Clinical Utility of Leukemia Cell Terminal Transferase Measurements

Ronald McCaffrey, Anne Lillquist, Stephen Sallan, Ellen Cohen, and Michael Osband

Abstract

Interest in the DNA-synthetic enzyme terminal deoxynucleotidyl transferase (TdT) has developed from two sets of observations: first, in normal animals, it occurs only in immature thymic lymphocytes and in a subpopulation of bone marrow lymphocytes; second, it is present in the blast cells of almost all patients with acute lymphoblastic leukemia. A prospective trial to evaluate blast cell TdT as a predictor of responsiveness to vincristine and prednisone in 30 Philadelphia chromosome-positive patients with blastic chronic myelogenous leukemia was undertaken. Eleven of 16 TdT-positive patients responded, whereas only one of 14 TdT-negative patients showed improvement. Among TdT-positive patients under the age of 50 years, the response rate was 76%. Enzyme-negative patients under the age of 50 had an 11% response rate. Blast cell morphology (i.e., lymphoblastic versus myeloblastic) had no significant correlation with either responsiveness or TdT activity. These results suggest that blast cell TdT activity may identify leukemic patients who are likely to respond to vincristine and prednisone irrespective of their conventional classification.

Introduction

The development of a variety of new techniques over the last decade to supplement traditional morphology has resulted in remarkable refinements in our ability to stratify patients with leukemia and lymphoma into therapeutically meaningful categories. Thus, for example, lymphoid cancers are now frequently subclassified on the basis of their expression of normal T- or B-cell surface properties on blast cell membranes (44, 45). Although the biological basis of using normal cell phenotypes for the classification of primitive, abnormal cells may be questioned, the clinical utility of such an approach has been repeatedly validated (10, 43).

Our work on the classification of leukemia and lymphoma cells developed from our accidental observation, made originally several years ago, that the blast cells of a child with ALL contained the unique DNA-synthetic enzyme TdT. In this report, we update our work on the significance of TdT expression in leukemia cells. Our data continue to suggest that TdT is of clinical value in the classification of patients with acute leukemia and diffuse lymphoma. We will restrict ourselves in this review to the role of TdT in the classification of acute leukemia.

Original Discovery of TdT

The original discovery of TdT 20 years ago was the result of a fortuitous choice of calf thymus gland as starting material for the purification of eukaryotic DNA polymerases. Krakow and Kamen (26) and Bollum (4) simultaneously reported that calf thymus extracts contained a DNA polymerase which could add deoxynucleoside monophosphates, from their triphosphate precursors, to performed DNA in an end-addition manner. Bollum (5) and his colleagues went on to purify and extensively characterize this polymerase and the name "terminal deoxynucleotidyl transferase" was coined to distinguish it from ordinary replicative deoxynucleotidyl transfersases.

TdT differs from replicative DNA polymerases in that it requires only a single-stranded DNA molecule upon which to initiate synthesis. Replicative polymerases require a double-stranded structure. One strand of the double-stranded structure serves as a template, to direct the addition of monophosphate residues at the 3'-hydroxy end of the other (primer) strand, according to Watson-Crick base pairing (Chart 1). Since TdT requires only a single-stranded structure, it can be unambiguously assayed by providing only single-stranded DNA initiator molecules in the assay system. Under such single-stranded conditions, replicative DNA polymerases fail to function.

Chang (9), working in Bollum's laboratory, reported in 1971 that TdT also had a unique anatomic compartmentalization. She showed that, among a variety of normal hematopoietic and nonhematopoietic tissues from several species, TdT-positive cells occurred only in the thymus gland. [It was later shown (13, 35) that normal bone marrow also contained TdT at a level too low to be detected in the original Chang survey.]

The restricted expression of TdT in normal animals to thymus and bone marrow received little attention from biologists until it was later established that TdT activity was a consistent feature of lymphoblasts from patients with ALL. The discovery of this fact was, like the original discovery of TdT in thymus tissue, also a fortuitous event. It occurred as the result of a 1972 study which asked whether human leukemia cells contained reverse transcriptase, the special DNA polymerase of animal RNA tumor viruses. Working with embryonic mouse bone marrow fibroblasts, we had developed a simple, chromatographic method for the identification of reverse transcriptase in mouse cells infected with Moloney leukemia virus (2). In that system, the synthetic template-primer combination of poly(C)-oligo(dG) showed specificity for the viral enzyme. Under the conditions used, the constitutive cellular DNA polymerases, polymerases α, β, and γ, all fail to function with poly(C)-oligo(dG). We extended this observation to show that all known RNA tumor virus polymerases could use this template-primer combination. We therefore embarked on a study of human leukemia and lymphoma cells to determine whether TdT was present in blasts of patients suffering from acute leukemia and lymphoma.
leukemia for the presence of viral reverse transcriptase, using poly(C)-oligo(dG) as a probe.

