Serial Observations on Terminal Deoxynucleotidyl Transferase Activity and Lymphoblast Surface Markers in Acute Lymphoblastic Leukemia

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SUMMARY

Terminal deoxynucleotidyl transferase activity and cell surface markers were measured in peripheral lymphoid cells from 27 children with acute lymphoblastic leukemia in various phases of their disease. Lymphoblasts from untreated patients had smooth surface ultrastructure but heterogeneous surface receptors. Greater than 60% of lymphoblasts from 4 of 7 untreated patients formed rosettes with sheep red blood cells. Transferase activity was variable, ranging from 8 to 210 units/10⁶ blasts, but it was consistently elevated at diagnosis and in relapse. Transferase levels did not correlate with the presence of lymphoblast surface receptors. During induction therapy transferase activity decreased rapidly, but it remained elevated in peripheral lymphoid cells even when blasts were not detectable in the peripheral blood smears. Patients in remission had normal surface receptors and undetectable or minimally elevated levels of transferase. Terminal transferase activity may be a sensitive biochemical marker for a primitive cell population and may be important in the evaluation of therapeutic effectiveness in acute lymphoblastic leukemia.

INTRODUCTION

A variety of methods that aid in classifying the cell type involved in lymphoproliferative diseases and that serve as indicators of disease activity have been described. These include morphological and histochemical analyses, determination of malignant lymphoid cell surface markers, cytotoxicity assays, and biochemical markers. Terminal deoxynucleotidyl transferase is a biochemical marker currently being evaluated for practical value. This enzyme is a DNA polymerase that catalyzes the addition of deoxynucleoside triphosphates to the 3'-hydroxyl ends of single-stranded oligodeoxynucleotide initiators. Terminal transferase is an unusual DNA-synthesizing enzyme because it does not use template information to copy deoxynucleotide sequences. The enzyme is present in high concentration in thymus (11) and in low concentration in normal bone marrow (15); no activity has been found in normal or phytohemagglutinin-stimulated blood lymphocytes (5). The enzyme has been identified in blood lymphoid cells of acute lymphoblastic leukemia (24), in cell lines derived from patients with lymphoblastic leukemia (33), and in cells from a rare patient with acute or chronic myelogenous leukemia in blast crisis (15, 30).

The purpose of this study was to carry out a simultaneous examination of cell surface receptors, surface ultrastructure, and terminal deoxynucleotidyl transferase activity in blood lymphoid cells from children with acute lymphoblastic leukemia. These studies were performed at the time of diagnosis, serially throughout induction chemotherapy, and at the time of hematological remission and relapse. Extensive studies will be necessary to assess the usefulness of transferase as a marker of leukemic cells and disease activity. This study provides preliminary evidence that this enzyme is a useful marker.

MATERIALS AND METHODS

Patients. Lymphoid cells from 27 children with acute lymphoblastic leukemia were examined during different stages of the disease process. In 7 patients, complete data from diagnosis and throughout remission induction were obtained. Peripheral blood lymphoid cells were also collected from 10 patients in disease remission on chemotherapy, 7 patients in long-term disease remission of chemotherapy, and 3 patients at the time of disease relapse.

Seven children ranging from 18 months to 10 years of age and 20 adults without evidence of hematological abnormalities served as sources of normal peripheral blood.

Therapy. Induction therapy in the 7 patients studied from diagnosis through treatment consisted of hydrocortisone sodium succinate, 150 mg/sq m i.v. every 6 hr for 8 doses, followed by prednisone, 40 mg/sq m/day p.o., and vincristine, 1.5 mg/sq m/week i.v. for 4 weeks. Following remission induction, all patients received prophylactic central nervous system therapy consisting of cranial irradiation to 2400 rads.
and 5 i.t.\textsuperscript{3} injections of methotrexate, 12 mg/sq m/dose. Maintenance chemotherapy initiated at approximately Day 30 was 6-mercaptopurine, 50 mg/sq m/day, and cyclophosphamide, 200 mg/sq m/week p.o. Methotrexate, 20 mg/sq m/week p.o. was added after completion of central nervous system therapy.

