Clonal Identification in Acute Lymphoblastic Leukemia

By A. H. Goldstone, B. A. McVerry, G. Janossy, and H. Walker

In a case of acute lymphoblastic leukemia, two distinct types of leukemic blast cells could be identified throughout the course of the disease. The initially dominant type of blast cell was sensitive to chemotherapy; the other was drug-resistant, gradually becoming dominant as the disease progressed. The cell types could be clearly separated by their morphologic and surface membrane marker characteristics. The same chromosomal constitution was present in both types of blast cells, indicating a common clonal origin. Additional chromosomal abnormalities were present in the later stages of the disease, demonstrating that a distinct subclone had proliferated. This study illustrates that in some cases of acute leukemia, disease relapse is caused by growth of drug-resistant subclones that may be clearly identified by changes in morphology and surface membrane marker characteristics.

The emergence of drug-resistant subclones within a leukemic cell clone has been well documented in patients with Philadelphia-chromosome-positive (Ph1-positive) chronic myeloid leukemia (CML). In this disease the acquisition of additional chromosomal abnormalities within the Ph1-positive clone is often accompanied by profound changes in cellular morphology, growth characteristics, and drug sensitivity.1-3

It has long been suspected that clonal evolution might also be responsible for most cases of disease relapse in acute myeloid and lymphoid leukemias. Indeed, evolution in these diseases (defined as "any departure from euploidy") can sometimes be demonstrated.4,5 However, difficulty arises when the leukemic cell populations that emerge during relapse show morphologic characteristics that are different from those of the initial leukemic cell type. In most of these cases, clonal markers (e.g., chromosomal abnormalities or a particular membrane immunoglobulin phenotype) are absent at the onset of the disease, and therefore the emergence of a new independent leukemia cannot be formally excluded.

In this report we demonstrate that during disease relapse in acute lymphoblastic leukemia (ALL) a distinct drug-resistant subclone emerged from within a chromosomally identifiable leukemia clone. This subclone could be identified by both its morphologic and surface marker characteristics.

CASE REPORT

A 24-yr-old pregnant woman was admitted to the hospital complaining of recent onset of nasal and gum bleeding. On examination marked sternal tenderness, splenomegaly, and purpura were found. Initial studies showed: hemoglobin 11.0 g/dl, white cell count 130 × 10⁹/liter, and platelet count 35 × 10⁹/liter; 90% of the white cells were blast cells. A bone marrow aspirate was very cellular. The normal marrow population was virtually replaced by blast cells (vide infra). Chest x-ray, liver and renal function tests, electrocardiogram, and electrolytes were normal. A diagnosis of ALL was made, and
treatment with vincristine and prednisone was started. Although an initial response to therapy was seen, complete remission was not obtained despite treatment with COAP (cyclophosphamide, vincristine, cytosine arabinoside, prednisone), TRAMPCO (daunorubicin, cytosine arabinoside, Adriamycin, prednisone, vincristine, cyclophosphamide, 6-thioguanine), and L-asparaginase.

Approximately 8 wk after the initial presentation, occipital-frontal headaches, photophobia, and neck stiffness occurred. Although a lumbar puncture examination was not diagnostic, a course of cranial irradiation and intrathecal methotrexate and dexamethasone produced symptomatic relief. Her further course was complicated by recurrent infections and severe localized bone pain in the lower limbs that responded to local radiotherapy. Twenty weeks after the initial presentation, the patient died from septicemia and uncontrolled leukemia.

MATERIALS AND METHODS

Studies were performed on samples of fresh peripheral blood and bone marrow. Leukocytes were prepared by Ficoll-Isopaque density-gradient centrifugation. The leukemic cells were characterized by a panel of membrane and enzyme markers. The membrane markers were the following: anti-ALL serum made against the “common” non-T non-B form of ALL, anti-Ia-like serum made against purified p28,33 antigens, anti-HuTLA serum, a thymocyte and T-lymphocyte marker, antiimmunoglobulin (SmIg), a B-lymphocyte marker; and antimyeloid (My) serum. E-rosetting was performed with neuraminidase-treated sheep erythrocytes. Acridine orange was added to visualize the size of rosetting cells.

