

# Absence of Terminal Transferase May Predict Failure of Remission Induction in Childhood ALL

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Two children with acute lymphoblastic leukemia (ALL), whose lymphoblasts lacked terminal deoxynucleotidyl transferase (TdT) by both enzyme and fluorescent antibody assay, responded poorly or not at all to vincristine and prednisone. Both patients had high presenting white counts and mixed L1-L2 morphology. Lymphoblasts from one patient, an adolescent boy with a mediastinal mass, possessed surface membrane receptors for sheep red cells (E) and for complement (EAC) and had elevated adenosine deaminase activity (ADA). Lymphoblasts from a 2.5-yr-old boy without a mediastinal mass did not form E or EAC rosettes and did not express the Ia-like antigen or carry surface immunoglobulin. The poor response to therapy and absence of TdT were associated with a lymphoblast pheno-

type suggestive of a highly differentiated T-cell-derived line in one instance and an undifferentiated cell in the other instance. It is postulated that absence of TdT may predict poor therapeutic efficacy of vincristine and prednisone in acute lymphoblastic leukemia in childhood. The absence of TdT may correlate with other developmental characteristics of lymphoblasts, such as altered function or low numbers of glucocorticoid receptors or resistance to lysis by steroid drugs. Determination of many parameters of lymphoblast phenotype at diagnosis to characterize the nature of the malignant cells more precisely may ultimately enhance our understanding of, and improve therapy for, the group of leukemic children who fail to respond to standard regimens.

**T**ERMINAL deoxynucleotidyl transferase (TdT) is an enzyme whose normal distribution is restricted to cortical thymocytes and a minor population of bone marrow lymphocytes.<sup>1</sup> It is expressed during early stages of development of certain normal thymus-derived lymphocytes and is found in the blasts of most patients with acute lymphoblastic leukemia (ALL) of T- or null-cell type. The virtual absence of the enzyme from normal mature tissues, including lymphoid cells, and its high frequency in lymphoblasts has made it a valuable marker for malignant lymphoid tissues.

In about one-third of patients with chronic myelogenous leukemia (CML) in blast crisis, the malignant cells contain elevated levels of TdT. Presence of this enzyme identifies a subgroup of patients who may have a clinical response to administration of vincristine and prednisone.<sup>2</sup> Although TdT is known to be present in the blasts of about 95% of children with ALL, its expression has never been correlated with the response to traditional induction therapy.<sup>3</sup> We report two children with ALL who presented with extremely high tumor burdens and unusual cell surface marker phenotypes, whose leukemic cells lacked TdT. The patients responded poorly to vincristine and prednisone. Several clinical characteristics at diagnosis have been useful for prediction of long-term prognosis, but no other characteristics of lymphoblasts have predicted patient response to induction therapy. Determination of TdT in leukemic blasts may identify a population of patients with ALL for whom alternate induction therapy may be indicated.

## MATERIALS AND METHODS

Newly diagnosed leukemic patients at Rainbow Babies and Childrens Hospital in Cleveland were studied prior to entry onto

Children's Cancer Study Group protocols for treatment of ALL. Immunologic markers were determined on lymphoblasts isolated by Ficoll-Hypaque density gradient centrifugation from blood and bone marrow by the Diagnostic Immunology Laboratory at University Hospitals. Parameters studied included: complement receptors determined by rosetting with complement-coated sheep erythrocytes (EAC);<sup>4</sup> spontaneous rosetting with sheep erythrocytes (E);<sup>5</sup> surface immunoglobulin by direct immunofluorescence;<sup>6</sup> and surface Ia-like antigen by indirect immunofluorescence (OKIa-1, Ortho Diagnostics, Raritan, NJ).<sup>7</sup> Quantitative determination of TdT activity was performed in the laboratory of Dr. M.S. Coleman at the University of Kentucky using d(pA)<sub>50</sub> as an initiator and <sup>3</sup>H-GTP as a substrate.<sup>1</sup> In addition, fluorescent antibody determination of intracellular TdT was performed using a qualitative method (Bethesda Research Laboratories, Bethesda, MD).<sup>8</sup> Adenosine deaminase activity (ADA) was determined by a radiochemical technique.<sup>9</sup>

Morphological classification of acute lymphoblastic leukemia was done using criteria established by the French-American-British group.<sup>10</sup> Bone marrow smears were classified as L1, L2, a mixture of L1 and L2, or L3 (Burkitt's). Sudan black, periodic acid-Schiff (PAS), peroxidase, and nonspecific esterase (NSE) stains were performed by standard techniques.

## Case 1, T.T.

This 16.5-yr-old white male was admitted to Rainbow Babies and Childrens Hospital on December 12, 1979, with superior vena cava obstruction, massive hepatosplenomegaly, generalized adenopathy, and enlargement of both testes. The hematocrit was 36%, platelet count 175,000, and white count 250,000, with 4% neutrophils, 3%

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*Supported in part by Grant R01-A1 16060 from the National Institutes of Health.*

*Submitted July 16, 1982; accepted January 5, 1983.*

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0006-4971/83/6106-0009\$01.00/0

lymphocytes, and 93% blasts. Chest film showed massive mediastinal lymphadenopathy.