**Lymphoblast and/or Lymphocyte Isolation.** Ten to 20 ml of heparinized peripheral blood were obtained for assays of cell surface markers and surface ultrastructure. Ten to 20 ml of peripheral blood were collected in 5 mM EDTA for terminal transferase determinations. Peripheral blood was diluted 5:1 with 6% dextran (M.W. 250,000) in 0.9% NaCl solution and allowed to sediment for 45 min at room temperature. Leukocyte suspensions were centrifuged, diluted in HBSS,\textsuperscript{3} and layered onto Ficoll-Hypaque gradients. The gradient was centrifuged at 250 \times 10^3 \text{g} for 35 min according to the method of Boyum (8). The mononuclear cell layer was aspirated with a curved Pasteur pipet and washed twice in HBSS. Carbonyl iron powder was used to remove contaminating monocytes, when present, as described by Raff (28). The lymphoid cells were resuspended to 5.0 \times 10^7/ml in HBSS. The viability of cells was greater than 95% as tested by trypan blue exclusion.

**E Rosette Assay.** E rosettes were assayed by the method of Bentwich et al. (3). Briefly, 0.1 ml of lymphoid cells (5 \times 10^6/ml) in HBSS was incubated with 0.1 ml of a 0.5% suspension of fresh SRBC in HBSS and 0.02 ml of human AB+ serum (previously absorbed with SRBC) at 37° for 5 min. The mixture was spun at 50 \times 10^3 g for 5 min and then incubated at 4° for 1 hr. The cells were gently resuspended and 200 cells were counted under oil immersion. An E rosette-forming cell was defined by the attachment of 3 or more SRBC.

**EAC Rosette Assay.** EAC rosettes were determined by the methods of Ross et al. (29). EA cells were prepared by mixing 0.5 ml of 5% suspension of fresh sheep erythrocytes in GVB with an equal volume of the IgM fraction of rabbit anti-sheep hemolysin (Baltimore Biological Laboratories, Cockeysville, Md.) diluted 1:20,000 in GVB and incubated at 37° for 30 min. The EA cells were washed twice, mixed with an equal volume of fresh human serum (diluted 1:20 in GVB), and incubated at 37° for 30 min to form EAC\textsubscript{w} cells. Equal volumes of 2 \times 10^6 lymphoid cells/ml and a 1% suspension of EAC\textsubscript{w} cells were gently mixed and incubated at 37° for 15 min on a circular rotator. After resuspension, 200 cells were counted at \times 400 and cells with 4 or more adherent EAC\textsubscript{w} cells were considered positive.

**Determination of Surface Immunoglobulins.** Lymphoblast and/or lymphocyte surface immunoglobulins were determined as previously described by Gajl-Peczalska et al. (18), using fluorescein-conjugated IgG, IgM, and IgA antisera supplied by Dr. Neil Davis, Cincinnati Children’s Research Foundation. Lymphoid cells in HBSS were incubated with a 1:20 dilution of type-specific antisera at 4° for 1 hr. After centrifugation the cell pellet was resuspended in 20 \mu l of 50% glycerol in phosphate-buffered saline. Two hundred cells were counted, using a Zeiss UV microscope equipped with a B-12 excitation filter and a No. 50 barrier filter. Percentage of cells with surface Ig represents the sum of percentages of cells reacting with antisera to IgA, IgM, and IgG.

**Preparation of Specimens for Scanning Electron Microscopy.** Cells were fixed for scanning electron microscopy by the method of Polliack et al. (27), with minor modification. Mononuclear cells (2 \times 10^6/ml) suspended in HBSS were spun down onto Kimble No. 1, 12-mm round coverglasses at 10 \times g for 5 min. The cells attached to the coverglasses were fixed in 2% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.3 to 7.4, 320 to 330 mOsmoles) for 30 min at room temperature. They were then rinsed 3 times with buffer and postfixed in 1% osmium tetroxide for 1 hr at room temperature. The cells were rinsed 3 more times with buffer and then dehydrated in graded ethanol and isoamyl acetate solutions for 5 min each. The specimens were dried by the critical point method of Anderson (2) (Ivan Sorvall critical point apparatus, Newtown, Conn.). The coverslips were attached to aluminum specimen holders and the specimens were coated with gold and carbon prior to examination at 20 kV in a Mark 2A Cambridge Stereoscan microscope.