Our first patient was a 5.5-year-old child with ALL (33). Her leukemic cells were homogenized and prepared for chromatography using the conditions established in the mouse model system. Fractions from the column eluate were assayed, using poly(C)-oligo(dG) as the template-primer combination.

The 2-peak pattern eluting from the column (Chart 2) when fractions were assayed with poly(C)-oligo(dG) was originally interpreted by us to mean that the child's cells contained RNA tumor virus reverse transcriptase (33). As we began to characterize this enzyme, however, we were surprised to find that it required only a single-stranded, preformed DNA initiator (34). It thus shared with calf thymus TdT the unique ability to polymerize DNA in an end-addition, template-independent manner. A similar activity was present in normal human thymus but not in other cells or tissues assayed (Table 1).

Based on the data from the Chang survey, which had established the restricted expression of TdT in normal animals, we interpreted the presence of TdT in the leukemic cells of our patient to mean that these cells developed from a clone of cells which were normally TdT positive (34). This line of thinking stimulated us to change the focus of our work from a study of reverse transcriptase in leukemic cells to an exploration of the significance of TdT expression in such cells.

**Procedures for the Identification of TdT**

TdT can be identified by biochemical assay, by radioimmunoassay, and by immunofluorescent assay.

For the biochemical assay, a modification of our standard procedure (34, 37) is recommended. Immediately before homogenization, phenylmethylsulfonyl fluoride, a serine protease inhibitor, and ethanol are added to achieve concentrations of 10 mw and 5%, respectively, in order to inhibit proteolytic degradation of TdT, which occurs when samples containing cells rich in proteolytic activity (e.g., phagocytic cells in normal bone marrow, or CML samples) are assayed. After detergent treatment, the crude homogenate is extracted with high salt, which results in more efficient enzyme solubilization (30). TdT activity is then identified following phosphocellulose chromatography of the crude homogenate. We have found direct assays on crude homogenates to be unreliable and have therefore used at least one column purification step for enzyme quantitation. This difficulty with crude extracts has not been experienced by others (11).

The assay involves providing the enzyme source with a radiolabeled deoxynucleotide triphosphate and a preformed DNA primer molecule with a 3'-hydroxy terminus onto which deoxynucleotide monophosphates are polymerized, releasing pyrophosphate. For human TdT, purified either from normal thymus or leukemia cells, a hierarchy of substrate and initiator preferences exists. The combination of dGTP and oligo(dA)₄ is superior to all others (37). We have avoided the use of nicked double-stranded DNA as an initiator with a single triphosphate as substrate, since the ubiquitous β-polymerase, in addition to TdT, can function under such conditions (11). One could therefore overestimate TdT both on a quantitative level and with regard to its absolute presence or absence by using such a relaxed assay system. Manganese is the preferred cation for human TdT in our hands (34, 37).

Rabbits and mice produce specific antibody when immunized i.m. with calf thymus TdT (6, 28). Anti-calf TdT antibody cross-reacts with both human thymic and leukemic TdT but does not recognize replicative DNA polymerases (e.g., retrovirus reverse transcriptase, DNA polymerases α and β) in competitive radioimmunoassays. Only tissues which have biochemically identifiable TdT have detectable TdT antigenicity by competitive radioimmunoassay. In the radioimmunoassay system developed by Kung et al. (28), 5 ng of TdT represented the lower level of sensitivity. The level of enzyme in calf thymus was determined to be 280 ng/mg soluble protein. On a per cell basis, this worked out to be 1 × 10⁶ molecules/calf thymocyte.
This calculation assumes equivalent enzyme expression in all thymocytes, which is probably an invalid assumption (see below). The TdT level in bone marrow was estimated to be 100-fold lower. However, if one calculates bone marrow activity on the basis of only 1 to 2% of the cells being positive (see below), the number of TdT molecules per TdT-positive bone marrow cell approaches the number per thymocyte.

