Adenosine Deaminase, Terminal Deoxynucleotidyl Transferase (TdT), and Cell Surface Markers in Childhood Acute Leukemia

By Mary S. Coleman, Martha F. Greenwood, John J. Hutton, Phillip Holland, Beatrice Lampkin, Carl Krill, and J. E. Kastelic

The purpose of this report is to compare measurements of enzymatic activities and cell surface markers as methods of distinguishing subtypes of lymphoid leukemias of childhood. Twenty-six children ages 2–15 yr were studied. Terminal deoxynucleotidyl transferase (TdT) activity was high in blasts from all 20 children with either null or T cell acute lymphoblastic leukemia. The activity of adenosine deaminase per cell was higher ($p < 0.005$) and that of TdT lower ($p < 0.05$) in T than in null cell lymphoblasts, although there was some overlap in values. Blasts from 3 children with acute lymphoid leukemia were positive for surface-associated immunoglobulins. The neoplastic lymphoid cells from these children differed from T and null cell leukemic lymphoblasts by having very low levels of TdT and adenosine deaminase activity. Measurements of adenosine deaminase and TdT may complement measurements of cell surface markers and distinguish biochemical subtypes of acute lymphoid leukemia.

CYTOLOGIC AND CYTOCHEMICAL criteria have proved useful in the classification of leukemias. With an increasing appreciation of the functional and biochemical heterogeneity of morphologically homogeneous lymphocytes, there have been efforts to measure simultaneously multiple immunologic and biochemical markers of cell type and to apply these to the classification of lymphoid malignancies. Measurements of cell membrane receptors have been successful in acute lymphoblastic leukemia (ALL) and are of clinical relevance because of the different prognoses of null and T cell disease. Quantiative measurements of the activity of terminal deoxynucleotidyl transferase (TdT) in neoplastic cells from bone marrow and peripheral blood can generally distinguish ALL from other types of acute leukemia. Measurements of the activity of adenosine deaminase (ADA) may help identify abnormal proliferations of B cells.

We report a systematic study of 26 children with acute leukemia. Our results indicate that measurements of ADA and TdT distinguish biochemical subtypes of lymphoproliferative disease. Combined cytochemical, immunologic, and bio-

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Supported by USPHS Grants CA19492, CA19842, and CA21435 and Contract CM67038 and Veterans Administration Research Service Project 596-3843. Dr. Coleman is the recipient of Research Career Development Award 1 KO4 CA00494.

Presented in part at the Annual Meeting of the American Society of Hematology, San Diego, California, December 6, 1977.

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chemical characterization of abnormal cells provides new objective tools for classification of these diseases.

**MATERIALS AND METHODS**

**Patients.** Nucleated cells from peripheral blood and/or bone marrow were examined in 26 children with acute leukemia at the time of diagnosis, prior to the institution of therapy. Patients with null, T, and B cell subgroups of ALL were selected from a large group of leukemic patients for enzyme assays and do not represent consecutive analyses. The protocol for the investigation was approved by the Committees on Human Investigation of the authors’ respective institutions.

**Isolation of cells.** Peripheral blood (20-40 ml) and/or 2.5-3 ml iliac crest bone marrow were collected in 5 mM EDTA for assays of cell markers and enzymatic activities. Nucleated cells were concentrated by dextran sedimentation followed in the case of peripheral blood by purification on Ficoll-Hypaque gradients. Contaminating monocytes were removed using carbonyl iron powder as described by Raff.20 The viability of cells exceeded 90% as assessed by trypan blue dye exclusion.

**Cell surface markers.** Cells forming E rosettes were quantitated as previously described by Bentwich et al.21 Lymphoid cells (0.1 ml, 5 x 10^6/ml) in Hanks’ balanced salt solution (HBSS) were incubated with 0.1 ml fresh sheep erythrocytes (0.5% suspension in HBSS) and 0.02 ml human AB+ serum (previously absorbed with SRBC) at 37°C for 5 min. After centrifugation at 50 g for 5 min, the mixture was incubated at 4°C for 1 hr. The cells were gently resuspended, and 200 cells were counted under oil immersion. Cells with three or more adherent SRBC were considered positive. A second aliquot of the cell suspension was collected on a glass slide by cytocentrifugation and stained with Wright-Giemsa to aid in assessing the morphology of reacting cells.

Surface immunoglobulins were determined by the method of Gajl-Peczalska et al.22 Lymphoid cells (0.1 ml) in HBSS (5 x 10^6/ml) were incubated with an optimal dilution of fluorescein-conjugated goat monospecific antisera to human γ, μ, and α heavy chains at 4°C for 1 hr. In some cases only polyvalent antisera was used. After centrifugation the cells were washed twice in cold phosphate-buffered saline (PBS), and the cell pellet was resuspended in 20 μl 50% glycerol in PBS. Two hundred cells were counted using a Zeiss fluorescent microscope equipped with a vertical illuminator.