**Enzyme Assays.** Lymphoid cells were suspended at a density of 1 to 2 \times 10^6 cells/ml in 0.25 M potassium phosphate buffer, pH 7.5, containing 1 mM mercaptoethanol. The cell suspension was sonicated 4 times in 15-sec bursts with cooling. The extract was centrifuged at 100,000 \times g for 60 min and the resulting supernatant fraction was assayed directly for terminal deoxynucleotidyl transferase.

Radioactive deoxynucleoside triphosphates were from New England Nuclear, Boston, Mass. Unlabeled deoxynucleoside triphosphates were from P-L Biochemicals, Milwaukee, Wis. Oligo d(pA)\textsubscript{10} was prepared in our laboratory (13). Assays for terminal deoxynucleotidyl transferase contain 0.2 M potassium cacodylate buffer (pH 7.0), 1 mM \textsuperscript{3}HdGTP with a specific activity of 100 or 500 cpm/pmole, 0.02 mM oligo d(pA)\textsubscript{10}, 10 mM MgCl\textsubscript{2}, 1 mM mercaptoethanol, and 15 \mu l of cell extract in a final volume of 125 \mu l. Processing of reaction products (4) and calculation of activities have been described (12). The assay used is specific for terminal transferase activity, even in crude tissue extracts (12). One unit of terminal transferase activity equals 1 n mole of nucleotide polymerized in 1 hr. Specific activities are expressed as units/10^6 cells. Assays were initially carried out at \textsuperscript{3}HdGTP specific activity of 100 cpm/pmole. Samples with less than 1 unit/10^6 cells are reassayed both at a \textsuperscript{3}HdGTP specific activity increased to about 500 cpm/pmole and in the presence of 10% ethanol and 10 mM N-ethylmaleimide. The combination of ethanol and N-ethylmaleimide completely inhibits transferase. This assay distinguishes transferase from the low-molecular-weight DNA-dependent DNA polymerase B and nonspecific binding of radioactivity to the GF/C disc.

\textsuperscript{3} The abbreviations used are: i.t., intrathecally; HBSS, Hanks’ balanced salt solution; SRBC, sheep RBC; E rosettes, sheep erythrocyte rosettes; GVB, gelatin-Veronal-buffered saline (0.005 M sodium barbital, pH 7.3; 0.2 M NaCl; 0.03 M CaCl\textsubscript{2}; 0.2 M MgCl\textsubscript{2}; and 0.1% gelatin); EAC\textsubscript{w}, human complement-coated sheep RBC.

\textsuperscript{1} Transferase and Surface Markers in Leukemia

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\textsuperscript{2} Transferase and Surface Markers in Leukemia
RESULTS

Acute Lymphoblastic Leukemia Prior to Chemotherapy. Initial observations in 7 patients with untreated acute lymphoblastic leukemia are summarized in Table 1. The children ranged in age from 3 to 17 years. The number of lymphoblasts in their peripheral blood ranged from 38,000 to 366,000/cu mm and constituted greater than 90% of the cell suspensions assayed. The "purity" of the cell population made the interpretation of cell surface markers and transferase activities much less subject to error than when the lymphoblast count is low or the cell population is mixed in type. Terminal transferase activity was markedly elevated in all patients, ranging from 8 to 210 units/10^6 lymphoid cells. Enzyme activity from these patients is comparable to or higher than that found in human thymocytes from 4 children ranging in age from 9 months to 15 years (20 to 60 units/10^6 cells), and much higher than transferase in normal blood lymphocytes (less than 0.05 unit/10^6 cells) in children from 1 to 10 years of age.