The anti-calf TdT antibody has also been adapted for use in an immunofluorescence assay (3, 14). By immunofluorescence, the assay for TdT is simple, rapid, requires only approximately 10^5 cells as starting material, and allows TdT to be specifically identified in single cells. In the Kung immunofluorescence assay (29), TdT-positive leukemia cells show granular staining of the entire nucleus; frequently, the entire cell appears stained. Bone marrow cells and thymocytes have similar patterns. Bollum and associates have described 2 immunofluorescence assays for TdT. In the first (14), using rabbit F(ab')2 antibody, the enzyme appeared to be present predominantly in thymocyte cytoplasm. In the second (20), using affinity column-purified nondigested antibody, 3 classes of thymocytes were identified: thymocytes with nuclear, thymocytes with cytoplasmic, and thymocytes with total cell fluorescence. In bone marrow cells, the fluorescence was restricted to the nucleus. Leukemia cells were not studied in the Gregoire system. In the Kung system, as noted above, thymocytes, marrow cells, and leukemia cells all show nuclear and cytoplasmic staining. This variation in subcellular distribution in the Gregoire assay may be physiologically significant; alternatively, it may result from technical factors in sample processing.

We have been able to adapt the Kung (29) immunofluorescence system for use in flow cytometry (Fig. 1). With the development of easy to use, relatively inexpensive flow cytometry equipment and commercially available TdT antibodies, analyses of this sort on as few as 1 x 10^4 blast cells could become routine measurements.

Expression of TdT in Human Leukemia Cells

Leukemic cells from almost every patient with ALL contain TdT. The enzyme has been identified in ALL cells from both children and adults, and from patients with null (common) cell ALL, T-cell ALL, pre-B ALL, and pre-T ALL (12, 17, 19, 22). A single case of B-cell ALL with TdT activity has also been reported (46). The 5% of patients clinically classified as ALL in our series were TdT negative have not been reviewed in detail. As a group, however, they had aggressive disease with initial remissions of less than 18 months.

TdT expression is not confined to blast cells from patients who have ALL by the usual clinical and morphological criteria (Table 2). About 5% of patients with AML, as conventionally defined, have TdT-positive blast cells. This subset of AML patients has not been analyzed in detail in terms of other clinical or laboratory features. A much larger subset of blastic CML patients (about 30% in most series) also have blast cells which are TdT positive. Not unexpectedly, about one-half of the cases of acute undifferentiated leukemia are TdT positive. TdT-positive blast cells also occur in patients who develop acute leukemia following several forms of myeloproliferative or lymphoproliferative disease. TdT-positive cells have been observed in some patients who developed acute leukemia following myeloid metaplasia with myelofibrosis, following polycythe-

### Table 2

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<tr>
<th>Clinical diagnosis</th>
<th>No. of cases</th>
<th>No. TdT-positive</th>
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<tr>
<td>ALL</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>AML</td>
<td>70</td>
<td>3</td>
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<tr>
<td>Acute undifferentiated leukemia</td>
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<td>6</td>
</tr>
<tr>
<td>Blastic CML</td>
<td>80</td>
<td>28</td>
</tr>
<tr>
<td>Post-polycythemia vera leukemia</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Post-myeloid metaplasia leukemia</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Post-chemo-radiotherapy leukemia</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Stable-phase chronic myelogenous leukemia</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>B-cell chronic lymphocytic leukemia</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>T-cell chronic lymphocytic leukemia</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Sézary syndrome</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Hairy cell leukemia</td>
<td>7</td>
<td>0</td>
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<tr>
<td>Multiple myeloma</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 1. Flow cytometric analysis of biochemically TdT-positive acute leukemia cells processed through the Kung (29) indirect immunofluorescent TdT assay system. A, flow cytometric analysis of cells incubated with rabbit antiserum to calf thymus TdT and fluoresceinated goat anti-rabbit IgG; B, cells incubated only with fluoresceinated goat anti-rabbit IgG. Pictures are direct polaroid photographs of oscilloscope screen. Ordinate, cell number; abscissa, relative fluorescent intensity; 10,000 cells were analyzed in both experiments using a Biophysics Model 4800A flow cytometer with a 10-milliwatt laser exciting at 4880 Å.
the term "lymphoid" in this nonmorphological way is obviously confusing. We have sought for another term to define the biochemical phenotype with which we are dealing but have been unable to find a suitable alternative to "lymphoid."

