CONCISE REPORT

A Unique Surface Marker Profile in T-Cell Acute Lymphocytic Leukemia

By E. R. Richie, M. P. Sullivan, and J. van Eys

A 5-yr-old girl with acute lymphocytic leukemia presented with moderate hepatomegaly, marked splenomegaly, but no evidence of a mediastinal mass. The peripheral blood white count was 270 × 10^9/liter with 99% leukemic cells. Surface marker analysis showed the lymphoblasts to be E-rosette negative and complement receptor positive. The patient's leukemic cells were unreactive with anti-p23,30, which detects Ia-like antigens, and strongly reactive with A99 anti-T-cell serum, which reacts with normal human thymocytes and peripheral blood T cells. The percentage of leukemic cells bearing complement receptors diminished during relapse. The leukemic cells obtained at diagnosis and during relapse were nonreactive to mitogens and alloantigens and failed to stimulate proliferation of normal lymphocytes in mixed lymphocyte culture. There was no evidence for active suppression of normal lymphocyte reactivity mediated by the leukemic cells. The surface marker and functional profile of these leukemic cells is consistent with that of an early stage in T-cell maturation.

IMMUNOLOGIC surface markers have been useful in identifying the cellular origin of neoplastic cells in lymphoproliferative malignancies. In childhood acute lymphoblastic leukemia (ALL), surface marker analyses demonstrate heterogeneity among individual patients regarding the cellular origin of the malignant cells. Approximately 10%-20% of childhood ALL patients have leukemic cells that express sheep erythrocyte receptors (ERs) and T-cell antigens. The leukemic cells from another subgroup of ALL patients contain cytoplasmic IgM indicative of a pre-B-cell origin. Still another subgroup (5% of patients studied) have leukemic cells that express surface membrane immunoglobulin (SmIg), complement, and/or Fc receptors. The remaining group of ALL patients are referred to as non-T, non-B type ALL, since their lymphoblasts fail to express conventional T- or B-cell surface markers. Although the majority of non-T, non-B ALL cells express Ia-like antigens, a component of B-lymphocyte membranes, the presence of Ia antigens on a variety of other cell types, including acute myelogenous leukemia cells and normal granulocyte progenitors, precludes definitive assignment of these ALL cells to the B-cell lineage. We recently reported that leukemic cells from 4 of 34 patients studied expressed complement receptors (C3Rs) without simultaneous expression of ERs, FcRs or SmIg. None of these patients showed evidence of mediastinal involvement at diagnosis. In this report further characterization of the lymphoblasts obtained from one patient at diagnosis and during relapse suggests that the neoplastic proliferation represents a very early stage in T-cell ontogeny and emphasizes the heterogeneity of surface marker profiles, which may be encountered within the subgroup of T-cell ALL.

CASE REPORT

A 5-yr-old Latin American girl had fever, anorexia, abdominal distention for 1 day prior to diagnosis of acute leukemia on May 15, 1977. Physical examination showed pallor and petechiae, small cervical nodes, enlargement of both parotid glands, moderate hepatomegaly (7 cm below the RCM) and marked splenomegaly (12 cm below the LCM). The peripheral blood showed hemoglobin 2.9 g/dl, WBC count 270,000/cu mm (99% unidentified cells), platelets 19,000/cu mm; 97% of the bone marrow cells were unclassified "compatible with acute leukemia." The cerebrospinal fluid WBC chamber count and protein and glucose levels were normal; the cytocentrifuge smear, however, showed 26% of cells to be undifferentiated blasts. Chest radiograph and skeletal survey were normal; intravenous pyelography was compatible with sinus. The bone marrow biopsy revealed acute lymphoblastic leukemia with involvement of all medullary elements. A complete remission occurred on January 9, 1979.