In 4 of 7 patients with acute lymphoblastic leukemia prior to treatment, the majority of blood lymphoblasts formed E rosettes; and in 3 of these 4 patients, radiological evidence of a mediastinal mass was present. In 3 of the 7 untreated patients less than 10% of the lymphoid cells formed E rosettes, whereas the mean for normal control lymphocytes from children was 55 ± 7%. In all patients the percentages of lymphoblasts bearing surface immunoglobulins (IgA, IgG, and IgM) or complement receptor sites were below the control range for normal lymphocytes. Thus, 4 of 7 patients prior to chemotherapy had T-cell surface markers as defined by E rosette formation, and in 3 of 7 a significant percentage of T- or B-cell surface markers was not demonstrated. There are no clear correlations between the levels of transferase activity and the types of surface receptors, ages of patients, or blast counts.

The surface ultrastructure of lymphoblasts as defined by scanning electron microscopy was similar in all 7 patients at the time of disease diagnosis. More than 80% of lymphoblasts were devoid of microvillous projections and had a characteristic "smooth" surface topography (Table 1; Fig. 1A). These surface features are identical to those of the majority of lymphoid cells obtained from the thymus glands of children (Fig. 1B). The smooth-surfaced lymphoblasts can be readily distinguished from the myelomonoblasts seen in acute myelomonoblastic leukemia, which have numerous elongated, thin ruffled processes (Fig. 1C). For comparison, a normal blood monocyte with extensive membrane ruffling and 2 normal lymphocytes with numerous microvilli are seen in Fig. 1D.

Acute Lymphoblastic Leukemia during Induction Chemotherapy. A favorable clinical and hematological response was noted in all 7 patients with the induction chemotherapy used. The absolute number of blood lymphoblasts declined precipitously during the first 7 days of chemotherapy. Terminal transferase activities in blood lymphoid cells serially assayed during induction therapy are summarized in Chart 1. The characteristics of the patients at the time of initial diagnosis are shown in Table 1. Transferase activity declined rapidly during the first few days of therapy and then stabilized between 0.1 and 0.7 unit/10^6 lymphoid cells following 30 to 45 days of induction chemotherapy. Decline of cells morphologically characterized as lymphoblasts was more rapid than the decline in transferase activities. In 4 of 7 patients in whom no blasts were identified by routine examination of the blood smears by Day 12 of chemotherapy, transferase values ranged from 1.2 to 9.4 units/10^6 lymphoid cells.

### Table 1

**Characteristics of patients at the time of initial diagnosis prior to chemotherapy**

Lymphoblasts were isolated and assayed for E, EAC rosettes, and surface Ig. Preparations of cells were examined using scanning electron microscopy for percentage of smooth-surfaced lymphoid cells. Cell extracts were assayed for terminal transferase activity, expressed as units/10^6 cells.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Mediastinal mass</th>
<th>Blasts/cu mm</th>
<th>Transferase (units/10^6 cells)</th>
<th>E rosettes (%)</th>
<th>EAC rosettes (%)</th>
<th>Surface Ig (%)</th>
<th>Scanning electron microscopy smooth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. C.</td>
<td>17</td>
<td>0</td>
<td>366,000</td>
<td>210</td>
<td>2</td>
<td>3</td>
<td>14</td>
<td>90</td>
</tr>
<tr>
<td>L. R.</td>
<td>4</td>
<td>0</td>
<td>116,000</td>
<td>98</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>81</td>
</tr>
<tr>
<td>D. S.</td>
<td>6</td>
<td>0</td>
<td>38,000</td>
<td>90</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>J. P.</td>
<td>5</td>
<td>+</td>
<td>127,000</td>
<td>50</td>
<td>92</td>
<td>1</td>
<td>4</td>
<td>90</td>
</tr>
<tr>
<td>M. B.</td>
<td>7</td>
<td>+</td>
<td>219,000</td>
<td>46</td>
<td>68</td>
<td>2</td>
<td>2</td>
<td>ND</td>
</tr>
<tr>
<td>L. B.</td>
<td>3</td>
<td>0</td>
<td>279,000</td>
<td>21</td>
<td>87</td>
<td>4</td>
<td>11</td>
<td>90</td>
</tr>
<tr>
<td>W. S.</td>
<td>8</td>
<td>+</td>
<td>70,000</td>
<td>8</td>
<td>75</td>
<td>2</td>
<td>6</td>
<td>90</td>
</tr>
</tbody>
</table>

Controls:
- Adults: <0.05, 65 ± 6, 13 ± 6, 25 ± 7, <40
- Children: <0.05, 55 ± 7, 17 ± 7, 30 ± 6, ND

*+, mediastinal mass on chest X-ray; ND, not done.

