Complement Receptor-positive, Sheep Erythrocyte Receptor-negative Lymphoblasts in Childhood Acute Lymphocytic Leukemia

Ellen R. Richie, Steven J. Culbert, Margaret P. Sullivan, and Jan van Eys

Department of Pediatrics, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

ABSTRACT

Lymphoblasts from 36 children with acute lymphocytic leukemia and two children with convoluted lymphoma were evaluated for expression of membrane receptors for sheep erythrocytes, complement, and the Fc portion of immunoglobulin G as well as for surface membrane immunoglobulin. Thirty patients had lymphoblasts that failed to express these membrane markers, four patients had sheep erythrocyte receptor-positive lymphoblasts, and a third group of four patients had lymphoblasts that displayed complement receptors only. The complement receptor-positive (CR⁺) lymphoblasts did not exhibit cytochemical, adherent, or phagocytic properties consistent with monocyte differentiation. The CR⁺ lymphoblasts formed rosettes with complement-bearing zymosan particles as well as complement-bearing immunoglobulin sensitized sheep erythrocytes. However, CR⁺ lymphoblasts from one patient were capable only of binding zymosan particles activated with mouse but not human serum. The CR⁺ lymphoblasts did not respond by [methyl-²H]thymidine incorporation to mitogens or allogenic cells in the mixed-lymphocyte reaction. Whereas the lymphoblasts from two of the CR⁺ patients stimulated lymphocytes from normal individuals to proliferate in a mixed-lymphocyte reaction the lymphoblasts from another CR⁺ patient were incapable of stimulating a proliferative response in the mixed-lymphocyte reaction. A distinctive clinical profile has not emerged. However, the finding that two of the four patients have relapsed within 1 year suggests that CR⁺ lymphoblasts may be indicative of an unfavorable prognosis.

INTRODUCTION

Childhood acute lymphocytic leukemia is a heterogeneous disease with respect to the cellular origin of the leukemic lymphoblasts and to prognosis. Two acute lymphocytic leukemia subgroups are distinguished when leukemic lymphoblasts are studied for expression of T- and B-lymphocyte surface markers. The majority of acute lymphocytic leukemia patients referred to as having null-cell or non-T-, non-B-cell leukemia since their lymphoblasts do not express receptors for sheep erythrocytes, complement, or the Fc portion of IgG and also lack detectable (SmIg⁺) (2, 5, 6). A smaller subgroup of acute lymphocytic leukemia patients (approximately 10 to 20%) have lymphoblasts that express E-receptors characteristically found on normal human T-lymphocytes (23, 24). Patients with T-cell leukemia generally have a poorer prognosis than do patients with non-T-, non-B-cell leukemia (23, 24). Since the non-T, non-B subgroup is still heterogeneous with respect to prognosis, lymphoblasts from this subgroup have been tested for expression of a variety of lymphocyte receptors and/or antigens in order to delineate further subgroups based on cellular origin or stage of differentiation. For example lymphoblasts that are E-receptor and SmIg negative may express T-cell antigens or B-cell (la-like) antigens (16, 17). Vogler et al. (25) have recently described a subgroup of acute lymphocytic leukemia patients whose lymphoblasts contain low amounts of cytoplasmic immunoglobulin and may represent a neoplastic proliferation of pre-B-cells.

Complement receptors have not been reported on E-receptor- and SmIg-negative lymphoblasts in childhood acute lymphocytic leukemia, although they have been observed on lymphoblasts from some patients with E-receptor-positive T-cell leukemia (3, 18). In this report we describe the surface marker and in vitro functional characteristics of leukemia cells from 4 acute lymphocytic leukemia patients whose lymphoblasts displayed complement receptors with no or minimal expression of SmIg, E, or Fc receptors.

MATERIALS AND METHODS

Patients. The study group consisted of 36 children (ages 7 months to 15 years) with previously untreated acute lymphocytic leukemia diagnosed on the basis of morphological and cytochemical characteristics of the bone marrow aspirate. Also included are 2 patients with convoluted lymphoma without bone marrow involvement at diagnosis. Blood and marrow cells, and in the 2 lymphoma cases pleural fluid, were obtained at the time of diagnosis. Peripheral blood was also obtained from 25 healthy adult volunteers. Appropriate informed consent was obtained from each patient and/or their parents for these studies.

Preparation of Lymphoid Cells. Mononuclear cells were isolated from heparinized peripheral blood by differential centrifugation on standard Ficoll-Hypaque gradients (4). Bone marrow cells were isolated by Ficoll-Hypaque centrifugation in part by Grant CA-20763 awarded by the National Cancer Institute and by Grant IN-43-0 from The American Cancer Society.

To whom requests for reprints should be addressed, at Department of Pediatrics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Received May 15, 1978; accepted July 21, 1978.

