Characterization of Lymphoblast Fc Receptor Expression in Acute Lymphoblastic Leukemia

By Gregory H. Reaman, Werner J. PiChler, Samuel Broder, and David G. Poplack

Lymphoblasts from 18 patients with untreated acute lymphoblastic leukemia were investigated for the presence of conventional cell surface markers (spontaneous sheep red blood cell rosette formation, complement receptors, and surface immunoglobulin). The expression of Fc receptors for both IgG and IgM was investigated using indicator bovine erythrocytes coated with rabbit anti-BRBC (IgG and IgM fractions). The leukemic cells of all patients in this study expressed Fc receptors for both IgG and IgM. In contrast with previous reports, null (non-T non-B) lymphoblasts as well as T lymphoblasts demonstrated Fc-IgG and Fc-IgM receptors. However, these two immunologic subclasses of leukemic cells demonstrated significantly different patterns of Fc receptor distribution. These data suggest that expression of Fc receptors is an early event in cellular differentiation in this lymphoid malignancy.

The study of cell surface membrane markers on normal human lymphocytes has resulted in the definition of distinct immunologic classes of these cells. Thymus-derived (T) lymphocytes form spontaneous rosettes (ER) with sheep red blood cells (SRBC). Bursa-equivalent-derived (B) lymphocytes bear complement (C') receptors and possess surface immunoglobulin (SIg.). The identification of similar cell surface markers on acute lymphoblastic leukemia (ALL) cells has allowed classification of this lymphoid malignancy into T-cell, so-called null-cell (non-T non-B), and pre-B-cell or B-cell types. In addition to confirming that ALL is an extremely heterogeneous disorder, such an immunologic classification has proved to be of prognostic significance.

Techniques have recently been developed that allow further subclassification of normal human lymphocytes. Specifically, T lymphocytes have been subclassified on the basis of their expression of membrane receptors for either the Fc fragment of IgG (Tg cells) or the Fc fragment of IgM (Tμ cells). Different functional specificities have been reported in these T-cell subclasses. Of special interest, T lymphocytes with Fc-IgG receptors may have suppressor cell activity in certain in vitro systems designed to assess B-cell differentiation and immunoglobulin synthesis.

An additional method of subclassifying T lymphocytes has been devised based on the relative affinity with which these cells form rosettes with SRBC, i.e., high- and low-affinity ER-forming lymphocytes. In this system, low-affinity rosette-forming lymphocytes possess the Fc-IgG receptor.
The present study was undertaken to investigate the expression of Fc-IgG and Fc-IgM receptors on leukemic lymphoblasts from patients with ALL.

MATERIALS AND METHODS

Patient Population

Eighteen newly diagnosed patients with ALL who were cared for by the Pediatric Oncology Branch of the National Cancer Institute were studied. The diagnosis of ALL was based on classic, clinically accepted criteria.20

Cell Collection

Leukemic cells were collected either from peripheral blood by leukopheresis or from bone marrow at the time of diagnosis. Cells were harvested by dextran sedimentation and stored in a Teflon bag in Roswell Park Memorial Institute 1640 medium (RPMI-1640) with 10% fetal calf serum (FCS) GIBCO, Grand Island, N.Y.) and 10% dimethyl sulfoxide at -170°C in the vapor phase of liquid nitrogen.

Mononuclear Cell Preparation

In preparation for assay procedures, cells were rapidly thawed, separated by Ficoll-Hypaque (LSM, Litton Bionetics, Kensington, Md.) density centrifugation, washed three times, and resuspended in RPMI-1640. Viability as assessed by trypan blue exclusion was greater than 90% in all instances.

ER

The method for detecting ER-forming cells has been previously described by Weiner et al.21 In brief, 5 x 10^7 leukemic lymphoblasts were exposed to 10^7 neuraminidase-treated SRBC in the presence of absorbed FCS at 37°C for 5 min and centrifugation at 100 g for 5 min. The lymphoblast-SRBC pellets were incubated at 4°C for 18 hr, then gently resuspended and counted in a hemacytometer. Cells binding three or more SRBC were considered ER-positive.