There is a significant percentage of smooth surfaced lymphoid cells as determined by scanning electron microscopy.

The control population comprised 20 normal adults and 7 normal children ranging from 18 months to 10 years of age. Results, mean ± SE.
Surface marker assays on the blood lymphocyte population returned toward normal during induction therapy. Following 1 month of chemotherapy, the mean value for E rosettes in the 7 patients was 52 ± 8%, EAC<sub>hu</sub> rosettes 15 ± 9%, and surface Ig 15 ± 7%.

**Acute Lymphoblastic Leukemia,** **Patients on Chemotherapy.** The initial observations and data obtained in 10 additional children with acute lymphoblastic leukemia in disease remission, but receiving chemotherapy, are summarized in Table 2. Initially, none of the patients had a mediastinal mass and blood lymphoblasts ranged from 0 to 230,000/cu mm. All patients were in continuous remission for 8 to 24 months when blood lymphocyte transferase and surface marker assays were performed. Transferase activity was less than 1 unit/10<sup>8</sup> lymphoid cells (range, less than 0.05 to 0.9 unit/10<sup>8</sup> cells) in the 10 patients we have studied at random from the clinic population of children in this stage of their disease. The mean value for E rosettes was 74 ± 6%, EAC<sub>hu</sub> rosettes 10 ± 7%, and surface Ig 8 ± 2%. These values appear abnormal, showing a deficiency of cells typing as B-lymphocytes and an excess of cells typing as T-lymphocytes (normal values are shown in Table 1). This observation of a relative deficiency of B-cells is in agreement with prior findings of Sen and Borella (31) for patients on chemotherapy.

**Acute Lymphoblastic Leukemia, Patients off Chemotherapy.** The initial observations and data obtained in 7 additional children with acute lymphoblastic leukemia who have completed 3 or more years of chemotherapy and remain in continuous remission are summarized in Table 3. At the time the blood transferase and surface marker assays were performed, each of the patients was in hematological remission and the time periods following discontinuance of drug therapy ranged from 2 to 52 months. Transferase levels in the 7 patients ranged from less than 0.05 to 0.5 unit/10<sup>8</sup> lymphoid cells. The mean value for E rosettes was 43 ± 11%, EAC<sub>hu</sub> rosettes, 18 ± 6%, and surface Ig 30 ± 10%. The distribution of surface markers on lymphocytes is within the normal range for children we have studied.

**Acute Lymphoblastic Leukemia in Disease Relapse.** The initial observations and data obtained in 3 children with acute lymphoblastic leukemia in relapse are summarized in Table 4. Relapse occurred following 16, 38, and 52 months of continuous remission. The blood transferase levels at the time of relapse were 9, 60, and 67 units/10<sup>8</sup> lymphoid cells and the absolute numbers of blood lymphoblasts were 1,500, 1,800, and 1,000/cu mm, respectively.