3616 CANCER RESEARCH VOL. 38
Cr+ Lymphoblasts in Acute Lymphocytic Leukemia

Cr+ lymphoblasts were obtained from 0.83% Tris-NH₄Cl lysing buffer. Cells were collected from pleural fluid by centrifugation. All lymphoid cell populations were washed 3 times with Ca²⁺ and Mg²⁺-free Hanks’ balanced salt solution and resuspended in RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) media supplemented with 10 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid buffer, 2 mM glutamine and gentamicin (50 U/ml).

**E-Receptors.** T-lymphocytes were identified by rosette formation with sheep erythrocytes at a lymphocyte:sheep erythrocyte ratio of 1:50 after 1 hr of incubation at 4° in RPMI containing 25% absorbed fetal calf serum (15). A rosette was defined as any lymphoid cell with 3 or more sheep erythrocytes attached. At least 200 lymphocytes were counted.

**SmIg.** B-lymphocytes were identified by the presence of SmIg after preincubation at 37° with latex particles to identify contaminating monocytes and release cytophilic immunoglobulin. The cells were then washed and resuspended in azide-containing phosphate-buffered NaCl, (8 mg/liter; Na,HPO₄·7H₂O, 2.16 g/liter) to which fluorescein-conjugated polyvalent goat anti-human immunoglobulin (Behring Diagnostics, Somerville, N. J.) was added. After incubation at 25° for 30 min the cells were pelleted through fetal calf serum, and washed, and smears were prepared. At least 200 cells were observed for positive membrane fluorescence with an A.O. microscope equipped with a UV vertical illuminator.

**Complement Receptors.** Two methods were used to detect complement receptors. EAC reagent was prepared by sensitizing sheep erythrocytes with the IgM fraction of rabbit anti-sheep erythrocytes (Cordis Laboratories, Miami, Fla.) and subsequent incubation with C5 deficient mouse serum (21). Zymosan particles were activated with fresh human or mouse serum according to the method of Mendes et al. (19). Mononuclear cells were incubated with the EAC and ZyC reagents as previously described (19, 21). A CR+ cell bound 3 or more EAC erythrocytes or ZyC particles.

**Fc Receptors.** Rabbit antiserum prepared against bovine erythrocytes (Eₐ) was used at a 1:1 dilution for sensitizing washed Eₐ (10). A 1% suspension of the resulting Eₐ A reagent (0.1 ml) was mixed with 10⁶ lymphoid cells (0.1 ml), and the mixture was centrifuged at 200 × g for 5 min. After incubation at room temperature for 30 min, rosette-forming cells were enumerated as previously described.

**Lymphocyte Cultures.** Normal or leukemic cells in supplemented RPMI 1640 media containing 2.5% AB plasma were cultured at 2 × 10⁶ cells/well in a microplate (Linbro Scientific, Hamden, Conn.). Cultures were incubated without mitogen or with several concentrations of phytohemagglutinin P (Burroughs Wellcome and Co., Research Triangle Park, N. C.), concanavalin A (Miles Laboratories, Inc., Elkhart, Ind.), or pokeweed mitogen (Grand Island Biological Co.) for 4 days in a humidified CO₂ incubator. For the MLR 10⁶ responding lymphoid cells were incubated with 10⁵ mitomycin C (Sigma Chemical Co., St. Louis, Mo.) (25 μg/10⁶ cells)-stimulating cells in microplate wells for 7 days. At 18 to 24 hr prior to harvest 0.1 μCi of [³H]dTdH (6 Ci/mmol) was added to each well for the mitogen cultures, and 1 μCi of the same [³H]dTdH was added per well for MLR cultures. The cells were harvested with an automated sample harvester, and [³H]dTdH uptake was assayed by standard liquid scintillation counting techniques.

**RESULTS**

The data in Table 1 summarize the patterns of surface marker expression that we have observed on leukemic blasts from 36 untreated acute lymphocytic leukemia patients and 2 patients with convoluted lymphoma. Thirty patients were classified as non-T, non-B acute lymphocytic leukemia because their lymphoblasts did not form E-ro-

---

**Table 1**

Percentage of acute lymphocytic leukemia cells bearing T- and B-cell membrane markers

<table>
<thead>
<tr>
<th>Acute lymphocytic leukemia classification</th>
<th>Source of leukemic cells</th>
<th>% of lymphoblasts positive</th>
<th>E-receptor</th>
<th>SmIg</th>
<th>C-receptor</th>
<th>Fc-receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-T, Non-B</td>
<td>Bone marrow</td>
<td>3⁶ (0-10)</td>
<td>0.6⁶ (0-5)</td>
<td>5⁶ (0-10)</td>
<td>5⁶ (0-9)</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Peripheral blood</td>
<td>91</td>
<td>&lt;1</td>
<td>19</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone marrow</td>
<td>59</td>
<td>&lt;1</td>
<td>4</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pleural fluid</td>
<td>83</td>
<td>&lt;1</td>
<td>29</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pleural fluid</td>
<td>84</td>
<td>&lt;1</td>
<td>10</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>CR+</td>
<td>Bone marrow</td>
<td>4</td>
<td>&lt;1</td>
<td>26</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone marrow</td>
<td>1</td>
<td>&lt;1</td>
<td>37</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone marrow</td>
<td>4</td>
<td>&lt;1</td>
<td>23</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone marrow</td>
<td>2</td>
<td>&lt;1</td>
<td>61</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