Total ER, High-Affinity ER, and Low-Affinity ER

These methods are described by West et al.22 in detail. Briefly, total ER-forming lymphoblasts were detected by incubation of 4 x 10^7 leukemic cells with 0.2 ml of SRBC (3 x 10^9/ml) in the presence of absorbed FCS at 37°C for 5 min and centrifugation at 100 g for 5 min. The lymphoblast-SRBC pellets were incubated at 29°C for 18 hr, then gently resuspended and counted in a hemacytometer. High-affinity ER-forming cells were determined using a lower concentration of SRBC (10^6/ml) and incubating the cell pellets at 29°C for 18 hr. The proportion of low-affinity ER-forming lymphoblasts was assessed by subtracting the percentage of high-affinity ER-forming cells from the total ER-forming lymphoblasts.

Complement Receptors

Erythrocyte antibody complement rosettes (EAC'R) were detected using SRBC coated with IgM antibody (19S rabbit anti-SRBC, Cordis Laboratories, Miami, Fla.) and fresh BALB/c mouse serum as a complement source. Equal volumes of lymphoblasts (4 x 10^6/ml) and EAC' (4 x 10^7/ml) were rocked and incubated at 37°C for 40 min, and EAC'R were counted immediately thereafter.

Fc Receptors

Erythrocyte antibody rosettes (EA-IgG R and EA-IgM R) were prepared as previously described.23 Bovine red blood cells (BRBC) were washed three times in Veronal-buffered saline containing 0.01-M EDTA, sensitized with anti-BRBC IgG fraction (EA-IgG), and diluted 1:50 (hemolysis titer 1:16,000, protein concentration 4 mg/ml), or sensitized with an anti-BRBC IgM fraction (EA-IgM) diluted 1:25 (hemolysis titer 1:128,000, protein concentration 5 mg/ml) by incubation at 37°C for 30 min. The IgG and IgM fractions were prepared from inactivated rabbit hyperimmune sera, and the purity was tested as previously described.23 Cells were washed three times and resuspended in medium 199 (GIBCO, Grand Island, N.Y.) with 1% bovine serum albumin to give a 1% suspension. Equal volumes of leukemic
Fc-lgG AND Fc-lgM RECEPTORS IN ALL

cells (10^7/ml) and coated BRBC were mixed, centrifuged at 200 g for 5 min, and incubated at 4°C for 1–3 hr. Pellets were gently resuspended, and 200 cells were counted. EA-lgG R and EA-lgM R were determined independently and without prior knowledge of results of other cell marker determinations.

**Cyto centrifuge Preparations**

To determine the number of lymphoblasts in the mononuclear cell suspensions as well as the morphology of rosette-forming cells, cyto centrifuge preparations were made using the Cytospin (Shandon Elliot, Sewickley, Pa.) and stained with Wright’s stain. The percentage of rosette-forming lymphoblasts was determined by multiplying the number of rosettes on wet preparations by the percentage of rosettes that had cytologically identifiable blasts at their centers.

**Slg**

Slg was assessed by direct immunofluorescence microscopy (Leitz microscope with Ploem illumination) of 5 × 10^6 cells incubated at 4°C with a fluorescein-conjugated goat antihuman polyvalent antibody (Cappel Laboratories, Downingtown, Pa.) in the presence of sodium azide.

**Statistical Methods**

Tests of significance were performed using the signed rank test of Wilcoxon.

**RESULTS**

**Morphology of Collected Cells**

Cytologic examination revealed that 88%–100% of the mononuclear cells collected from patients prior to therapy were leukemic lymphoblasts.

**ER**

Between 12% and 82% of lymphoblasts from 8 patients formed ER (Table 1) and were considered to represent T-cell lymphoblasts.