A simple inspection of Table 2 shows how such a biochemical definition of "lymphoid" damages traditional morphological concepts. The established categories into which leukemic patients are now classified on the basis primarily of morphology imply a homogeneity which does not, in fact, exist. The revision of certain established concepts may therefore not be without benefit. For example, is it more appropriate to say that if 50% of AML patients achieve a remission on a certain regimen then this represents a 50% response rate for that regimen than is to say that a subset of AML patients (equal to 50% of the entire group) has a 100% response rate? There can be little argument about the need for a classification system which results in homogeneous grouping of patients into therapeutically meaningful categories.

This line of reasoning led us to test our speculation that TdT-positive leukemias are lymphoblastic in nature, irrespective of their conventional classification, in a therapeutic trial in a TdT-positive leukemia which has not been traditionally considered to be lymphoblastic. Blast crisis CML, which is usually considered to be a variant of AML, was selected for study (40). The drug combination of vincristine and prednisone, which results in high initial remission rates in lymphoblastic leukemia and in low rates in myeloblastic leukemia, was selected as therapy (21). Canellos et al. (8) in 1971 had in fact shown that about 30% of patients with blast CML were initially responsive to vincristine and prednisone, a response rate that correlated closely with the overall percentage of TdT-positive cases seen in this disease (Table 2). Our hypothesis predicted that all the vincristine-prednisone responders should fall in the TdT-positive group.

### Blastic CML Trial

A multiinstitutional cooperative clinical trial was established to test our speculation (32). A total of 30 patients with Philadelphia chromosome-positive blast CML have now been studied. Blast crisis was defined by the presence of at least 30% blast cells in the bone marrow or peripheral blood. Two patients had de novo Philadelphia chromosome-positive acute leukemia; the remaining 28 had a well-defined preceding CML in a stable phase, ranging in duration from 8 months to 10 years. Patient ages ranged from 3 to 78 years; 13 were below the age of 50 years. All 30 patients were treated only with vincristine sulfate, 1.5 mg/m² (with a maximum dose of 2 mg), given i.v. each week, and prednisone, 60 mg/m², given p.o. each day. In all cases, at least 2 doses of vincristine were administered before a patient was considered to be nonresponsive. The results of the TdT assays were not known to the physicians caring for each patient until the chemotherapy had been completed. We defined complete response as complete clearing of blast cells from the peripheral blood, return of peripheral blood counts to normal, and return of normal marrow cellularity with less than 5% blast cells. Patients were considered to be nonresponsive if, after a minimum of 14 days of prednisone and 2 doses of vincristine, their peripheral blood or marrow blast cell count were unchanged or increased.

By Wright-Giemsa morphological criteria, 13 of the 30 were lymphoblastic; the remaining 17 were myeloblastic. Sixteen were TdT positive; 14 were TdT negative. (This series is biased toward TdT-positive cases; a random series of 30 blast CML patients would contain approximately 10 TdT-positive cases.)

Responsiveness to the vincristine-prednisone therapy is summarized in Table 3. Only one of the 14 TdT-negative cases responded, whereas 11 of the 16 TdT-positive cases achieved remission. In all of the responders, the responsiveness, in terms of a decrease in absolute peripheral blood blast cells, was evident by the second week of treatment. Three of the TdT-positive responders had "flawed" responses. In one patient, who at the beginning of vincristine and prednisone treatment had a WBC of 72,000 with 30% blast cells, platelets of 6,000/cu mm, and hematocrit of 25%, the blood was cleared of blast cells and the marrow was back to normocellularity with less than 5% blast cells by the fourth week of treatment. However, leukopenia and thrombocytopenia were present for an additional 6 weeks, before the peripheral blood returned to normal. In the 2 other patients, peripheral blood blast cells also cleared by the fourth week, in association with a normocellular marrow, but in both patients a persistent thrombocytopenia developed.

The association of several patient characteristics with responsiveness were analyzed (Tables 4 and 5). TdT positivity alone predicted a 67% response rate (11 of 16 TdT-positive patients responded). Only 1 of 14 (7%) TdT-negative patients responded. When age and TdT status were considered together, 78% of TdT-positive patients under age 50 years responded, whereas only 11% of TdT-negative patients under age 50 responded. Thus, age and TdT status considered together were extremely significant (p < .008). Morphology alone was not significantly predictive for either responsiveness or TdT status, a result which requires emphasis.

