Solid-Phase Enzyme Immunoassay of Terminal Deoxynucleotidyl Transferase Evaluated
Martin Fiebiger, Reise Stankovic, Delia Schwartz, and Morton K. Schwartz

We evaluated a newly developed solid-phase immunoassay (EIA) of terminal deoxynucleotidyl transferase (TdT, EC 2.7.7.3) and compared it with the enzymatic assay of TdT involving DNA polymerase. We assessed the precision, performance characteristics, and clinical efficacy of the EIA procedure, using 249 specimens of peripheral blood and bone marrow and 118 specimens of whole blood. On linear regression analysis of results for these 249 samples as measured by the two procedures, the correlation coefficient was 0.87. Distribution of TdT in mononuclear cells isolated from whole blood and bone marrow of subjects in several disease categories indicated good concordance between the two assay procedures. The EIA procedure is precise, can be performed on whole blood without first isolating mononuclear cells, is nonisotopic, and shows potential as a quantitative indicator for the differential diagnosis and monitoring of human leukemia.

Additional Keyphrases: bone marrow • leukemia • enzyme activity • reference interval

Terminal deoxynucleotidyl transferase (TdT, DNA nucleotidyltransferase, EC 2.7.7.31; nucleoside-triphosphate:DNA deoxynucleotidyltransferase) is a DNA polymerase that catalyzes end-addition of deoxynucleoside monophosphates to primer DNA. Unlike most DNA polymerases, TdT does not require a template (1–4).

In a study of the classification of leukemia and lymphoma cells, McGaffrey et al. (5) observed that blast cells of a child with acute lymphocytic leukemia (ALL) contained a unique DNA polymerase, which was TdT. By either enzymatic (6–9) or immunocytochemical (6, 10–13) assays for TdT, nonleukemic individuals are seen to have low or undetectable amounts of the TdT in their lymphocytes, whereas greater amounts are generally present in certain leukemias and lymphomas (6–8, 14, 15). The presence of TdT-positive blasts is a useful discriminator between acute lymphoid leukemia and acute myeloid leukemia (6, 11, 15, 17).

TdT activity has been measured by biochemical assays (1, 5, 8, 14), solid-phase enzyme immunoassays (15–20), and immunofluorescence assays (17, 21, 22). The biochemical assay has received the greatest attention, both technically and clinically; extensive modifications in the methodology by several investigators (4, 5, 8, 9, 23–25) have resulted in a lack of standardized assay conditions. The associated differences in reference values have contributed to the complexity of clinical interpretation and concerns as to the clinical efficacy of TdT as a lymphoblastic phenotype.

We have investigated a newly developed solid-phase enzyme immunoassay (EIA) for TdT (Abbott Laboratories, North Chicago, IL), evaluating its precision and comparing its clinical utility with that of the TdT DNA polymerase assay that has been routinely used in our laboratory for more than five years, and verifying reference interval limits for the EIA procedure. Our findings are reported here.

Materials and Methods

Enzyme immunoassay for TdT: All reagents needed to perform this assay are supplied in kit form by Abbott Laboratories. The procedure has been previously described (20) and is summarized in the package insert supplied by Abbott Laboratories.

Subjects: Using the Abbott TdT-EIA procedure, we assayed TdT in 249 blood lymphocyte and bone-marrow samples and 118 whole-blood specimens. Patients under 15 years of age provided approximately 85% of the ALL samples, 50% of the AML samples, and 50% of the normal and nonleukemic blood-disorder samples.

Peripheral blood and bone-marrow specimens for confirmation of reference interval limits were respectively specimens from normal donors to the bloodbank and specimens collected from patients with nonleukemic disorders and sent to the laboratory for purposes other than TdT evaluation. Bone-marrow specimens from normal volunteers were unavailable. We collected 26 whole-blood specimens from normal volunteers and from seven patients with nonleukemic disease. Bone-marrow specimens from eight patients (seven myelodysplasia and one autoimmune disease) with nonmalignant disease were collected and assayed for TdT by EIA.

Isolation of mononuclear cells from bone marrow and peripheral blood: Mononuclear cells (MNC) were isolated from bone marrow and peripheral blood by a standard flotation technique (26). We aspirated the cells from the interface, washed, and resuspended them in isotonic saline (NaCl, 150 mmol/L), then made the cell counts. Details of the MNC preparation and analysis of whole-blood samples are described in the package insert supplied by Abbott Laboratories. Cell preparation for assay of TdT by the enzymatic polymerase procedure has been previously described (8, 9).

Comparison assay: We compared results by EIA with those by the DNA polymerization assay already in use in our laboratory. This assay is based on the method of Mertelsmann et al. (8) but includes an ATP inhibition modification (9). All chemicals were AR grade or better.

Results

Precision: Seven control samples were each analyzed in replicates of five over a four-assay period, i.e., 20 assays of each (Table 1). For TdT in concentrations ranging from 3.0 to 23 μg/L, the within-assay CV was approximately 5%. The between-assay CV ranged from 6.0 to 15.7%.

Reference interval: TdT by EIA and by the biochemical assay with isolated MNC from patients with nonmalignant disease yielded <60 ng per 10^9 MNC and <0.1 U per 10^9 MNC, respectively. For a small group of patient controls

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A preliminary report of this work was presented at the AACC national meeting, Atlanta, GA (abstract: Clin Chem 1985;31:1982).

Nonstandard abbreviations: TdT, terminal deoxynucleotidyl transferase; ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia; MNC, mononuclear cells.