- a >90% lymphoblasts.
- b Mean of 30 specimens (S.E., 0.6).
- c Mean of 30 specimens (S.E., 0.5).
- d Mean of 22 specimens (S.E., 0.8).
- e Mean of 12 specimens (S.E., 0.8).
- f Numbers in parentheses, range.
- NT, not tested.
settes and failed to express SmIg, complement receptors, and Fc receptors. A second group of 4 patients, classified as having T-cell acute lymphocytic leukemia, had E-rosette-positive lymphoblasts. In 3 patients the E-rosette-positive lymphoblasts also displayed complement and/or Fc receptors. In a third group of 4 patients, the lymphoblasts were distinguished by the presence of complement receptors on a proportion of leukemic cells with minimal expression of other surface markers. There was no overlap in the range of complement receptors on non-T, non-B lymphoblasts (0 to 10%) with that observed on the CR⁺ lymphoblasts (23 to 61%). We therefore consider the 4 CR⁺ patients as distinct from the non-T, non-B subgroup.

**Cytochemical and Surface Marker Characteristics of CR⁺ Lymphoblasts.** As cells of the monocytic series possess complement receptors, we believed that it was imperative to verify the lymphoid origin of the CR⁺ leukemic blasts. Cytochemical stains revealed that the CR⁺ lymphoblasts were negative when tested for peroxidase and nonspecific esterase. None of the CR⁺ blasts were capable of phagocytosis with both ZyC and EAC reagents. Since zymosan activates complement receptors, we believed that it was imperative to verify the lymphoid origin of the CR⁺ lymphoblasts.

Cytochemical stains revealed that the CR⁺ lymphoblasts expressed both immune adherence and C3d receptors, ZyC reagent prepared with absorbed zymosan particles and subsequently incubated with fresh zymosan at 37°C. The ZyC reagent prepared with absorbed serum retained the capacity to bind to normal lymphocytes and CR⁺ lymphoblasts.

In order to determine whether the CR⁺ lymphoblasts expressed both immune adherence and C3d receptors, ZyC reagents were prepared with human or mouse serum. Mouse serum contains a potent C3b inactivator enzyme yielding C3d-bearing ZyC reagent (8), whereas zymosan incubated with human serum also displays noncleaved C3b components (confirmed by immune adherence assay). An interesting disparity was noted (Table 3) when lymphoblasts from 2 CR⁺ patients were tested with zymosan particles activated with mouse versus human serum. Whereas CR⁺ lymphoblasts from Patient 2 as well as mononuclear cells from normal individuals bound both types of reagent, lymphoblasts from Patient 4 reacted only with ZyC prepared with mouse serum. This suggested that C3d but not immune adherence receptors were present on leukemic cells from Patient 4. As expected lymphoblasts from 3 representative non-T, non-B acute lymphocytic leukemia patients did not bind zymosan activated with mouse or human serum.

**Functional Characteristics of CR⁺ Lymphoblasts.** Bone marrow lymphoblasts from the 4 CR⁺ patients were cultured with several concentrations of phytohemagglutinin, concanavalin A, and pokeweed mitogen. Leukemic cells from all 4 patients failed to demonstrate a positive response to any of these mitogens (data not shown). Similarly, the leukemic blasts were nonresponsive to allogeneic stimulation in mixed-lymphocyte culture. Chart 1 shows that, although the bone marrow cells from Patient 3 responded poorly to allogeneic cells, the mitomycin C-blocked lymphoblasts were a potent target for stimulating [³H]dThd incorporation by allogeneic responding lymphocytes. Similar results were obtained with lymphoblasts from Patient 2 (data not shown). In contrast the lymphoblasts from Patient 4 neither responded to nor stimulated allogeneic normal lymphocytes in culture.

**Clinical Characteristics of CR⁺ Patients.** Clinical characteristics of the 4 patients with CR⁺ leukemia are presented in Table 4. A distinctive clinical picture has not evolved. Hepatomegaly was present in 3 of 4 patients, and meningeal involvement was present at the time of diagnosis in 2 of 4 patients. None of the patients had evidence of mediastinal involvement. A prolonged period of time was required to achieve complete remission in Patients 1 and 4. Patients 1 and 3 underwent bone marrow relapse 7 and 8.5 months after achieving complete remission.

