Two New Acute Lymphoblastic Leukemia Cell Lines With Early B-Cell Phenotypes

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Two leukemic cell lines (697 and 207) were established from bone marrow cells obtained from children with ALL in relapse. These cell lines were positive for the common-ALL antigen (CALLA), the HLA-DR (i.e., la-like) antigen, and for cytoplasmic and surface IgM heavy chains. The lines were negative for other immunoglobulin heavy chains and light chains. The lines had elevated levels of terminal deoxynucleotidyl transferase enzyme and expressed surface antigens found on normal myeloid-macrophage cells (MMA) and on natural killer cells (HNK-1). A minority of cells in line 207 expressed the T-1, T-6, and Leu-1 antigens as detected by monoclonal antibodies. Line 697 was positive for Epstein-Barr virus (EBV), while line 207 did not possess EBV. Line 697 carried a marker chromosome (identified as a translocation between chromosomes 7 and 19), which was also present in the patient's fresh leukemic cells. The leukemic origin of the cell lines was further indicated by their morphological, cytochemical, and immunologic similarity to the patients' leukemic cells. Phenotypically, both cell lines appear to be arrested in a transitional stage of development between pre-B and B cells and express surface antigens usually found on normal and fresh leukemic cells of non-B-cell lineages.

ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) in children has been found to consist of several immunologic subtypes. Among these are the pre-B-cell ALL subtype (approximately 20% of total cases), in which the leukemic cells contain immunoglobulin M heavy chains (\( \mu \)) in their cytoplasm and lack surface immunoglobulin (slg), \(^1\) \(^3\) and the rare B-cell ALL subtype (1%-3% of total cases), in which the leukemic cells possess slg. \(^4\) \(^5\) Three leukemic cell lines of the pre-B phenotype have been described. These were derived from two children and a young adult with ALL, lymphoblastic lymphoma, and Ph\(^1\)-chromosome-positive chronic myelogenous leukemia in blast crisis, respectively. \(^6\) \(^7\) There are no cell lines derived from children with B-ALL, although two B-ALL cell lines have been established from affected adults. \(^8\) \(^9\) \(^10\) We report here the establishment and characterization of two leukemic cell lines (697 and 207) from children with ALL. These lines appear to be in a transitional stage of development between pre-B and B cells, and they express cell surface antigens that are not usually seen on fresh leukemic cells of B lineage.

MATERIALS AND METHODS

Establishment of Cell Lines

Cell line 697 was established in November 1979 from a bone marrow aspirate obtained from a 12-year-old boy originally diagnosed as having null cell (non-T, non-B) ALL. The cells were cultured at the time of the patient's first relapse, when the bone marrow was found to contain 99% blasts, the majority of which had the common-ALL phenotype (i.e., non-T, non-B, and common-ALL antigen positive); the cells were not examined for cytoplasmic immunoglobulin (Table 1). Cell line 207 was established in September 1980 from a bone marrow aspirate (78% blasts) obtained from a 10-year-old boy with ALL during a second relapse. The phenotype of the relapse cells was common-ALL, with the exception that 9% of the cells were positive for cytoplasmic \( \mu \) chains when examined using the immunofluorescence assay described below (Table 1). Since a minimum of 10% cytoplasmic \( \mu \) cells in the bone marrow is required for classification as pre-B-ALL, the cells were considered to have a common-ALL phenotype.