Bone marrow contained 90% lymphoblasts of mixed L1 and L2 morphology by the French-American-British (FAB) classification. These blasts were negative with nonspecific esterase, Sudan black, and peroxidase stains. Surface and enzyme markers are shown in Table 1.

The patient was started on vincristine (2 mg/sq m/dose q wk), prednisone (40 mg/sq m/day), and l-asparaginase (6,000 U i.m. every other day) on a Children's Cancer Study Group protocol. After 6 days of therapy, the white count had risen to 270,000, and there was no relief of superior vena cava obstruction or diminution of hepatosplenomegaly. Cyclophosphamide (1.2 g/sq m/dose) and 600 rad of mediastinal radiation therapy were added, and vincristine, prednisone, and l-asparaginase continued. The bone marrow contained 100% lymphoblasts 27 days after initiation of chemotherapy, and hepatosplenomegaly had not changed. Another dose of cyclophosphamide and 2 doses of adriamycin (60 mg/sq m/dose) were given, and asparaginase was discontinued.

Two months after presentation, high-dose cytosine arabinoside (3 g/sq m/dose every 12 hr for a total of 12 doses) was begun. He developed total marrow aplasia, but hepatosplenomegaly persisted and circulating lymphoblasts reappeared 1 mo later. He died 5 mo after diagnosis, with extensive leukemic infiltration of the bone marrow, liver, spleen, lymph nodes, kidneys, adrenals, and testes at autopsy.

### Case 2, R.C.

This 27-mo-old white boy was admitted to Rainbow Babies and Childrens Hospital on April 18, 1980, with a 3-day history of fever, diarrhea, and pallor. His liver was palpable 2 cm below the right costal margin, and spleen 6 cm below the left costal margin. There was no adenopathy, and chest film showed no mediastinal mass. His hematocrit was 26%, platelet count 52,000, and white blood count 95,800, with 10% neutrophils and 61% lymphoblasts. Serum LDH was 1,470.

The bone marrow contained 75% lymphoblasts of mixed L1 and L2 morphology. The blasts were PAS-positive and negative for nonspecific esterase, Sudan black, and peroxidase. Surface and enzyme markers are shown in Table 1.

The child was begun on vincristine, prednisone, and l-asparaginase on the same schedule and study as the first patient. The marrow was hypocellular with 20% blasts after 4 wk. After 6 wk, the marrow

contained 2% blasts and 5% smudge cells. Peripheral counts were normal, hepatosplenomegaly had resolved, and he began maintenance chemotherapy after receiving 1,800 rad cranial irradiation and 6 doses of intrathecal methotrexate. Bilateral testicular wedge biopsies performed 6 mo after diagnosis demonstrated no leukemic involvement.

Ten months after initial presentation, 9% blasts were noted in his peripheral blood smear. Marker studies, including TdT assay, repeated on bone marrow containing 62% blasts gave identical results as at diagnosis.

Reinduction was accomplished with 2 courses of daunorubicin (30 mg/sq m/day for 3 days) and cytosine arabinoside (100 mg/sq m/day continuous infusion i.v. for 7 days).

Allogeneic bone marrow transplantation was performed in second remission, following high-dose cytosine arabinoside (3 g/sq m/dose every 12 hr for 12 doses) and fractionated total body irradiation (200 rad twice a day for 3 days). He developed graft-versus-host disease and radiation pneumonitis, confirmed by lung biopsy, and died of respiratory complications 3 wk after bone marrow transplant.

## DISCUSSION

Both the patients presented here fell into high-risk categories by all criteria.<sup>11-13</sup> Both had extremely high peripheral blood white counts, mostly lymphoblasts, and both were male. One was less than 3 yr of age, and the other was adolescent with a mediastinal mass. Both had organomegaly, with what may have been largely extramedullary disease, since the presenting hematocrit, platelet count, and absolute neutrophil count were close to normal despite extensive marrow infiltration and massive leukocytosis. Both had a substantial proportion of marrow lymphoblasts with L2 morphology by the FAB classification. None of these factors is thought to predict failure of remission induction, although all are associated with a high incidence of relapse and poor long-term prognosis.<sup>14</sup>

Both these patients had highly unusual cell surface and enzyme marker phenotypes.<sup>3,14</sup> The blasts obtained from T.T., case 1, expressed two T-cell markers (E receptors and elevated adenosine deaminase), but not TdT, a marker of immature T cells. These blasts also carried surface receptors for complement (EAC), which may be present on immature T cells,<sup>15</sup> but is more commonly considered a B-cell marker.<sup>4</sup> Several other patients with leukemia and lymphoma have been found to have blasts possessing both E and EAC receptors.<sup>16-21</sup> Of the reported leukemic patients, none with both markers has done well. Blasts from 2 of 14 reported patients with lymphomas were positive for TdT, E and EAC receptors,<sup>22</sup> so the presence of both E and EAC receptors does not imply that TdT is not also expressed. Ninety-three percent of patients with ALL enter remission within 4 wk on the induction therapy administered to these patients.<sup>23,24</sup> The probability that the failure of both these patients to enter remission was a random event unrelated to their unusual lymphoblast phenotype is less than 0.5%.