**Enzymatic assays.** Purified cells were suspended at a density of 1–2 x 10^8 cells/ml in 0.25 M potassium phosphate buffer pH 7.5 containing 1 mM mercaptoethanol. The cell suspension was sonically disrupted by four 15-sec bursts with cooling. The extract was centrifuged at 100,000 g for 60 min, and the resulting supernatant fraction was assayed for TdT and ADA. Our assay of TdT has been described.16 Reaction mixtures contained 0.2 M potassium cacodylate buffer pH 7.0, 1 mM 3H-dGTP with a specific activity of 100 cpm/pmoles, 0.02 mM poly d (pA)50, 1 mM mercaptoethanol, and 15 μl cell extract in a final volume of 125 μl. One unit TdT activity equals 1 nmole dGTP polymerized in 1 hr at 35°C. Specific activities are expressed as U/10^8 nucleated cells. Reaction mixtures for ADA contained 0.05 M potassium phosphate buffer pH 7.0, 0.40 mM 14C-adenosine with a specific activity of 3 cpm/pmoles, and 20 μl tissue extract in a final volume of 100 μl.23 One unit ADA activity equals 1 nmole 14C-inosine produced per minute. Specific activities are expressed as U/10^8 cells.

**Statistics.** Unless otherwise noted, tests of statistical differences were performed using the Mann-Whitney test. Average values are expressed as mean ± SE.

**RESULTS**

Observations in 23 children with ALL at the time of initial diagnosis are summarized in Table 1. The majority of lymphoblasts from the peripheral blood and bone marrow of patients 1–13 did not form rosettes with sheep erythrocytes or have surface immunoglobulin. The leukemia was classified as null cell ALL. The median age of this group was 4 yr. The peripheral leukocyte counts were 6.9–299 x 10^9/liter (mean 64 ± 22 x 10^9). The percentages
Table 1. Cell Surface Markers and Enzymatic Activities in Acute Leukemia at Diagnosis

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<th>Blasts (%)</th>
<th>Enzymatic Activities (U/10⁸ cells)</th>
<th>Surface Markers (%)</th>
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*Percentage of blasts bearing SigM.
†Mean ± SE, n = 11, ages 1 wk–14 yr.

of blasts in peripheral blood and bone marrow were 16%–93% and 22%–99%, respectively. Activities of TdT in the peripheral lymphoid cells of patients 1–13 were 31–299 U/10⁸ cells (mean 95 ± 22 U). Activities of ADA were 85–1136 U/10⁸ cells (mean 365 ± 87 U). Activities of TdT and ADA in lymphoid cells from the peripheral blood of normal children were 0.34 ± 0.18 and 95.9 ± 5.1 U/10⁸ cells, respectively (n = 11).

Greater than 50% of lymphoblasts from the peripheral blood and bone marrow of patients 14–20 formed rosettes with sheep erythrocytes but did not have surface immunoglobulin. The leukemia was classified as T cell ALL. The median age of patients with T cell ALL was 6 yr and did not differ significantly (p > 0.2) from the median age of patients with null cell ALL. The peripheral leukocyte counts were 11.6–655.0 × 10⁹/liter (mean 299 ± 87 × 10⁹), which was significantly higher by Student’s two-tailed t test (p < 0.025) than the peripheral leukocyte count in patients with null cell ALL. Activities of TdT in the peripheral lymphoid cells of patients 14–21 were from 8.0–50 U/10⁸ cells...
(mean 30.7 ± 6.1 U), which did differ significantly ($p < 0.01$, Mann-Whitney test) from values in null cell ALL. Activities of ADA in peripheral lymphoid cells of patients 14–21 were 502–10,689 U/10$^8$ cells (mean 2800 ± 1400 U), which did differ significantly ($p < 0.005$, Mann-Whitney test) from the mean activity of ADA in cells from patients with null cell ALL.

Three patients (21–23, Table 1) had acute leukemia of lymphoid origin with greater than 50% of blasts bearing surface immunoglobulin. The leukemia was apparently of B cell origin. The male:female ratio was 2:1, and the median age was 8 yr. All had widespread extramedullary disease as evidenced by marked hepatosplenomegaly and lymphadenopathy. Central nervous system disease was present at onset in two. Therapeutic efforts were unsuccessful, and median survival was 5 mo. Cytologic features were indistinguishable from those of Burkitt leukemia cells. The majority of blasts from both peripheral blood and bone marrow samples of these three patients stained positively for surface-associated immunoglobulin of different classes, an intense multifocal pattern with anti-IgM and a fainter, more diffuse pattern with anti-IgG and -IgA. The nonuniform reactivity with IgG and/or IgA antisera was interpreted as antibody coating. In peripheral blood samples containing 46%–82% blasts the activities of TdT and ADA were low, 0.27 ± 0.02 and 51.3 ± 5.5 U/10$^8$ cells, respectively. The activity of TdT did not differ significantly from mean values found in normal lymphocytes but did differ from mean values found in active T or null cell ALL ($p < 0.01$, Student's $t$ test). The activity of ADA was significantly lower than mean values found in normal lymphocyte populations ($p \leq 0.001$, Student's $t$ test) and T and null lymphoblasts ($p < 0.01$, Student's $t$ test). Extracts of the leukemic cells that had surface immunoglobulin did not inhibit the activities of ADA or TdT in extracts from typical leukemic lympho-