**Summary of Observations on Transferase Activities.** A summary of the terminal transferase activities in peripheral

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**Table 2**

| Transferase activities in lymphoid cells of 10 patients in clinical remission on chemotherapy |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| Lymphocytes from peripheral blood of patients in clinical remission on chemotherapy were purified and assayed for terminal transferase activity. | | | |
| **Initial diagnosis** | **Present status** | | |
| **Patient** | **Age (yr)** | **Mediastinal mass** | **Blasts/cu mm** | **Duration of remission (months)** | **Blasts/cu mm** | **Transferase (units/10<sup>8</sup> cells)** |
| L. S. | 3 | 0 | 230,000 | 24 | 0 | 0.9 |
| R. H. | 4 | 0 | 0 | 22 | 0 | 0.7 |
| K. H. | 3 | 0 | 3,000 | 20 | 0 | 0.6 |
| J. U. | 4 | 0 | 158,000 | 19 | 0 | <0.05 |
| D. D. | 3 | 0 | 16,000 | 18 | 0 | 0.5 |
| J. L. | 4 | 0 | 143,000 | 18 | 0 | <0.05 |
| D. H. | 5 | 0 | 67,000 | 18 | 0 | 0.3 |
| B. S. | 3 | 0 | 0 | 14 | 0 | 0.8 |
| M. C. | 3 | 0 | 2,000 | 10 | 0 | 0.6 |
| E. M. | 5 | 0 | 0 | 8 | 0 | 0.4 |

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lymphoid cells of 27 children with acute lymphoblastic leukemia at various stages of the disease process is presented in Chart 2. Transferase was markedly elevated in the peripheral blood of all leukemic patients prior to treatment and when in relapse. Transferase activity declined rapidly during induction therapy but remained elevated (0.35 to 0.7 unit/10⁸ cells) in 6 or 7 patients at the time initial hematological remission was achieved. Lymphocyte transferase activity was detectable (0.3 to 0.9 unit/10⁸ cells) in patients with longer durations of remission both on and off chemotherapy, 8 of 10 and 3 of 7, respectively. We believe that transferase values are slightly elevated in these patients since we have not detected transferase activity in peripheral lymphocytes from 20 normal adults and 7 normal children studied (Table 1). Since the number of children examined is small, it is possible that an occasional normal child may have detectable transferase activity (greater than 0.05 unit/10⁸ cells).

**DISCUSSION**

The differentiation of B- and T-lymphocytes utilizing characteristic surface markers has recently found wide application in the investigation and classification of various lymphoproliferative disorders. The analysis of neoplastic lymphoid cells on the basis of B- or T-cell properties has led to the speculation that this type of characterization may have important prognostic and therapeutic implications in acute lymphoblastic leukemia. Lymphoblasts in the majority of leukemic patients have not been found to possess identifiable surface markers and have been designated "null cells." However, lymphoblasts bearing T-cell markers (E rosettes)
have been described in acute lymphoblastic leukemia and the incidence varies among different series, depending on the stage of disease and the percentage of blood lymphoblasts. Review of previously reported series (1, 6, 7, 9, 14, 16, 17, 21, 22, 25) identified 46 cases of untreated acute lymphoblastic leukemia having greater than 80% lymphoblasts at the time of marker analysis. Thirteen cases, or approximately 20%, were designated as examples of T-cell leukemia. In our experience, analyzing serial admissions of 12 children with untreated lymphoblastic leukemia having greater than 90% peripheral blood blasts, the majority of lymphoblasts (greater than 60%) formed E rosettes in 5 patients, but in 7 patients no lymphoblast markers were identified. In the 5 patients with demonstrable T-cell markers 3 had evidence of a mediastinal mass and all had initial blast counts greater than 50,000/cu mm. Of the 7 patients in whom no lymphoblast markers were found none had evidence of a mediastinal mass, and in 5 patients the initial blast count was greater than 50,000/cu mm. This suggests that the presence of T-lymphoblast markers in acute lymphoblastic leukemia may be associated with bulk disease and possibly with a more aggressive clinical course, as others have described (10, 32).

Scanning electron microscopy allows differentiation of the surface ultrastructural features of specific cell types. In all 12 cases of untreated acute lymphoblastic leukemia that we have examined to date, the lymphoblast surface was characteristically smooth (20). Polliack and De Harven (26) also recently reported the generally smooth surface features of lymphoblasts in acute lymphoblastic leukemia. The lymphoblast surface closely resembles the normal human thymocyte and the normal smooth surfaced blood lymphocyte. Malignant cells appear to retain surface characteristics of the normal cell line involved; therefore differentiation of malignant from normal lymphoid cells by scanning electron microscopy observations is not possible. Scanning electron microscopic observations have, however, proved useful in classifying the leukemic cell type involved, since one can readily distinguish lymphoblasts from myelomonoblasts on the basis of surface ultrastructure. The smooth surface of the lymphoblast differs markedly from the undulated, ruffled membrane surface of the myelomonoblasts observed in 5 patients with acute myelomonoblastic leukemia (20).

