Cytogenetic and Immunoglobulin Markers of Human Leukemic B-Cell Lines

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SUMMARY

This study describes the establishment of two permanent leukemic B-cell lines (BALM-1 and -2) originating from the blood cells of a patient with a B-cell type of acute lymphoblastic leukemia. The cells of BALM-1 and -2 exhibited cell surface markers compatible with B-cell origin. The identity between the leukemic cells in vivo and those of the BALM lines was established on the basis of the karyotypic picture, including marker chromosomes 14q+ and t(12;17;?) and cell surface immunoglobulins (κ, δ and μ chain determinants).

INTRODUCTION

A number of lymphoid cell lines have been established in the past from the cells of patients with a variety of diseases, including malignant ones (26, 27, 32). The majority of these lines are known to be immunoglobulin-producing lymphoblastoid cell populations (B-cell lines), and their overall similarities have been well characterized. Indeed, B-cell lines established from the cells of healthy donors are indistinguishable from B-cell lines established from leukemic blood (14, 22, 28, 29). Except for B-cell lines of Burkitt's lymphoma origin, there is little evidence indicating that they are possibly of malignant origin (2, 31).

The successful establishment and characterization of leukemic T-cell lines has led to a renewed look at homogeneous T- and B-cell lines (23, 24). One of the most remarkable leads came from studies that indicated that ALL2 can be subgrouped phenotypically into 3 categories, i.e., null-cell (non T/non B) (about 75% incidence), T-cell (about 20% incidence), and B-cell (about 5% incidence) types of ALL (5–9, 20, 30). A poor prognostic outcome of B-cell ALL has been noted among the 3 subgroups of ALL (1, 3, 13, 19, 36).

To date, only those lymphoblastoid cell lines derived from T-cell ALL have been successfully established and proved to represent permanent leukemic cell lines. We report the successful establishment of 2 permanent leukemic B-cell lines, in which it was demonstrated that the cytogentic and cell surface immunoglobulin markers were identical with those of the "fresh" leukemic blasts in vivo. To our knowledge, this is the 1st report in which established human cell lines of acute leukemic B-cell origin have been thus characterized.

CLINICAL DATA

A 56-year-old male patient was admitted on March 11, 1976, to the Roswell Park Memorial Institute with the diagnosis of acute leukemia. The peripheral WBC was 43,500/cu mm with 90% leukemic blasts. Examination of the leukemic blasts for cell surface markers and immunological characteristics (see below) indicated that they were most compatible with B-cell-type ALL. Following antileukemic therapy, the patient entered complete remission with a WBC of 2800/cu mm without leukemic blasts. Nevertheless, clinical and hematological status worsened subsequently, and the patient expired on May 10, 1976. Autopsy findings showed leukemic involvement of the bone marrow, spleen (2.5 kg), and paratracheal and paraesophageal regions.

MATERIALS AND METHODS

Cell and Culture Preparations. A portion of the fresh leukemic cell population obtained prior to antileukemic therapy, isolated on Hypaque-Ficoll discontinuous density gradient centrifugation at 400 x g for 30 min at room temperature and washed 3 times with PBS (pH 7.2), was set up in 2 plastic flask cultures (Falcon 3024, 250 sq cm) with approximately 25 ml of Roswell Park Memorial Institute Nutrient Medium 1640 supplemented with 10% (v/v) heat-inactivated fetal calf serum and antibiotics (penicillin, 100 μg/ml; streptomycin, 50 μg/ml). The cultures were incubated at 37° in a 5% CO2-humidified incubator. An immediate cell proliferation in the cultures was noted, and subculturing of the cell populations, which began to form cell aggregates of various sizes, was possible by 10 days. By the end of 1 month in culture, the leukemic cell populations in both flasks were considered to be established. The general appearance of these 2 cell lines was indistinguishable from that of the many SmIg-positive B-lymphoblastoid cell lines previously established. The 2 cell lines were designated BALM-1 and -2.