Received September 18, 1986; accepted November 17, 1986.
Table 1. Within- and Between-Assay Precision of EIA for TdT

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control value, ng/mL</th>
<th>Within assay</th>
<th>Between assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD, ng/mL</td>
<td>CV, %</td>
<td>SD, ng/mL</td>
</tr>
<tr>
<td>Panel A</td>
<td>3.0</td>
<td>0.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Panel B</td>
<td>3.3</td>
<td>0.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Panel C</td>
<td>6.3</td>
<td>0.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Panel D</td>
<td>11.3</td>
<td>0.5</td>
<td>4.8</td>
</tr>
<tr>
<td>Panel E</td>
<td>10.7</td>
<td>0.5</td>
<td>4.7</td>
</tr>
<tr>
<td>Panel F</td>
<td>12.2</td>
<td>0.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Panel G</td>
<td>23.4</td>
<td>1.4</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Panels A–E are TdT-containing extracts; panel F and G were prepared by suspending TdT-containing human leukemia cells in whole blood then extracting, n = 20 each.

from whom we had bone-marrow specimens, the TdT concentration (by EIA) was <160 ng per 10⁶ MNC. The observed upper reference interval limits of 50 and 160 ng per 10⁶ MNC for whole blood and bone marrow, respectively (in normal and nonleukemic lymphocytes), are in agreement with previous findings (20). However, the number of patients in our control group was too small for the data to be used in establishing reference intervals. Therefore we used the upper reference interval limits recommended by Abbott Laboratories for whole blood and bone marrow: 100 and 320 ng per 10⁶ MNC, respectively. The upper reference limit of normality for bone-marrow specimens as determined by enzymatic DNA polymerase assay was 0.2 U per 10⁶ MNC.

Correlation of TdT as measured by enzymatic DNA polymerase and EIA: MNC isolated from 142 samples of bone marrow and 107 samples of peripheral blood were analyzed by both the enzymatic and EIA procedures. All points represent average values for duplicate analyses. The overall correlation coefficient for the combined 249 samples was 0.87. Figure 1 indicates the linear regression analysis of the TdT by EIA and enzymatic DNA polymerase assay.

Clinical distribution of data: Table 2 indicates the distribution of TdT (as measured by EIA) in MNC isolated from samples of whole blood and bone marrow of patients in several disease categories. Increased concentrations (>100 ng per 10⁶ MNC) were seen in almost all extracts of blood from patients with active ALL, except for four patients in ALL remission. Only occasionally was TdT increased in acute myelocytic, acute myelomonocytic, or chronic lymphocytic and myelogenous leukemia. In three of 16 patients with lymphoma, the TdT exceeded 100 mg per 10⁶ MNC. For all 28 specimens from normal individuals, TdT concentrations were 100 ng per 10⁶ MNC. TdT in bone-marrow samples from patients with newly active (diagnosed or relapsed) ALL was increased; in most patients with CML none was detected, as was also the case for 20 of 22 patients with lymphoma.

Figure 2 shows values for TdT, by both the polymerase assay and EIA, in MNC isolated from peripheral blood and bone marrow, respectively, in various diseases. By either assay, nine of 10 patients with active ALL showed increased values for TdT in peripheral blood. In patients with ALL and CML, at various stages of disease, there was excellent concordance between the two procedures, as also was true in general for TdT in bone marrow.

Discussion

TdT, a normal constituent of hematopoietic cells, has been utilized as a specific intracellular marker for immature lymphocytes and is a useful diagnostic and management aid for various types of leukemia (7, 10, 25, 27). High concentrations of TdT have been reported in peripheral blood lymphocytes, as well as in the marrow of patients with ALL (7, 11, 12, 14, 16). Increases have also been noted in patients with CML in blast phase and in approximately 10% of patients with acute myelocytic leukemia (23, 28, 29).

Bollum (1) first demonstrated the non-template-directed DNA polymerase activity of TdT. Over the years, numerous modifications of the extraction and assay procedure have been made, but have not totally eliminated the technical difficulties involved in specimen handling, enzyme extraction, and isolation of radiolabeled polynucleotide. The lack of standardization of assay techniques and conditions is related to the latter difficulties, the interassay CVs for the enzymatic DNA polymerization procedure with thymic extracts being two- to threefold that of the EIA. It is this...
isolated polymerase

The increases of TdT measured by EIA in the bone marrow of two of nine patients with acute myelomonocytic leukemia and in one of six whole-blood specimens, are in accord with previous reports (11, 12). It has been suggested that the relatively small number of patients in this subset may actually represent an aberrant expression of myeloid markers or a mixed lymphoid myeloid leukemia (37). Similar observations have been made regarding the small number of patients with lymphoma (2/22 bone marrow and 3/16 whole blood) who had increased concentrations of TdT. Murphy and Jaffe (32), underscoring the controversy involving TdT-positivity in lymphoma, suggested that the presence of TdT may represent a useful marker for the diagnosis and staging of lymphoblastic lymphoma.

Our evaluation of the newly developed solid-phase immunoassay, in comparison with the TdT enzymatic DNA polymerization assay, demonstrates the clinical utility and technical acceptability of the EIA procedure. The limitations of the enzymatic DNA polymerase assay are several: inadequate standardization, the use of radioactive substrate, marginal precision, and the requirement for isolation of MNC from peripheral-blood specimens. The EIA procedure is quantitative and reproducible, does not involve isotopes, permits the use of positive and negative quality-control materials that can be stored frozen for long periods (20), and can be performed on whole-blood samples. We conclude that the TdT-EIA procedure is a useful analytical tool for the differential diagnosis and monitoring of human leukemia.

References

9. Bhalla RB, Schwartz MK, Modak MJ. Selective inhibition of terminal deoxynucleotidyl transferase (TdT) by adenosine ribonucleoside triphosphate (ATP) and its application in the detection of