Quantitative determinations of terminal transferase activity may prove to be a sensitive indicator of disease activity in acute lymphoblastic leukemia, since the enzyme is present in very high activity in blood lymphoblasts (23) but is not detectable in normal peripheral lymphocytes (15). Transferase levels in lymphoblasts are variable but markedly elevated at the time of initial diagnosis and disease relapse in all patients with acute lymphoblastic leukemia that we have studied to date. Transferase activity did not correlate with the presence or absence of lymphoblast surface markers or ultrastructure. The level of transferase activity at diagnosis does not appear to be predictive of initial responsiveness to therapy, since all patients readily achieved a complete remission status. The relationship of initial transferase activity to long-term prognosis remains to be determined.

This study indicates that terminal transferase activity may be a biochemical marker of a primitive cell population important both in the identification of the leukemic cell type and in evaluation of effectiveness of therapy. The continued elevation of terminal transferase activity in the absence of identifiable blasts in the blood smears of 4 patients following 10 to 14 days of induction therapy indicates that transferase assays may be more sensitive than morphological criteria in monitoring disease activity. The significance of low but detectable levels of transferase activity in some patients in disease remission, but not in others, can be determined only after a longer follow-up period.

To achieve maximal usefulness, it is necessary that the method of assaying terminal transferase be both sensitive and specific. The activities of transferase that we observe in leukemic lymphoblasts range from 8 to 210 nmol nucleotide incorporated per hr per 10^9 cells at 37°. Values observed by McCaffrey et al. (23) in lymphoblasts range from 0.3 to 5.0 nmol nucleotide incorporated per hr per 10^9 cells at 37°, which is approximately 4% as high as the activities we find. In myeloblasts from patients with acute myelogenous leukemia, we usually find approximately 0.3 to 1.0 unit of transferase per 10^9 cells (15), whereas McCaffrey et al. (23) report less than 0.01 unit/10^9 cells. The level of terminal transferase found in all untreated children with acute lymphoblastic leukemia is markedly higher than that found in our assays of 20 adults with acute myeloblastic leukemia. Our assay is sensitive enough to quantitate transferase in specimens of low activity such as normal bone marrow. The improved sensitivity and specificity of the assay procedure is due to the use of assay conditions optimal and specific for transferase. This assay was developed for estimation of transferase in crude calf thymus extracts and makes fractionation of extracts by column chromatography of sucrose gradients unnecessary as a routine procedure. Although some reservation was expressed in the original use of this assay, subsequent experience has shown that values obtained in crude extracts correlate well with those from sucrose density gradients and column chromatography (12, 15). The homopolymer oligo d(pA)6 is used as an initiator to minimize the effect of endonucleases, since cleavage products still score in the assay. The concentration of the nucleotide, [3H]dGTP, is maintained at 1 mm, well above the Km of transferase, giving both maximal velocity and linear kinetics. Cacodylate is used as a buffer and is optimal for transferase, while inhibiting DNA-dependent DNA polymerases α and β. The nucleotide dGTP is used in the polymerization reaction since the product is relatively resistant to exonuclease.

The quantitative measurement of terminal transferase activity in peripheral blood cells appears to be a sensitive method of assessing disease activity in acute lymphoblastic leukemia. Serial terminal transferase determinations obtained during the course of the disease may allow earlier detection of relapse and indicate the need for modification of chemotherapy. The ultimate clinical value of terminal transferase measurement in classifying populations of leukemic cells and in predicting long-term prognosis can be established only with further experience.
ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. Scanning electron photomicrographs. A. lymphoblasts from the peripheral blood of a child with acute lymphoblastic leukemia. x 4,000. B. thymocytes from a normal child. x 7,000. C. myelomonoblasts from the peripheral blood of a patient with acute myelomonocytic leukemia. x 4,000. D. monocyte from normal peripheral blood with 2 microvillous-covered lymphocytes in background. x 10,000.
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