**Table 1. Surface and Enzyme Markers**

Feature	Case 1	Case 2	Childhood ALL
slg-positive	0%	0%	1%-3% <sup>11</sup>
la-positive	0%	0%	95% <sup>14</sup>
E rosettes	72%	0%	15%-20% <sup>11</sup>
EAC rosettes	61%	0%	
TdT immunofluorescence	0%	0%	95% <sup>8</sup>
TdT enzyme activity (U/10 <sup>8</sup> cells)	2.91	0.45	9-299 (mean 72) <sup>3</sup>
ADA enzyme activity (nmole/10 <sup>8</sup> cells/min)	828	ND	85-10,689 (mean 1,197) <sup>3</sup>

Values are given for bone marrow lymphoblasts from both of the present cases. For comparison, the percentage of children with ALL whose leukemic cells express these markers and the range of enzyme activities in patients with T- and null-cell ALL are given. To ensure a large comparison population, values are derived from the literature cited. TdT-positive cells are normally less than 1% of peripheral blood lymphocytes, and the enzyme activity less than 5.9 U/10<sup>8</sup> cells.<sup>1</sup> ADA activity in normal lymphocytes is 86-106 nmole/10<sup>8</sup> cells/min.<sup>3</sup>

The blasts from R.C., case 2, had none of the surface or enzyme markers examined. The absence of the Ia-like antigen is associated with a poor long-term prognosis,<sup>15</sup> but has not been shown to predict failure of induction therapy. This child did enter remission after 6 wk of treatment with vincristine, prednisone, and l-asparaginase, but induction was prolonged and remission tenuous at best.

Several studies have confirmed the correlation of the presence of TdT in blasts with the response of patients with CML in blast crisis to therapy with vincristine and prednisone.<sup>2,25,26</sup> Absence of TdT appears to be a reliable predictor of failure to respond and an indication that alternative drugs should be tried.<sup>2</sup> A subgroup of adult patients with diffuse histiocytic lymphoma responded extremely well to therapy and have tumor cells that possess TdT.<sup>22</sup> TdT-positive lymphoblasts are found in 94% of children with null-cell ALL.<sup>3,8,27</sup> Most lymphoblasts that express T-cell surface characteristics (E receptors) also possess TdT. Approximately 95% of patients have Ia-positive lymphoblasts, with the majority of the Ia-negative blasts having T-cell surface characteristics.<sup>14</sup> The incidence of both TdT-negative and Ia-negative lymphoblast phenotype is not known, but should not exceed 0.3% (6% TdT-negative times 5% Ia-negative).

TdT is present during thymic development in fetal and neonatal animals and humans.<sup>28-30</sup> In young mice, the major class of thymic lymphocytes that contains TdT is cortisone-sensitive and immunoincompetent.<sup>23</sup> Dexamethasone treatment of juvenile rats eliminates TdT-positive lymphocytes transiently from blood and liver.<sup>28</sup> Low numbers of glucocorticoid receptors on the

surface of lymphoblasts correlates with poor clinical response to steroid therapy in ALL,<sup>23</sup> but the presence of substantial numbers of steroid receptors does not reliably predict a good clinical response.<sup>31-34</sup> Mature lymphocytes in humans are not steroid-sensitive. Steroid sensitivity may depend on the exact stage of lymphoblast maturation, as well as the ability of the cell to bind the drug. The correlation between the presence of TdT and the response to vincristine and prednisone therapy may be related to functional properties of these lymphoblasts, which are expressed simultaneously with TdT synthesis, rather than to the activity of TdT itself.<sup>35</sup>

The widespread availability of reagents and simplified techniques for identification of surface antigens, glucocorticoid receptors, cytoplasmic immunoglobulin, ADA, and TdT should facilitate examination of all of these parameters in all patients. Data on large populations of patients followed prospectively may permit identification of patients for whom standard therapy is not optimal and encourage design of alternative protocols for these patients. In addition, detailed characterization of malignant lymphoid cells using multiple parameters should provide valuable information about normal lymphocyte ontogeny because of the clonal origin of the lymphoblasts.<sup>36-38</sup>

#### ACKNOWLEDGMENT

We are grateful for the contributions of the following colleagues: Elizabeth M. Kurczynski, M.D., for caring for case 1; Richard Wamsley, M.D., for referring case 2; Glenn Pierce, Ph.D., for performing the assays of adenosine deaminase activity; and Dr. M.S. Coleman at the University of Kentucky for performing biochemical assays of TdT.

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