*Table 1. Cell Surface Markers in ALL*

| Patient | Age | Initial WBC (cells/cu mm) | Source of Cells Examined | Lymphoblasts in Cell Suspension (%) | Total ER (%) | High-Affinity ER (%) | Low-Affinity ER (%) | EAC'R (%) | Slg (%)
|---------|-----|--------------------------|--------------------------|-----------------------------------|-------------|---------------------|---------------------|-----------|------
| 1       | 16 yr | 76,800 PB | 98 | 82 | 84 | 2 | 2 | 0
| 2       | 6 mo | 1,080,000 PB | 88 | 36 | 43 | 40 | 3 | 1 | 0
| 3       | 5 yr | 11,400 PB | 95 | 12 | 14 | 14 | 0 | 2 | 2
| 4       | 12 yr | 103,000 PB | 95 | 43 | 51 | 50 | 1 | 0 | 0
| 5       | 10 mo | 315,000 PB | 95 | 100 | 46 | 46 | 45 | 1 | 0 | 0
| 6       | 16 yr | 35,500 PB | 90 | 32 | 34 | 33 | 1 | 2 | 0
| 7       | 6 yr | 27,500 PB | 100 | 54 | 54 | 52 | 2 | 1 | 0
| 8       | 19 yr | 12,000 BM | 93 | 29 | 30 | 29 | 1 | 0 | 0
| 9       | 13 yr | 5,900 PB | 98 | 28 | 32 | 30 | 2 | 12 | 12
| 10      | 25 yr | 22,300 PB | 95 | 0 | 0 | 0 | 0 | 0
| 11      | 13 yr | 7,600 PB | 100 | 0 | 0 | 0 | 0 | 0
| 12      | 6 yr | 30,000 PB | 98 | 1 | 2 | 1 | 1 | 3 | 0
| 13      | 9 yr | 45,000 PB | 98 | 0 | 2 | 0 | 2 | 1 | 1
| 14      | 16 yr | 23,400 PB | 88 | 0 | 2 | 1 | 1 | 0 | 1
| 15      | 22 yr | 22,000 PB | 92 | 0 | 0 | 0 | 0 | 1 | 1
| 16      | 12 yr | 3,800 PB | 97 | 0 | 1 | 0 | 1 | 1 | 0
| 17      | 4 yr | 1,600 BM | 94 | 0 | 1 | 1 | 0 | 0 | 0
| 18      | 7 yr | 31,000 BM | 100 | 0 | 0 | 0 | 0 | 0 | 0

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**Total ER, High-Affinity ER, and Low-Affinity ER**

The percentage of total ER-positive lymphoblasts was comparable to the percentage obtained using neuraminidase-treated SRBC. Of the population of lymphoblasts forming ER, nearly all formed high-affinity rosettes. In all cases studied, 3% or less of the T lymphoblasts formed low-affinity ER (Table 1).

**EACR and Slg**

Twelve percent of the lymphoblasts from 1 patient (patient 9) formed EAC'R and were positive for Slg. Twenty-eight percent of the lymphoblasts from this same patient also formed ER. None of the lymphoblasts from the other patients studied expressed B-cell surface markers.

**EA-IgG R**

Some percentage of lymphoblasts from each of the patients studied demonstrated receptors for the Fc fragment of IgG (Table 2). Between 18% and 63% of lymphoblasts that formed ER possessed Fc-IgG receptors. Null lymphoblasts similarly demonstrated Fc receptors for IgG; however, only 4%–25% of these cells were EA-IgG-R-positive. Thus, a quantitative difference in number of Fc-IgG receptors was observed between these two groups.

**EA-IgM R**

Receptors for the Fc fragment of IgM were demonstrated on lymphoblasts from each of the patients studied regardless of T-cell or null-cell status (Table 2). Between 4% and 23% of the T-cell lymphoblasts demonstrated Fc receptors for IgM. In contrast, a larger portion of null lymphoblasts (16%–66%) expressed Fc-IgM receptors. As shown in Table 2, the ratio of T lymphoblasts with Fc-IgM receptors to those with Fc-IgG receptors ranged from 0.07 to 0.56, whereas the same ratio for null lymphoblasts ranged from 1.2 to 6.0. When these data were

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analyzed by the Wilcoxon signed rank test, this difference in Fc-IgM/Fc-IgG receptor expression between T-cell and null-cell lymphoblasts was significant ($p < 0.01$).