Fig. 1. Activities of TdT and adenosine deaminase in lymphoid cells from peripheral blood of children with ALL. Both abscissa and ordinate are logarithmic scales. Each point represents a different patient. Surface characteristics of blasts: $\triangledown$, T cell, formed E rosettes; $\Box$, null cell, did not form E rosettes or have Slg; $\blacksquare$, B cell, had Slg but did not form E rosettes.
blasts. There is no evidence that the surface immunoglobulin was directed against either of these enzymes or that other types of inhibitors of ADA and TdT were present.

When the TdT and ADA activities present in peripheral leukemic lymphoid cells were plotted against one another on a logarithmic scale (Fig. 1), T, null, and B cell ALL fell into separate biochemical groups. B cell disease was very distinct and characterized by low TdT and low ADA activities. T cell and null cell ALL can be characterized as high TdT–high ADA and high TdT–intermediate ADA, respectively. Points representing patients with these two subtypes of ALL did not intermingle. There was a weak but statistically significant correlation between the logarithms of ADA and TdT activities in null ($r = 0.73, p < 0.01$) but not in T ($r = 0.33, p > 0.1$) or B ($r = -0.97, p > 0.1$) cell disease.

We also studied three children with acute myelogenous leukemia (Table 1). Blasts from these children had low levels of TdT, high levels of ADA, absent sheep cell receptors, and absent Slg.

**DISCUSSION**

Measurements of TdT activity are of established value in distinguishing null or T cell ALL from other types of acute leukemia. TdT activity is generally much higher in blasts from patients with typical ALL than in other leukemias. Blasts in ALL can be classified by null, T, or B cell on the basis of surface membrane markers, and these three categories of disease have distinctly different prognoses. Average TdT activity is lower in T-marked lymphoblasts than in null-marked lymphoblasts and is much lower in B than in either T or null lymphoblasts. Patients with null cell ALL respond more readily to treatment and survive longer than patients with T cell ALL. Patients with B-marked lymphoblasts generally respond poorly to treatment and die within a few months of diagnosis. Both B- and T-marked ALL characteristically have more tumor or bulk disease than null cell ALL. They may be closely related to the lymphomas.

ADA is another enzyme that may serve as a biochemical marker in leukemia. Adults with chronic lymphocytic leukemia have very low levels of ADA in their neoplastic lymphocytes. Decreased activities of ADA are associated with other types of abnormal proliferations of B cells, such as multiple myeloma. We cannot confirm a report that ADA activity is generally decreased in leukemic lymphoblasts. Indeed, in our experience ADA activity in leukemic lymphoblasts is very high in those marked T, intermediate in those marked null, and low in those marked B. When measurements of TdT and ADA activities are combined, three biochemical subtypes of ALL are identified; these subtypes correlate with those defined by membrane markers (Fig. 1).

ADA activity in T lymphoblasts is similar to that found in thymus and in cell lines of T lymphoblastoid type. B lymphoblasts have ADA activity very similar to that found in spleen cells. A severe deficiency in ADA activity causes abnormal deoxynucleotide metabolism with selective toxicity to lymphoid cells. Purine and pyrimidine analogues affecting deoxynucleotide metabolism are available. Some of these should be carefully evaluated for effec-
tiveness in the treatment of lymphoid malignancies. Variations in ADA activity in cells may be of relevance in planning such chemotherapy, since this enzyme is involved in the metabolism of many of these drugs.

The continued development of biochemical markers of leukemic cells should provide clinically useful information relevant to differential diagnosis. Whether or not these techniques can be used to predict the response of disease to treatment or to assess adequacy of treatment will require improvement in methods of biochemical measurement and extensive clinical trial. Of particular relevance are flow cytofluorometric techniques permitting the biochemical characterization of large numbers of individual cells in a mixture of cells such as is obtained from bone marrow. Detection of very small numbers of residual leukemic blasts in marrow may be possible using fluorescein-labeled antibody to enzymes such as TdT.29

REFERENCES

Adenosine deaminase, terminal deoxynucleotidyl transferase (TdT), and cell surface markers in childhood acute leukemia

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