**DISCUSSION**

The major finding from this study is that complement receptors may be expressed independently of SmIg, E-receptors, and Fc receptors on acute lymphocytic leukemia...
lymphoblasts. To our knowledge this is the first report of a group of childhood acute lymphocytic leukemia patients without mediastinal involvement whose lymphoblasts at diagnosis express complement receptors exclusively. In 21 patients with childhood acute lymphocytic leukemia studied by Barrett et al. (3), none of the patients had lymphoblasts that displayed complement receptors only. Kadin and Billing (16) described 3 acute lymphocytic leukemia patients (2 adults and 1 child) whose lymphoblasts were B-cell antigen, E-receptor, and SmIg negative but positive for complement receptors. These patients differed from the 4 described in this report in that all of the former patients presented with a mediastinal mass. Similarly, Jaffe et al. (14) described 2 patients with lymphoblastic lymphoma associated with a mediastinal mass whose malignant cells displayed complement receptors in the absence of E-receptors.

Complement receptors have been associated with B- and null lymphocyte populations from normal individuals. Although Ehlenberger et al. (9) reported that all complement receptor-positive peripheral blood lymphocytes bear SmIg, others have observed a small proportion of circulating normal lymphocytes that display complement receptors in the absence of detectable SmIg (1, 20). In particular, studies reported by Ross (20) have confirmed that approximately one-half of the CR+ lymphocytes in peripheral blood do not contain SmIg as detected by (Fab')2 antiimmunoglobulin reagents. A similar subpopulation of CR+, SmIg-negative lymphocytes occurs in tonsil and splenic tissue (20). Chess et al. (7) have also reported a "null" subset of CR+, SmIg-negative peripheral blood lymphocytes that develops SmIg and the capacity to secrete immunoglobulin after 6 days of in vitro culture.

The population of CR+, SmIg-negative lymphocytes is normal or expanded in patients with Bruton's type agammaglobulinemia. Several investigators have found that these patients lack SmIg-positive lymphocytes but maintain a population of CR+ lymphocytes that do not form E-rosettes (12, 22).

Although complement receptors are not found on normal peripheral blood T-lymphocytes, malignant T-cells may express E- and complement receptors simultaneously (3, 14, 18). This observation emphasizes the need for caution in assigning a lymphoid neoplasm to either the T- or B-cell series. Malignant cells may express membrane markers that are present early in ontogeny but are not found on corresponding mature cells. Complement receptors have been found on E-receptor-negative human fetal thymocytes at 8 to 10 weeks of gestation (11). In vitro culture of the CR+ thymocyte population gave rise to a population of complement receptor-negative, E-receptor-positive cells (11). In keeping with these observations, Molt-4, a lymphoid cell line established from a T-cell leukemia, lost the capacity to...
express E-receptors after repeated subcultivation but retained complement receptor expression (13).

Since complement receptors may be found on null, T-, or B-lymphocytes, depending on the stage of differentiation or maturation as well as whether neoplastic transformation has occurred, we cannot yet predict the cellular origin of the CR+ leukemic cells described in this report. The leukemic cells from each of the CR+ patients were nonresponsive to mitogens, and none of the 3 tested responded to allogeneic stimuli in MLR. However, whereas lymphoblasts from Patients 2 and 3 were excellent stimulators when mixed with normal lymphocytes in MLR, lymphoblasts from Patient 4 did not elicit an MLR response. This finding suggests either that the lymphoblasts from Patient 4 do not express appropriate HLA-stimulating antigens or that the leukemic cells suppress MLR reactivity.

The prognostic significance of lymphoblasts bearing complement receptors is not entirely clear. However, since the time required for remission induction was prolonged in 2 of the patients and 2 patients have relapsed within 1 year, CR+ lymphoblasts may indicate a poor-risk group. Clinical comparison of patients with T-cell, non-T, non-B-cell, and CR+ cell leukemia will be presented in a subsequent report. The association of complement receptors with lymphoblasts that do not express other typical T- or B-lymphocyte membrane markers is another indication of the heterogeneity in surface marker profiles that may be encountered in childhood acute lymphocytic leukemia. Additional studies are needed more clearly to define the cellular origin and prognostic significance of CR+ lymphoblasts.

ACKNOWLEDGMENTS

The authors are grateful to Diana Gillespie and Pat Cox for their excellent technical assistance.

REFERENCES


Complement Receptor-positive, Sheep Erythrocyte Receptor-negative Lymphoblasts in Childhood Acute Lymphocytic Leukemia

Ellen R. Richie, Steven J. Culbert, Margaret P. Sullivan, et al.


Updated version Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/38/11_Part_1/3616](http://cancerres.aacrjournals.org/content/38/11_Part_1/3616)

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.