The results partially validate our hypothesis that TdT positivity is a "lymphoblastic" phenotype. We are, of course, aware that responsiveness to vincristine-prednisone therapy may be determined by factors unrelated to either TdT positivity or the "lymphoblastic" nature of cells. Nevertheless, we think that this result is a partial validation of our TdT-lymphoblast hypothesis.

A practical consequence of these observations is that TdT

<table>
<thead>
<tr>
<th>Table 3</th>
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<tbody>
<tr>
<td>Responsiveness to vincristine-prednisone therapy in blastic CML</td>
</tr>
<tr>
<td>Cases</td>
</tr>
<tr>
<td>Terminal transferase-negative</td>
</tr>
<tr>
<td>Terminal transferase-positive</td>
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<th>Table 4</th>
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<tr>
<td>Blastic CML study: Morphological characteristics of responders and nonresponders to vincristine-prednisone therapy</td>
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<tr>
<td>Morphology</td>
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<tr>
<td>TdT-positive Responders</td>
</tr>
<tr>
<td>Failures</td>
</tr>
<tr>
<td>TdT-negative Responders</td>
</tr>
<tr>
<td>Failures</td>
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</tbody>
</table>
activity can now be used a priori to recognize the subgroup of blastic CML patients who are most likely to respond to vincristine-prednisone therapy. It is also possible that those other patients with TdT-positive leukemias, which are not usually considered to be lymphoblastic (Table 2), will also be similarly responsive. The antisera to various ALL cells developed by Greaves et al. (18), Kung et al. (27), and Schlossman et al. (27, 42) should also be useful in identifying these same subgroups.

Our interest in the 30 patients in this trial was focused on initial responsiveness to vincristine and prednisone. After the induction of remission, further therapy was at the discretion of the several individual physicians caring for the patients and thus varied from patient to patient. The effect of initial responsiveness to vincristine-prednisone on overall survival was small (median survival of 20+ weeks for responders compared to 12 weeks for nonresponders). It is to be hoped that the development of consolidation and maintenance regimens, which now require our attention, will affect overall survival.

**TdT in Leukemia in Remission**

There exists in normal bone marrow a population of lymphoid cells which express TdT activity. In humans, this normal marrow TdT activity is biochemically indistinguishable from leukemic marrow TdT or from TdT from relapsing ALL (36). By immunofluorescence in the Kung assay (29), the glowing cells in normal marrow are not noticeably different from TdT-positive leukemia cells. As defined by both the biochemical assay and the immunofluorescent assay, the TdT-positive normal marrow cell population can increase dramatically in response to a variety of stimuli, such as febrile illness, nonspecific viral disease, and association with idiopathic thrombocytopenic purpura (19). TdT levels in morphologically normal remission ALL marrow vary markedly. By immunofluorescence, most remission patients show 1 to 2% TdT-positive marrow cells. In a few remission patients, we have noted transient increases to 10 to 15% TdT-positive cells in marrows which have been otherwise normal. Follow-up on these patients for periods of more than 1 year has not shown any prognostic significance to such fluctuation in TdT. Mertelsmann et al. (39), using biochemical assays, also reported elevated bone marrow TdT activity in remission patients. They describe 10 patients with ALL in morphological remission who had marrow TdT levels 3 to 40 times normal, overlapping with levels found at the time of initial diagnosis. With follow-up periods of up to 1 year, no relapses occurred in any of the 34 patients.

In marrow from patients with leukemia in remission, at least 2 classes of TdT-positive cells are potentially present: TdT-positive normal cells; and residual TdT-positive leukemic cells. In assessing a marrow specimen either biochemically, by radioimmunoassay, or by immunofluorescence, the relative contribution of either class of cells to the TdT activity observed is not known at present. Only when marrow is totally replaced by leukemic cells or when leukemic cells are obtained from peripheral blood can the observed TdT activity be termed leukemia associated. It must be stressed that our inability at present to distinguish the TdT of normal marrow from that seen in relapsing patients prevents the use of TdT as a monitor for remission or for predicting relapse.

**Anatomy and Physiology of Normal TdT-positive Cells**

Chang, working in Bollum’s laboratory at the University of Kentucky, was the first to show the unique anatomic compartmentalization of TdT-positive cells (9). She showed that, among a variety of tissues from several species (Table 2), TdT-positive cells occurred only in the thymus gland. Coleman et al. (35) and McCaffrey et al. (13, 37) later showed that normal bone marrow also contained TdT at a level so low that it was not detected in the original Chang survey.