Bone marrow cells from these patients were collected in preservative-free heparin and centrifuged over Ficoll-Hypaque (Histopaque, Sigma, St. Louis, Mo.) at 1000 g for 15 min. \(^11\) The mononuclear cell fraction, which contained the leukemic cells and residual normal bone marrow cells, was recovered from the interface. The cells were washed and inoculated in tissue culture flasks (Corning, Corning, N.Y.) containing culture media consisting of McCoy's 5A modified media (GIBCO, Grand Island, N.Y.), supplemented according to the method of Pike and Robinson. \(^12\) Also added were ribosides and deoxyribosides (10 mg/liter, Sigma), fetal calf serum (20% final concentration), 2-mercaptoethanol (2-ME, 5 \( \times \) \( 10^{-5} \) M, Sigma), penicillin (100 IU/ml, GIBCO), and streptomycin (100 mg/ml, GIBCO). The cells were cultured at a concentration of 2 \( \times \) \( 10^6 \) cells/ml in a humidified 37°C incubator containing 7.5% CO\(_2\) in air. The cell densities remained constant during the first week of culture and then increased to approximately 4-6 \( \times \) \( 10^6 \) cells/ml by the third week, at which time the cultures were split.

Both cell lines are presently growing with a doubling time (i.e., the time required for the total cell number to double) of approximately 60-70 hr in liquid medium consisting of Iscove's modified Dulbecco's medium (IMDM, GIBCO) supplemented with fetal calf serum, 2-ME, and antibiotics as described above. Line 697 cells tend to adhere to one another and grow in clumps of up to several hundred cells, while line 207 grows as a single-cell suspension. The lines have been found to be mycoplasm-free by the agar culture technique. \(^13\)

The cell lines have also been grown as colonies in semisolid...
efficiency of the cell lines in methylcellulose medium ranges from 0.5% to 1.0% when cultured at cell concentrations of 10^5 cells/ml. The cloning efficiency of the cell lines in methylcellulose medium (DOW, 4000 cps; 0.8% final concentration) containing IMDM, 20% fetal calf serum, and 2-ME. Other experiments were done on marrow cells that had been cryopreserved in liquid nitrogen prior to testing. Other markers were examined with several cytochemical stains, including acid phosphatase, PAS, peroxidase, ASD chloroacetate and nonspecific esterases, and products were labeled by growing the cell lines in medium containing ^35S-methionine, and the cell lysates were incubated with goat anti-human μ or (kappa + lambda) antibodies and S. aureus protein A. The precipitates were then reduced, alkylated, and separated by sodium dodecyl sulfate polyacrylamide-gel electrophoresis.

Receptors for the Fe portion of IgG (FCR) were detected with heat-aggregated human IgG and FITC-conjugated anti-human IgG antibody (Meloy Laboratories, Springfield, Va.). Receptors for activated C3 components of human complement were assayed by rosette formation with complement-coated zymosan particles.

T-cell markers included erythrocyte-rosette (ER) formation with sheep erythrocytes at 4°C and the presence of T-cell antigens detected by indirect immunofluorescence with the OKT monoclonal antibodies T1, 3, 4, 5, 6, and 9 (Ortho Pharmaceuticals, Raritan, N.J.) and the Leu-1, 2, and 3 monoclonal antibodies (Becton-Dickinson); fluorescein-conjugated goat antibody to mouse immunoglobulin was used as the secondary antibody. Other monoclonal antibodies were used to detect a myeloid-macrophage antigen (MMA) and a human natural killer cell antigen (HNK-1). Epstein-Barr virus capsid antigen and nuclear antigen were tested for with immunofluorescence methods by Dr. Paul Feorino (Center for Disease Control, Atlanta, Ga.).

Enzyme Studies
Terminal deoxynucleotidyl transferase (TdT) levels were determined enzymatically by Dr. Mary S. Coleman (University of Kentucky, Lexington, Ky.) as previously described. Values are given as units per 10^6 cells, where 1 unit equals 1 nmole substrate polymerized per hour. Indirect immunofluorescence assays for cellular expression of TdT were also performed using rabbit anti-calf TdT antibody and fluorescein-conjugated goat anti-rabbit antibody (Bethesda Research Laboratory, Gaithersburg, Md.).

RESULTS

Cell lines 697 and 207 had similar morphology with Wright's stain and consisted of medium-sized lymphoid cells with a large nuclear:cytoplasmic ratio. The nucleus was frequently indented and contained a fine chromatin network; nucleoli were not prominent (Fig. 1). Cytochemical studies showed that lines 697 and 207 were slightly positive for acid phosphatase and PAS, while they were negative for peroxidase and esterases. Vacuoles in both lines were shown to contain lipid by the oil-red-O staining.

