive Leukemic Cells (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudan black B</td>
<td>31</td>
</tr>
<tr>
<td>DAB-Myeloperoxidase</td>
<td>39</td>
</tr>
<tr>
<td>Periodic-acid Schiff</td>
<td>negative</td>
</tr>
<tr>
<td>Alpha-naphthyl esterase</td>
<td>negative</td>
</tr>
<tr>
<td>butyrate esterase</td>
<td>negative</td>
</tr>
<tr>
<td>Alpha-naphthyl acetate esterase</td>
<td>negative</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>90 (diffuse)</td>
</tr>
<tr>
<td>E rosettes</td>
<td>4</td>
</tr>
<tr>
<td>Surface immunoglobulins</td>
<td>3</td>
</tr>
<tr>
<td>Cytoplasmic immunoglobulins</td>
<td>1</td>
</tr>
<tr>
<td>Ia-like antigen</td>
<td>96</td>
</tr>
<tr>
<td>Fc receptors</td>
<td>9</td>
</tr>
<tr>
<td>Terminal deoxynucleotidyl transferase</td>
<td>90</td>
</tr>
<tr>
<td>T cell antigen</td>
<td>6</td>
</tr>
<tr>
<td>Common ALL antigen</td>
<td>84</td>
</tr>
<tr>
<td>Monocyte antigen</td>
<td>2</td>
</tr>
</tbody>
</table>

localized to the nonspecific granules in the myeloid cell cytoplasm. Myeloperoxidase activity is best visualized by using 3,3'-diaminobenzidine (DAB) as substrate and DAB myeloperoxidase activity is usually present in blasts from cases of acute myelogenous leukemia, acute myelomonocytic leukemia, and acute megakaryocytic leukemia. Myeloperoxidase activity is negative in blasts from cases of ALL. Flandrin and Daniel indicate that complete absence of myeloperoxidase activity is the best practical criterion for diagnosis of ALL.

Sudan black B stains a variety of lipids including neutral fats, phospholipids, and sterols. In leukocytes, Sudan black B positive material is usually present in granulocyte precursors and immature monocytes but not in lymphoid cells. To our knowledge, there are only two reported cases of sudanophilia in lymphoblasts collected from cases of ALL. Lymphoblasts from these two cases were positive for common ALL antigen, Ia antigen, and TdT.

In normal tissues, TdT positive cells are observed in approximately 80 percent of cortical thymocytes and two to five percent of bone marrow cells. Also, TdT is present in the majority of neoplastic cells in 90 percent of cases of ALL and in most cases of lymphoblastic lymphoma. In addition, TdT is positive in blasts from approximately one-third of the cases of chronic myelogenous leukemia in blastic crisis. Approximately
six percent of cases of acute myelogenous leukemia have an elevated number of TdT positive cells. In some of these cases, features of both myeloid and lymphoid leukemias (i.e., mixed myeloid-lymphoid leukemias) are present with TdT negativity occurring in myeloid cells and TdT positivity in lymphoid cells. However, in a few cases, TdT activity was demonstrated in cells which were myeloid by other criteria suggesting an aberrant TdT expression in malignant myeloid cells.

The Ia (HLA-D, DR) antigens are present on stem cells, B lymphocytes, monocytes, and activated T lymphocytes. However, the Ia antigen is present on neoplastic cells from most cases of acute leukemia, chronic lymphoid leukemias and non-Hodgkin's lymphomas of B cell lineage. Thus the presence of Ia antigen on the neoplastic cells from the current case is of little help in determining the lineage of these leukemic cells.

In normal adult individuals the common ALL antigen is present on two percent of bone marrow cells and normal neutrophils. In the fetal liver, five to 10 percent of cells are positive for the common ALL antigen. Normal peripheral blood T and B lymphocytes, null cells, immature granulocytes, monocytes, or platelets do not possess the common ALL antigen. The common ALL antigen is usually present on blasts from non B, non T ALL, pre B cell ALL, some cases of undifferentiated acute leukemias, 40 percent of cases of chronic myelogenous leukemia in blastic crisis, and a few cases of non-Hodgkin's lymphoma. However, the common ALL antigen is virtually absent in cases of acute myeloid leukemia. Thus, the presence of the common ALL antigen on the neoplastic cells from our patient suggests a lymphoid origin to at least a subpopulation of the leukemic cells.

In the current report, neoplastic cells possessed characteristics of both myeloid and lymphoid cells. The exact interpretation of these findings suggests at least four explanations.

The first possibility is that the patient may have been in the blastic phase of chronic myelogenous leukemia. Although no karyotype was performed in this patient, the presentation and past history make this an unlikely possibility. In addition, blasts from patients in blastic phase of chronic myelogenous leukemia are not simultaneously positive for DAB myeloperoxidase, Sudan black B, TdT, and the common ALL antigen.

As a second possibility, derepression of a marker genome of leukemic cells from either myeloid or lymphoid origin could allow the expression of an antigen or enzyme of the opposite line. Bettelheim et al demonstrated the expression of a myeloid-specific antigen (VIM-DS) on TdT positive blast cell populations from two cases of childhood ALL. Furthermore, leukemic cells from one of their cases possessed Ia and common ALL antigens. These investigators suggested that the manifestation of the VIM-DS antigen was the result of a derepression of the genome in ALL blasts. Genome derepression is probably not operative in our case because two myeloid (SBB and DAB myeloperoxidase) and two lymphoid (TdT and common ALL antigen) markers exist on these leukemic blasts. Thus, more than one genome would need to be derepressed and this is an unlikely event.

Thirdly, this case may represent a true mixed myeloid-lymphoid leukemia with two separate malignant clones, a smaller (30 to 40 percent) population of myeloblasts and a larger (60 to 70 percent) group of lymphoblasts. The apparent overlap of myeloid and lymphoid characteristics on 20 to 30 percent of the blasts could be an artifact secondary to the counting of only 200 cells for each marker assay.

Finally, it is possible that multiple my-
eloid and lymphoid markers exist on a common myeloid-lymphoid stem cell which has not yet been fully characterized. Perentesis et al. described a five year old girl with acute leukemia. Her blasts initially appeared lymphoid and they contained numerous surface antigens typical of ALL. However, after six days of therapy, the malignant cell had a myelomonocytic morphology. Although new surface antigens were demonstrated on the myelomonoblasts, some of the monoclonal antibodies which reacted with the original lymphoid blasts continued to react with new myelomonocytic blasts. These findings argue in favor of the existence of a stem cell leukemia with multiple myeloid and lymphoid cell characteristics. The present authors believe that the immunologic and cytochemical data from the current case could best be explained by either of the latter two hypotheses. However, it seems evident that further ontogenic, immunologic and cytochemical studies on additional cases are needed to establish the exact mechanism of the biphenotypic markers in these cases of acute leukemia.

References