Therapy was initiated May 18, 1977, with conventional doses of vincristine sulfate (VCR) and prednisone (Pred), high doses of cyclophosphamide (Cytoxan, CYT) alternating with high doses of methotrexate (MTX), and "triple-v" intrathecal (IT) therapy. Hematologic improvement was prompt and extramedullary disease regressed. Bone marrow improvement, however, was minimal. Therapy was changed to an adriamycin-VCR-Pred regimen on August 8, 1977, and a partial remission of 6 wk duration was obtained. "Sandwich" Cytosar-V-asparaginase-Cytosar therapy was initiated October 19, 1977. Five-day courses of Cytosar were repeated monthly, together with "triple" IT therapy. A complete remission was sustained until December 12, 1978. Investigative therapy, AMSA [NSC 24992, methanesulfon-anisidide, 4'-(-acridinyl amino)] was given September 26-30, 1978, with no response. Further chemotherapy was declined. The liver, spleen, and kidneys became enlarged, but the CNS remained relapse free. Death occurred on January 9, 1979.
assays were performed as previously described for detection of T cells/mL.3

rosette formation with ox erythrocytes sensitized with CS-deficient mouse serum.4 Laboratories) and subsequent incubation receptors. EAC reagent was prepared by sensitizing sheep B and

Preparation of Lymphoid Cells

Mononuclear cells (MNC) were isolated from heparinized peripheral blood and bone marrow by centrifugation on Ficoll-Hypaque gradients. After washing with Ca++ and Mg++ free Hank’s balanced salt solution, the MNC were resuspended in RPMI 1640 (GIBCO, Grand Island, N.Y.) media supplemented with 10 mM Hapes buffer, 2mM glutamine, and gentamycin (50 μg/ml).

Cryopreservation of Lymphoid Cells

Cells were cryopreserved in RPMI 1640 media containing 20% fetal calf serum (FCS) and 10% dimethyl sulfoxide at 2 × 10^7 cells/ml according to the method of Holden et al.5 Viability of the recovered cells, as assessed by trypan blue dye exclusion, exceeded 85%.

Cell Surface Markers

Spontaneous rosette formation with sheep erythrocytes and SmIg assays were performed as previously described6 for detection of T and B lymphocytes, respectively. Two methods were used to detect C3 receptors. EAC reagent was prepared by sensitizing sheep erythrocytes with the IgM fraction of rabbit anti-E (Cordis Laboratories) and subsequent incubation with C5-deficient mouse serum.7 Zymosan particles (Zy) were activated with mouse serum according to the method of Mendes et al.8 Fc receptors on MNC were detected by rosette formation with ox erythrocytes sensitized with a 1:100 dilution of rabbit anti-ox antisera.9 Mouse erythrocyte rosette-forming cells (MRFC) were assayed according to Gupta and Good.10

Cell Surface Antigens

Surface antigenic analysis of leukemic cells was kindly performed by Dr. Stuart Schlossman utilizing heteroantisera in an indirect immunofluorescence assay. Anti-T-cell sera (A99) were prepared by immunizing rabbits with purified plasma membrane fractions from CEM, a human T-lymphoblastoid cell line. The resulting antisera were rendered T-cell-specific by multiple absorptions with normal B cells, null cells, macrophages, and non-T leukemic cells. Antiserum specific for Ia-like antigens (anti-p23,30) was prepared and tested for specificity as previously described.11 Analysis of fluorescence-staining cells was performed with a fluorescence-activated cell sorter (Becton-Dickinson).12,13

Mixed Lymphocyte Culture (MLC)

Lymphocyte cultures containing 10^5 responding and 10^5 mitomycin-C-treated stimulating cells were established and harvested as described previously.7

RESULTS

The surface marker and antigenic profile of leukemic cells obtained at diagnosis is presented in Table 1. Leukemic cells from peripheral blood and bone marrow expressed C3Rs but showed minimal or no expression of other surface markers. Although C3Rs are considered to be a characteristic membrane component of B lymphocytes, the antigenic profile exhibited by the leukemic cells clearly demonstrated that these cells were of T as opposed to B-cell origin. Thus, the leukemic cells failed to react with anti-p23,30 antisera, which detect Ia-like antigens,15 while reacting strongly with antisera specific for human cells of T-lineage.14

Peripheral blood obtained during a subsequent relapse contained 70% leukemic cells. The proportion of cells bearing C3Rs had diminished to 22% using either the ZyC or EAC reagent. That the relapse cells expressing C3Rs were leukemic blasts was verified by morphological examination of cytocentrifuge slide preparations. The data in Table 2 show that relapse lymphoblasts are poor stimulators in MLC. In order to determine whether this deficiency was the result of active suppression, leukemic cells were added to mixtures of normal responding and stimulating lymphocytes. No inhibition of the MLC response occurred in the presence of leukemic cells. In addition, leukemic cells obtained at diagnosis and relapse were poorly responsive to T-cell mitogens and alloantigens (data not shown).