Results of immunologic and terminal transferase testing are summarized in Table 1. Both lines were positive for CALLA and for the HLA-DR antigen. Furthermore, both lines expressed μ heavy chains but not light chains or any other heavy chain classes. Mu chains were easily detected by immunofluorescence in the cytoplasm, while the cell-surface staining for μ chain determinants was distinct but faint. In biosynthetic studies, newly synthesized μ chains were precipitated by anti-μ but not by anti-(kappa + lambda) antibodies; no light chains were precipitated by either set of antibodies.

Additional cell markers identified on both cell lines were myeloid-macrophage antigen (MMA), the

| Table 1. Phenotypic Features of Cell Lines 207 and 697 and of Pre-B-Cell ALL |
|-----------------------------|-----------------------------|
|                            | 207 | 697 | Pre-B-Cell ALL* |
| **Surface markers**         |     |     |                 |
| Common ALL antigen          | 70  | 70  | 60  |
| HLA-DR                      | 80  | 47  | 82  |
| IgM heavy chain             | 70  | 90  | 11  |
| IgD, G, A, heavy chains     | < 1 | < 1 | 2   |
| Kappa, lambda light chains  | < 1 | 1   | 1   |
| FcR                         | 29  | 3   | 2   |
| CR                          | < 1 | 1   | 0   |
| E rosettes                  | < 1 | < 1 | 1   |
| OKT-1                       | 45  | 45  | 98  |
| OKT-3, 4, 5, 8              | < 1 | < 1 | 1   |
| OKT-6                       | 13  | 13  | 1   |
| OKT-9                       | 95  | 98  | 1   |
| Leu-1                       | 8   | 8   | 1   |
| Leu-2, 3                    | < 1 | < 1 | 1   |
| MMA                         | 87  | 81  | 1   |
| HNK-1                       | 64  | 69  | 1   |
| **Intracellular markers**   |     |     |     |
| IgM heavy chain             | 95  | 87  | 1   |
| IgD, G, A, heavy chains     | < 1 | < 1 | 1   |
| Kappa, lambda light chains  | < 1 | < 1 | 1   |
| TdT                         | 95  | 70  | 90  |

*Typical phenotypic features for pre-B-ALL are shown.
†Percent positive cells.
Numbers in parentheses are values (percent positive cells) for relapse bone marrow cells from patients 207 and 697. CALLA, HLA-DR, and TdT determinations were done on marrow cells that had been cryopreserved in liquid nitrogen prior to testing. Other markers were done on fresh marrow.
human natural killer cell antigen (HNK-1), and the transferrin-receptor antigen (T9). Some of the cells of line 207 were also positive for the T-cell antigens T-1, T-6, and Leu-1. Fc receptors could be detected on very few of the cells, and thus could not provide an artifactual basis for the detection of unusual antigens on the cell lines.

Both cell lines had elevated levels of TdT (>10 U/10⁶ cells). Enzymatic assay for TdT revealed 157 and 23 U/10⁶ cells for lines 207 and 697, respectively. When the immunofluorescence assay for TdT was performed, approximately 95% of the cells in both lines were found to be positive.

Line 697 was found to contain the Epstein-Barr virus nuclear and capsid antigens. Line 207 was negative for both of these antigens. Cytogenetic studies demonstrated that line 697 was pseudodiploid with a marker chromosome consisting of a translocation between chromosome 7 and 19: t(7;19) (q11;q13). This finding has been reported in a separate publication. Line 207 was found to have a normal karyotype (R. Green, personal communication); it appears to be the first reported leukemia cell line without a chromosomal abnormality.