Enzyme markers included terminal deoxynucleotidyl transferase (TdT), acid phosphatase, and nonspecific alpha-naphthol esterase. The preparation of reagents and their reactivity patterns on normal and leukemic cells have been described previously. Romanowsky, Sudan black, and periodic acid–Schiff (PAS) stains were standard techniques.

Direct bone marrow preparations for chromosomal analysis were made by a modification of the technique of Tjio and Whang. Unstimulated blood cultures were set up for 24 hr in TC-199 medium with fetal calf serum. Giemsa banding techniques were performed using a modification of the Summer method. Slides were incubated at 60°C for 90 min in 2 × SSC, followed by rinsing in distilled water and Giemsa staining.

In this study, vacuolated cells were blast cells with one or more definite cytoplasmic vacuoles present on light microscopy (May-Grunwald-Giemsa stain).

RESULTS

At presentation, a bone marrow aspirate was very cellular. The normal marrow cell populations were virtually replaced by blast cells. Most blast cells (99%) were intermediate in size, showed a smooth outline, a high nuclear-cytoplasmic ratio, and fine nuclear chromatin (type 1 cells, Fig. 1). These cells strongly reacted with anti-ALL serum but were unreactive with anti-Ia-like serum (Table 1).

During the terminal stages of the disease, most blast cells in bone marrow preparations (95%) showed prominent cytoplasmic vacuoles (type 2 cells, Fig. 2). Other morphologic features were similar to those of type 1 cells. At that time the blast cells were unreactive with anti-ALL serum but they stained strongly with anti-Ia-like serum.

Occasional type 2 cells (1%) were seen at presentation (Fig. 3). During the course of the disease there was a gradual change in the relative proportions of the two types of blast cells (Fig. 4): 6 wk after presentation 30%, 8 wk after presentation 65%, and terminally (20th wk) virtually all blasts (95%) were of type 2.

Both blast cell populations showed elevated levels of TdT; they failed to react with thymocyte and T-lymphocyte markers (e.g., E-rosetting and anti-HuTLA serum) and were negative for surface immunoglobulin and myeloid antigens.

These observations confirmed the diagnosis of “common” non-T non-B ALL,
with the proviso that some membrane determinants might have been selectively lost at certain stages of the disease (see Discussion).

Chromosomal analysis of bone marrow at presentation showed that, on examination of 25 cells, the karyotype was 46XX,1q−,7q−,t(1:19) in all cells. No normal cells were seen. In the terminal phase, 105 cells were examined; these were from an unstimulated peripheral blood culture. Ninety-two metaphases showed a karyotype of 55XX,+1q−,+7q−,+t(1:19),+C+C+D+D+19+22. Thirteen cells had a normal karyotype (46XX). No cells with the initial abnormal karyotype were seen.

Table 1. Membrane and Enzyme Marker Observations*

<table>
<thead>
<tr>
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<th>Presentation</th>
<th>Terminal Stage</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
<td>Bone Marrow</td>
</tr>
<tr>
<td>Anti-ALL serum</td>
<td>83</td>
<td>64</td>
</tr>
<tr>
<td>Anti-la-like serum</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Anti-HuTLA serum</td>
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<td>NT</td>
</tr>
<tr>
<td>Anti-myeloid serum</td>
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<td>0</td>
</tr>
<tr>
<td>E-rosettes</td>
<td>2</td>
<td>NT</td>
</tr>
<tr>
<td>Anti-SmIg</td>
<td>NT</td>
<td>1</td>
</tr>
<tr>
<td>TdT (units/10⁶ cells)†</td>
<td>6.2</td>
<td>NT</td>
</tr>
<tr>
<td>Nonspecific alpha-naphthol esterase</td>
<td>Negative</td>
<td>Weak granular positivity</td>
</tr>
<tr>
<td>Acid phosphatase‡</td>
<td>Occasional cell showed Block positivity</td>
<td>Occasional cell showed Block positivity</td>
</tr>
<tr>
<td>PAS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudan black</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

*Percentages of reactive cells are shown unless otherwise stated. NT = not tested.
†Normal range <0.5 unit/10⁶ cells.
‡ Majority of blast cells were positive.
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DISCUSSION