Surface Marker Assays. Details of the methodologies used have been described previously (17, 25, 37). In brief, the appearance of nonimmune rosettes with sheep RBC was used as a T-cell surface marker. The fresh leukemic and cultured cells were washed twice with PBS and then suspended in 0.9% NaCl solution at a cell density gradient of 1 x 107 cells/ml. Two-tenths of the cell suspension was mixed...
with 0.2 ml of either untreated sheep RBC suspension (1% suspension in PBS by volume) or 2-aminoethylisothiouronium bromide-treated sheep RBC, followed by centrifugation at 200 × g at room temperature for 3 min. The cell pellets were incubated undisturbed for 1 to 3 hr at either 4° or room temperature. The cells were then gently dispersed, and rosette-forming cells were determined under microscope at ×500. As B-cell surface markers, both rosette formation with EAC and immunofluorescent staining of SmIg were employed. The EAC-rosetting procedure was the same as that of T-cell rosetting, except that centrifugation was not required and the incubation of the EAC reaction was carried out at room temperature for 1 hr. The percentage of rosette-forming cells was obtained by counting at least 200 cells under the microscope. Direct membrane immunofluorescent staining for the SmIg was carried out using monospecific fluorescein isothiocyanate-isothiocyanate-labeled goat anti-human immunoglobulin chain reagents (κ, λ, α, δ, γ, and μ chain specific). Staining was carried out on washed viable cells with fluorescein isothiocyanate-conjugated anti-human immunoglobulin reagent at room temperature for 30 min. The percentage of fluorescein cells was determined by counting at least 200 cells on a Leitz UV fluorescent microscope with incident illumination at ×1000.

**Immunofluorescent Staining for Membrane Antigens.** A panel of specific rabbit xenontisera to various antigenic determinants (17, 25, 37), defined on human lymphocyte subpopulations, was used to identify respective antigens on the cells by an indirect membrane immunofluorescent test and a complement-dependent cytotoxic test. Details of immunization and absorption schedules have been previously described (25, 37).

**Chromosome Studies.** Examination of bone marrow cells for their chromosome constitution was performed by a direct technique previously described (33). Banding analysis was done with the G- and Q-methods (33).

**RESULTS AND DISCUSSION**

Table 1 summarizes the results of various determinations on the fresh leukemic blasts on March 11, 1976, and on the cells of the established cell lines BALM-1 and -2. Examination of the isolated leukemic blasts from the blood of the patient for cell surface markers revealed the presence of SmIg. Lambda light chain and α and γ heavy chains of immunoglobulins were not detected; however, the leukemic blasts bore α κ light chain and δ and μ heavy chains, shown by a direct immunofluorescent test with fluorescein-conjugated goat anti-human immunoglobulin chain-specific reagents. The cell surface markers of all cell populations matched, indicating possible clonal origin of the leukemic cells. The immunoglobulin chain specificity of the SmIg of the fresh leukemic cells and the established cell lines was the same and consisted of κ-type IgD and IgM. Thus, it is most probable that the BALM-1 and -2 cells were of the same origin as the leukemic B-cells. The antigenic makeup, determined by the complement-dependent cytotoxic and membrane immunofluorescent tests, with the use of a panel of specific xenontisera, demonstrated an identity in phenotype. There were B-cell- but no T-cell-associated antigens. Cross-absorption of the anti-B-cell serum with cells from BALM-1 and -2 showed complete removal of the anti-B-cell-associated antibodies. It was thus concluded that the BALM-1 and BALM-2 cells were B-cell-type lymphoblastoid cells. Among numerous B-lymphoblastoid cell lines previously established and characterized, there are a number of B-cell lines with a phenotype similar to that found on BALM-1 and -2. However, it is extremely rare for 2 B-cell lines that were derived from 2 separate flasks and originally from the same blood sample to have identical phenotypes, particularly in terms of SmIg and κ, δ, and μ chains. It has not been determined at this time whether the idiotypic determinants on these SmIg between BALM-1 and BALM-2 cells are the same.

When fresh leukemic blasts of the bone marrow of the patient were analyzed cytogenetically, there were distinct and common abnormalities in most of the metaphases examined. Direct marrow preparations for chromosome studies were made on 3 occasions (Table 2). The marrow cells from 3 aspirations showed a mode of 46 chromosomes. Analysis with Q- and G-banding techniques revealed the presence of normal and abnormal karyotypes; all chromosomally abnormal cells had 2 common marker chromosomes characterized as 14q+ and a complex translocation among Chromosomes 12 and 17 and an unidentified one. The appearance of the 2 marker chromosomes is shown in Fig. 1.

Accordingly, cytogenetic analysis of the cells of the established cell lines, BALM-1 and -2, was performed to ascertain whether the abnormalities seen in the leukemic cells in vivo persisted in the cultured cells. The modal chromosome number of the BALM-1 and -2 lines was 47, with considerable scattering in counts. Not a single normal metaphase was observed. Karyotypes of the metaphases obtained with banding procedures revealed the presence of the 2 distinct marker chromosomes observed in the marrow cells (Fig. 1). A few additional chromosomal abnormalities were also noted in the cells of BALM-1 and -2.