DISCUSSION

In this study we have observed that leukemic lymphoblasts express both Fc-IgG and Fc-IgM receptors regardless of their B-cell, T-cell, or null-cell status in other classification schema. These receptors were detected on T lymphoblasts and null lymphoblasts as well as on leukemic cells possessing both B-cell and T-cell markers. In an earlier study, Moretta et al. demonstrated that Fc-IgM receptors were present on the cell membranes of T lymphoblasts and suggested that expression of the Fc-IgM receptor was a reliable indicator of T-cell ALL. More recently, Beck et al. were unable to detect Fc receptors for either IgG or IgM on the surface of null lymphoblasts, thus suggesting that Fc receptor expression was a phenomenon restricted to lymphoblasts with T-cell characteristics. In contrast, the findings in our study indicate that Fc receptors for both IgM and IgG are expressed on T lymphoblasts as well as on those leukemic cells devoid of conventional B- or T-cell markers, referred to as null cells.

The experiments reported here used indicator erythrocytes coated with a high concentration of antibody (just below the agglutination point). This method increases the sensitivity of the Fc receptor assay and may explain the failure of earlier studies to detect Fc receptors on other than T lymphoblasts. Using the same sensitive assay, Fc-IgM receptors have been demonstrated on leukemic cells in chronic lymphocytic leukemic (a malignancy of monoclonal B-cell origin) as well as on human lymphoblastoid B-cell lines. In addition, both Fc-IgG and Fc-IgM receptors have been detected on normal human B lymphocytes, and, in fact, most of these cells express both receptors simultaneously. Therefore, expression of either Fc-IgG or Fc-IgM receptors cannot be considered specific for cells of T-cell lineage. This evidence supports our findings of Fc receptor expression on lymphoblasts that lack the capacity to form ER. Furthermore, the demonstration of both Fc-IgG and Fc-IgM receptors on lymphoblasts that expressed both B-cell and T-cell markers, coupled with the evidence cited previously, suggests that it is likely that these same receptors may be present on B-cell lymphoblasts as well.

The previous finding that normal T lymphocytes form ER with variable affinity has allowed the subclassification of these cells into two populations. Low-affinity ER-forming lymphocytes bear Fc-IgG receptors, whereas high-affinity ER-forming lymphocytes do not. However, in contrast to normal T lymphocytes, leukemic T lymphoblasts form only high-affinity ER and still express Fc-IgG receptors. In addition, although presumably of thymic origin, T lymphoblasts manifest a different pattern of cell surface markers than that seen in normal thymocytes, which form exclusively high-affinity ER but do not express Fc-IgG receptors.

In the majority of the experiments described in this report we used leukemic lymphoblasts isolated from peripheral blood. Fc receptors were determined only on cells that were cytomorphologically identified as lymphoblasts. In those patients in whom leukemic cells were isolated from bone marrow, similar percentages of lymphoblasts were observed to express both Fc-IgG and Fc-IgM receptors.

In this study leukemic lymphoblasts manifested both Fc-IgG and Fc-IgM
receptors regardless of their immunologic classification. However, there was a significant difference in the ratio of Fc-IgM to Fc-IgG receptor expression between null and T lymphoblasts. In patients with null-cell ALL, a greater number of lymphoblasts bore Fc-IgM receptors than receptors for IgG. This pattern of lymphoblast Fc receptor distribution was reversed in patients with T-cell ALL.

We have demonstrated the presence of Fc receptors on lymphoblasts that lack conventional B- or T-cell surface markers. Thus, in contrast to previous suggestions in the literature, the presence of Fc receptors does not appear to be a marker for a particular immunologic type of leukemic lymphoblasts. The observation that null lymphoblasts possess both Fc-IgM and Fc-IgG receptors with a characteristic pattern of distribution distinct from that of T lymphoblasts might suggest that null lymphoblasts represent malignant transformation of a lymphoid progenitor cell that has not completed differentiation along either the B- or T-cell axis. Further assessment of the dynamics of Fc receptor expression may result in increased understanding of cellular differentiation in ALL.

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REFERENCES


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