Barr et al. (7) partially characterized the nature of the TdT-positive marrow cells in humans. Using Ficoll-Hypaque density sedimentation and gravity velocity sedimentation sucrose gradients, this group determined that TdT was selectively expressed in small lymphoid cells in the marrow. Later, Silverstone et al. (47) showed that the major fraction of murine TdT-positive marrow cells had prothymocyte characteristics, as defined by Komuro et al. (24, 25).

In the thymus, the majority of TdT-positive cells are recovered in the upper layers of a 17 to 33% discontinuous bovine serum albumin gradient and are thus physically separable from cells capable of mature T-lymphocyte functions (11). In frozen sections of normal rat thymus, Goldschneider et al. (14) have shown that cortical thymocytes fluoresce brightly, while medullary lymphoid cells and thymic epithelial cells fail to stain when exposed to the Goldschneider F(ab')2 anti-terminal transferase immunofluorescent assay system.

These data are consistent with a model which states that TdT positivity develops in primitive marrow lymphoid cells, possibly in cells which are initially capable of either B- or T-cell differentiation. The TdT-positive marrow lymphoid cells leave the marrow and migrate to the cortical thymus, where they begin to differentiate to mature functional T-lymphocytes. As they differentiate, they migrate from the cortical thymus to the medulla, where TdT expression is shut off. No cells exit from the thymus while expressing TdT. The factors regulating TdT expression are unknown; thymic humoral factors are possibly involved.

Migration of TdT-positive cells in neonatal animals have recently been described by Bollum et al. (7). Similar migrations probably occur in older animals but have not yet been documented (38).

Although the physiological function of TdT is unknown, its major limitation to cells obviously committed to T-cell differentiation suggests that it has a role in generating the functional

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### Table 5

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Range</th>
<th>Median</th>
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<tr>
<td>TdT-positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>11</td>
<td>3–78</td>
</tr>
<tr>
<td>Failures</td>
<td>5</td>
<td>23–75</td>
</tr>
<tr>
<td>TdT-negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>1</td>
<td>47</td>
</tr>
<tr>
<td>Failures</td>
<td>13</td>
<td>5–62</td>
</tr>
</tbody>
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5 S. Salian and R. P. McCaffrey, unpublished observations.
properties of T-cells. Baltimore (1) suggested that those specificities of T-cell classes might arise on the basis of somatic mutation. TdT has the properties of an enzyme which would be obligate in any system involving mutations of DNA. It might therefore have such a function in the generation of differentiated, functionally mature T-cells, if such differentiation occurs on the basis of somatic mutation.

The Baltimore suggestion that TdT might serve as a somatic mutator in T-cell differentiation derives from the postulations of Jerne (23) on the generation of immunoglobulin specificities. The evidence is now reasonably compelling that immunoglobulin specificity is generated by repeated mutation of a small number of germ line V genes (31, 48). TdT has not been identified, however, in differentiating B-cells, including cells from the chick bursa of Fabricius studied from the hour of hatching to the eighth week posthatching. It is possible, however, that in mammalian species some of the bone marrow TdT-positive cells are pre-B cells (3, 47). The occurrence of TdT in leukemic cells which have a pre-B cell phenotype supports this possibility (18, 49).

The relationship of the 2 normal TdT-positive cell compartments to TdT-positive leukemic cells is a subject of intense interest. We have hypothesized that neoplastic cells with this enzyme are clonal expansions of one or another of these normal cell populations. Since the factors which regulate the differentiation of prothymocytes to thymocytes and then to mature T-cells (or pre-B cells to B-cells) are presently obscure, one cannot begin to identify a lesion or lesions in the differentiation network that might be responsible for the disordered proliferative state which results in leukemia or lymphoma. Humoral factors are likely to be involved; one can construct models involving either overproduction or underproduction of such putative factors. The thymic epithelial monolayer experiments of Pyke et al. (41) and the work of A. L. Goldstein et al. (15) and G. Goldstein et al. (16) suggest that some might profitably begin to examine the role of these factors as differentiation inducers in TdT-positive cells. The availability of several cultured cell lines which express TdT makes experiments of this sort feasible.

References


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