The presence of the 2 distinct marker chromosomes, seen in both the fresh leukemic cells and in the BALM-1 and -2 lines, strengthens our conclusion that these lines represent permanent, homogeneous populations of cells from a B-cell-type of acute leukemia. The ALL-specific antigen present in null-cell ALL, in undifferentiated leukemia, and in some cases in the blastic crisis of chronic myelocytic leukemia (6, 15, 16, 18) was not detected in the BALM-1 and -2 cells. Furthermore, the thymus leukemia antigen was also not detected. The presence of the Epstein-Barr virus genome in the BALM-1 and -2 cells was found by an immunofluorescent Epstein-Barr-associated nuclear antigen test (35).

Concerning the existence of B-cell ALL, Seligmann et al. (36), Kersey et al. (20), and Davey and Gottlieb (9) have reported evidence that this rare type of ALL occurs in 1 to 3% of all morphologically diagnosed ALL. Our experience is in agreement with these reports. B-cell ALL seems to be a more aggressive disease than the other types of ALL, leading to a poor prognosis, as in the case reported in this paper.

The detection of both IgD and IgM on the leukemic cells in this study appears to be the 1st for B-cell ALL, since previous reports (4, 9, 20) described a single immunoglobulin...
Table 1

<table>
<thead>
<tr>
<th>Cells from</th>
<th>SmIg</th>
<th>Rosette assays (% positive)</th>
<th>Membrane immunofluorescent test (% stained)</th>
<th>Complement-dependent cytotoxic test (% killed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh leukemic blasts in Peripheral blood</td>
<td>EAC 95</td>
<td>(43) 95 0 0 95 0 95</td>
<td>Normal T-cell antigen +</td>
<td>Normal B-cell antigen +</td>
</tr>
<tr>
<td>Bone marrow Cells from BALM-1</td>
<td>E520</td>
<td>(3) 95 0 0 98 0 98</td>
<td>Normal blast leukemia-associated antigen +</td>
<td>plus specific ALL antigen +</td>
</tr>
<tr>
<td>BALM-2</td>
<td>0 42</td>
<td>30 0 0 30 0 30</td>
<td>plus specific ALL antigen +</td>
<td>plus specific ALL antigen +</td>
</tr>
</tbody>
</table>

(a) Specific common ALL antigens. Specific rabbit antiserum was provided by Dr. M. F. Greaves, University College London, London, England (16).

(b) B-cell-associated antigens include both normal B-cell antigen and blast leukemia-associated antigens (25).

Table 2

<table>
<thead>
<tr>
<th>Chromosome number distribution</th>
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<tbody>
<tr>
<td>Material</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Bone marrow</td>
</tr>
<tr>
<td>3/25/76</td>
</tr>
<tr>
<td>4/16/76</td>
</tr>
<tr>
<td>BALM-1</td>
</tr>
<tr>
<td>BALM-2</td>
</tr>
<tr>
<td>6/10/76</td>
</tr>
<tr>
<td>3/01/77</td>
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(a) Numbers in parentheses, number of normal cells.

(b) A total of 120 additional cells with a chromosome number in the 2S range was observed; these cells contained 2 sets of the markers.

(IgM or IgG) as SmIg of leukemic cells. Our findings are similar to those reported for chronic lymphocytic leukemia, where SmIg was commonly characterized to be both IgD and IgM (10, 11). Apparently, studies of the cytogenetic constitution of cell lines derived from B-cell ALL have not been reported previously.

Abnormalities of Chromosome 14 have been noted often in a variety of lymphoid cancers (12, 21, 34, 38), including lymphomas, multiple myeloma, and plasma cell leukemia. In these studies, as well as in the present report, the No. 14 chromosome abnormality seems to be restricted only to those lymphoid neoplasias with B-cell characteristics. The significance, implication, and clinical importance of these cytogenetic abnormalities, as well as the selected immunoglobulin chains, in B-cell cancers remain to be determined in future studies. Established and homogeneous B-cell leukemic cell populations, as reported here, may be a great value for such studies.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Q- and G-banded partial karyotypes of the fresh leukemic cells and those of the cell line BALM-1 containing 2 common marker chromosomes (M1 and M2). The origin of M1 appears to have involved translocation of unknown segments to the long arm of Chromosome 14. Marker M2 was probably composed of most of Chromosome 17, the long arm of Chromosome 12, and part of an unknown chromosome.
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