DISCUSSION

The pre-B phenotype defined for fresh ALL cells consists of lymphoblasts that contain cytoplasmic IgM heavy chains and lack surface Ig. An occasional patient with pre-B-cell ALL has been found to have blasts with surface µ chains detectable by faint immunofluorescent staining. Except for the presence of cytoplasmic µ, the pre-B phenotype is typically identical to the common-ALL phenotype, i.e., CALLA+, HLA-DR+, ER−, TA− (T-antigen), and slg−; the leukemic cells of the pre-B phenotype have also been shown to express B-cell antigens and to have rearrangements of their immunoglobulin genes.

The phenotype of cell lines 697 and 207 corresponds most closely to the pre-B-ALL phenotype. The present lines also resemble the pre-B-ALL cell line (NALM-6) derived by Hurwitz et al. from a 19-yr-old man with ALL in relapse. All three lines are more than 90% cIgM+ and are negative for other cytoplasmic heavy-chain immunoglobulin classes. However, the present cell lines differ from NALM-6 in that the latter produces both µ heavy and lambda light chains.

Additionally, all three lines are CALLA+, HLA-DR+, TdT+, and CR−. Line 697 and 207 are ER−, while about 45% of NALM-6 cells are ER+. However, some of the cells in line 207 express the T-cell antigens T-1, T-6, and Leu-1, which are found on some T-ALL lymphoblasts and on normal peripheral blood T cells (T-1 and Leu-1) and common thymocytes (T-6). The Leu-1 and other "T-cell" antigens have previously been noted on some fresh B-ALL cell lines and on normal B-ALL cell lines but not in the cell lines described here. Lines 697 and 207 also express the T-9 antigen, which has been recently defined as the transferrin receptor and has been found on rapidly dividing normal and malignant cell populations belonging to a variety of cell lineages.

Lines 697 and 207 resemble the two previously established B-ALL cell lines BALM-1, 2 and BALL-1, in that they express surface µ chains and HLA-DR antigen. An additional similarity between lines 697 and BALM-1, 2 is the presence of EBV in these lines. This suggests the existence of EBV receptors on these cell lines, but direct evidence for this is lacking. All other reported ALL cell lines (including line 207) have lacked EBV (using the EBV nuclear-antigen detection method), while all normal B-cell lines have contained the virus. Also, CALLA and elevated levels of TdT have been found in lines 697 and 207 but not in EBV-transformed normal B-cell lines. The leukemic origin of the present cell lines is indicated by their morphological, cytochemical, and immunologic similarity to the patients' fresh leukemic cells. The leukemic cells from the patients and from the corresponding cell lines were slightly positive for PAS and negative for the other cytochemical stains. The immunologic phenotypes of the patients' leukemic cells (CALLA+, HLA-DR+, TdT+, CR−, and ER−) also matched that of the cell lines; an exception was the low percentage of cells positive for cytoplasmic µ chains (9%) in the patient from whom line 207 was established. However, it is possible that many of the leukemic cells in this patient had rearranged their immunoglobulin µ heavy-chain gene in preparation for µ chain expression. An additional important leukemic feature of line 697 was the presence of the same
marker chromosome (a translocation between 7 and 19) in both the patient's fresh leukemic cells and the cell line.

A remarkable finding was the detection of the myeloid-macrophage antigen (MMA) and the human natural killer cell antigen (HNK-1) on the cell lines; they have not been identified on fresh leukemic cells from other ALL patients with or without the pre-B phenotype.\(^25\) MMA is an antigen found on human peripheral blood monocytes, polymorphonuclear cells, and their colony-forming precursors. It has also been found on a myeloid cell line (K-562), on some T-cell lines (MOLT-4), and on a subpopulation of T cells activated in vitro, but not on circulating T or B cells or on EBV-transformed normal B-cell lines.\(^25\) The HNK-1 antigen has been found on a subset of granulocytes that can function as natural killer (NK) and antibody-dependent killer (K) cells; this antigen has not been found on normal pre-B or B cells.\(^26\)

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TWO NEW ALL CELL LINES

Characterization of a new chromosomal marker for acute lymphoblastic leukemia from a long-term cell line. (submitted for publication)


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