It has been well established that most cases of ALL of the non-T non-B type (common ALL) react with anti-ALL and anti-la-like sera and have high levels of TdT, but fail to express membrane antigens of thymocytes or myeloid cells (ALL+,Ia+,TdT+, HuTLA−, My−). Thus a triad of markers (ALL+, Ia+, TdT+) is available that positively identifies common ALL and so-called lymphoid blast crisis of chronic myeloid leukemia.10 This panel of markers helps to differentiate these diseases from leukemias of thymocyte phenotype (ALL−, Ia−, TdT−,
HuTLA\(^+\), My\(^-\)) and from myeloid leukemias (ALL\(^-\), Ia\(^-\), TdT\(^-\), HuTLA\(^-\), My\(^+\)).

In our patient two distinct subclones were identified. Both expressed the typical common ALL phenotype, with the notable exception that most blast cells at presentation failed to express Ia-like antigens (type 1 blasts: ALL\(^+\), Ia\(^-\), TdT\(^+\), HuTLA\(^-\), My\(^-\)), whereas most blast cells in the terminal stages, although failing to express the All antigen, did react strongly with anti-Ia-like serum (type 2 blasts: ALL\(^-\), Ia\(^-\), TdT\(^+\), HuTLA\(^-\), My\(^-\)). In addition, the two cell types could be clearly separated morphologically.

These findings could represent either two independent leukemic cell lines or two different cell populations arising from a common precursor. Chromosomal analysis excluded the possibility that these were independent leukemias, insomuch as the same chromosomal constitution was present in both cell populations. The additional abnormalities found in the type 2 cells were evidence of clonal evolution. For this reason the probable explanation of our findings is that the two subclones had derived from a common leukemic precursor with an ALL\(^+\), Ia\(^+\), TdT\(^+\) phenotype (Fig. 5). Whereas one subclone had selectively lost the expression of Ia-like antigen, the other had apparently lost the ALL antigen.

Selective loss of membrane antigens does occur. In a study of 131 patients with common ALL,\(^21\) 3 patients failed to express the Ia-like antigen (ALL\(^+\), Ia\(^-\), TdT\(^+\)), and 20 patients did not react with anti-ALL sera (ALL\(^-\), Ia\(^+\), TdT\(^-\)). The loss of Ia-like antigens during long-term culture of a human B-lymphoblastoid cell line has been reported.\(^22\) In addition, we have recently seen a patient with Ph\(^1\)-positive chronic myeloid leukemia in “lymphoid” blast crisis who had apparently lost the ALL antigen. Whereas blast cells in the cerebrospinal fluid were ALL\(^+\), blast cells in the bone marrow and peripheral blood failed to express the ALL antigen, although they had the morphologic appearance of lymphoblasts and had an ALL\(^-\), Ia\(^+\), TdT\(^+\), HuTLA\(^+\), My\(^-\) phenotype.\(^23\)

It may be argued that in our patient one subclone had developed from the other and not directly from a leukemic precursor (Fig. 5). We do not think this is likely. We have studied large numbers of leukemic patients, both initially and during the course of their disease. We have never seen a lymphoid Ia cell population become Ia\(^+\). In addition, recent information suggests that Ia-like antigens reflect normal
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Fig. 5. Postulated relationship between a leukemic precursor cell and the two subclones (see Discussion).

...making the acquisition of an Ia antigen in an Ia− cell population unlikely. Likewise, we have not seen ALL− cells subsequently express the ALL antigen.

An important observation was that whereas type 1 blast cells appeared to be sensitive to chemotherapy, type 2 cells were resistant, and gradually became the dominant subclone. Therefore this study shows that in at least some cases of ALL, disease relapse and further resistance to chemotherapy are direct results of the growth of drug-resistant leukemic subclones. In addition, these subclones may be identified by repeated observations using a panel of membrane surface markers.

ACKNOWLEDGMENT

We are indebted to Professor A. V. Hoffbrand and Dr. K. Ganeshaguru, Department of Haematology, Royal Free Hospital, London, for TdT analysis.

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