Table 1. Surface Marker and Antigenic Profile of Leukemic Cells

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Percent Leukemic Cells</th>
<th>Percent of Positive Cells</th>
<th>Anti-p23,30</th>
<th>A99 Anti-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>95</td>
<td>61</td>
<td>9</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>97</td>
<td>55</td>
<td>10</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*Not tested.

Table 2. MLC Stimulating Capacity of Leukemia Cells

<table>
<thead>
<tr>
<th>Source of Responding Cells</th>
<th>Source of Stimulating Cells*</th>
<th>Normal Donor 1</th>
<th>Normal Donor 2</th>
<th>Relapse Leukemia Cells</th>
<th>Normal Donor 1 + Relapse Leukemia Cells</th>
<th>Normal Donor 2 + Relapse Leukemia Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal donor 1</td>
<td></td>
<td>475 ± 10†</td>
<td>65,741 ± 5,446</td>
<td>3,573 ± 580</td>
<td>--</td>
<td>66,784 ± 3,416</td>
</tr>
<tr>
<td>Normal donor 2</td>
<td></td>
<td>41,034 ± 6,303</td>
<td>1,420 ± 330</td>
<td>5,531 ± 1,143</td>
<td>41,783 ± 87</td>
<td>--</td>
</tr>
</tbody>
</table>

*Stimulating cells were treated with mitomycin-C prior to culture.
†Average cpm ± SE of ^3H-dTdT uptake in triplicate cultures.

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DISCUSSION

It is well established that childhood ALL is a heterogenous disease in which patients may be subgrouped according to the immunologic markers expressed in or on the leukemic cells. The present findings emphasize that within a given subgroup (i.e., T-cell leukemia), further heterogeneity exists at least with respect to the pattern of surface marker expression. T-cell lymphoblasts in certain patients express both ERs and C3Rs, while in other patients only ERs are expressed on the leukemic cells.2,16-18 This report shows that C3Rs may be expressed in the absence of ERs on T-antigen-positive leukemic cells. To our knowledge this surface marker phenotype has not been previously reported in a patient without a mediastinal mass. Since a given lymphoid neoplasm probably represents malignant expansion of a normal lymphocyte clone at a particular stage of differentiation, one might expect leukemic T cells from different patients to vary in surface marker expression depending on the maturation stage of the target cell for the neoplastic event.

The unusual surface marker profile of the leukemic cells described in this report emphasizes the need for caution in assigning a T- or B-cell origin to malignant lymphoid cells on the basis of surface markers commonly associated with mature T or B lymphocytes. Complement receptors are a characteristic membrane component of normal B lymphocytes and have also been found on “null” lymphocytes that lack Smi and ERs.19 Although the major population of peripheral blood T cells do not express C3Rs, a small subpopulation of T cells in peripheral blood and a larger number in thoracic duct express both ERs and C3Rs.18 The number of C3R-positive T cells declines after incubation with thymic factors that induce differentiation.18 The concept that C3Rs are normally expressed during an early stage of T-cell maturation is supported by the observation that human fetal thymocytes at 8–10 wk gestation are C3R positive and ER negative but convert to the opposite phenotype as gestation proceeds presumably via an intermediate cell bearing both receptors.20 Indeed, Stein found that 30% of thymocytes at 12 wk gestation bear both C3Rs and ERs.21 It seems likely that the leukemic cells described in the present report represent expansion of an immature T-cell subset that has not yet acquired E-rosetting capacity.

The poor MLC-stimulating capacity of the leukemic cells is consistent with the deficiency of cell surface Ia-like antigens and is not a result of active suppression mediated by the leukemic cells. Han and Minowada recently speculated that leukemic null cells lacking MLC-stimulating activity represent early stages of T-cell differentiation whereas those that stimulate MLC reactivity may be of B-cell lineage.22 The reduction in the percentage of C3R-positive leukemic cells obtained during relapse may have been a consequence of the chemotherapeutic regimen, which adversely affected C3R expression. Alternatively, a selective growth advantage may have been associated with C3R-negative leukemic cells. Under these circumstances, leukemic cells may have undergone further differentiation in vivo during which C3R expression was lost. In this regard, Goldstone et al. described a case of adult ALL in which a drug-resistant leukemic subclone with a distinct antigenic phenotype emerged during the disease course.23

ACKNOWLEDGMENT

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REFERENCES

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