Use of Terminal Deoxynucleotidyl Transferase in the Diagnosis of Leukemia

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ABSTRACT

Terminal deoxynucleotidyl transferase (TdT) was determined by immunofluorescence in 30 patients with leukemia. In acute lymphocytic leukemia the proportion of cells positive for TdT was 19 to 77 percent during relapse (12 cases) and less than one percent during remission (3 cases). In seven cases of myeloproliferative disease and two cases of lymphoma, the TdT was less than one percent. In one case of generalized lymphoblastic lymphoma and five cases of chronic myelocytic leukemia with “lymphoblastic” crisis, the cells positive for TdT were moderately increased. The presence of TdT in blast cells appears to have diagnostic, therapeutic, and prognostic significance.

Introduction

Terminal deoxynucleotidyl transferase (TdT) activity was discovered in calf thymus gland during isolation studies of deoxyribonucleic acid replication enzymes. It is now known that the purified enzyme has a molecular weight of approximately 58,000 daltons and consists of a single polypeptide chain.

The enzyme, TdT, catalyzes polymerization of deoxynucleoside triphosphates and does not require a template, unlike the replicative deoxynucleotidyl transferases. However, TdT does require an initiator molecule containing a free 3' hydroxyl group to which the 5' deoxynucleotides are added. While the enzyme has no proven role, it is found only in immature lymphocytes and is thought to be involved with the immune process, possibly by producing diversification of antigen receptors in T and B cells.

Its specific activity can be measured using tritiated guanosine triphosphate and polydeoxyadenosine with at least three residues as initiator. The nanomoles of tritium are measured after incorporation into trichloroacetic acid precipitable material.

Functional enzyme activity, found in different locations such as thymus, bone marrow and spleen, correlated well with enzyme localized cells by fluorescent antibody studies.

Fluorescent antibody, specific for TdT, has demonstrated localization of the enzyme in the majority of cortical thymo-
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cytes and in some prothymocytes in spleen and bone marrow. There is also evidence that TdT is present in some pre B cells. In rodents during late embryonic development and shortly after birth, postthymic TdT positive cells can be found transiently in the peripheral blood.

A number of authors have reported that TdT is a useful marker in the classification of leukemic cell lines. Our experience has been reviewed in 30 patients seen over a one year period in which TdT assay by immunofluorescent antibody was carried out.

Materials and Methods

Patients were included in this report if they had TdT measured as part of their diagnostic workup for lymphoproliferative or myeloproliferative disease.

Terminal deoxynucleotidyl transferase was determined by immunofluorescent staining technique, and differential counts were performed. Bone marrow and peripheral blood were treated with 0.1 M NH₄Cl to lyse erythrocytes, and the mononuclear fraction was spread on a glass slide by cytocentrifuge. In a few cases, smears of cerebrospinal fluid cells were also prepared (red blood cell lysis was not required). All smears were fixed and maintained at room temperature in a dessicator for 48 to 72 hours before staining.

Rabbit antibody* to bovine TdT (15 μl) was layered over the cytocentrifuge smear and incubated for 30 minutes. The slides were washed three times in phosphate buffered saline (PBS) and 15 μl fluorescein tagged goat antirabbit IgG was added for 30 minutes. The slides were again washed three times in PBS and mounted with FA mountf at pH 9.5 for counting. The TdT positive cells were expressed as a percent of mononuclear cells in the preparation.

Results

Twelve patients with acute lymphocytic leukemia (ALL) by morphologic criteria showed TdT positive staining with 18 to 77 percent of the cells being fluorescent. Three patients studied during remission showed less than one percent TdT positive cells. Eight patients with chronic myelocytic leukemia (CML) in blast crisis were observed. In three, myeloblasts predominated and the TdT activity was not observed. The other five had blasts with lymphoid characteristics and demonstrated eight percent to 80 percent TdT positive cells. Only one patient showed less than 10 percent positive cells.

Four patients with myeloproliferative disorders and two patients with lymphoma showed less than one percent TdT positive cells. One patient with generalized lymphoma showed 10 percent TdT positive cells in the bone marrow.

One patient with CML illustrated the value of the TdT test in determining therapy. A 15-year-old white male, JP,

<table>
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<th>Diagnosis</th>
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<th>M/F</th>
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<tr>
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<td>26</td>
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<tr>
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<td>19-26</td>
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<td>36-65</td>
<td>2/2</td>
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*Acute lymphocytic leukemia
†Chronic myelocytic leukemia
‡Acute nonlymphocytic leukemia

* A kind gift of Dr. F. Bollum, Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD.
† Difco Laboratories, Detroit, MI.
developed Ph¹ chromosome positive chronic myelocytic leukemia in late 1979 and responded to busulfan and hydroxyurea. In mid 1981, he experienced an episode of blast crisis with white blood cells (WBC) 95,900 (22 percent blasts, and a predominance of myeloblasts, myelocytes, and promyelocytes) while TdT was negative. The patient showed a gradual response to cytosine arabinoside (ARAC). In January 1982, he again developed a blast crisis with WBC 109,000 (30 percent blasts of which 65 percent were TdT positive. He showed a partial response to vincristine and prednisone with WBC decreasing to 2,400 with eight percent TdT positive cells in February. In March, his white count increased to 232,000 with 84 percent TdT positive cells in the peripheral blood. Again, he showed a partial response to vincristine and prednisone.

A second patient, SG, a 16-year-old white male, presented with bone pain and a WBC of 38,000 with 14 percent blasts. Bone marrow showed many blasts of the L₁ type. He responded initially to the usual therapy for lymphoid leukemia. Three years later he showed relapse in marrow and cerebrospinal fluid (CSF) with larger blasts suggesting L₂ lymphoblasts or myeloblasts. In addition, TdT was present in 30 percent of the blasts. He was treated with vincristine, prednisone, cyclophosphamide and daunomycin as well as intrathecal methotrexate. The CSF did not clear completely nor did the bone marrow show remission until he was given intrathecal ARAC and hydrocortisone, as well as systemic ARAC, thioguanine and L-asparaginase.

While the bone marrow remained in remission, the patient showed two more CSF relapses, one year and one-and-a-half years later. Thirty to 50 percent CSF cells were TdT positive. At each remission, the patient responded to intrathecal ARAC and hydrocortisone.

A third patient SS, showed evidences of a mixed variety of leukemia and was a 17-year-old white male who presented with fever and pneumonia. The marrow showed 64 percent blasts which appeared to be myeloblasts and were TdT negative. He failed to achieve remission on daunomycin, ARAC, prednisone and vincristine. He was sent home for terminal care on low dose (0.7 mg per kg) of ARAC. He developed marked megaloblastic changes after two months and, supported with transfusions, attained complete remission. Three months later he showed a relapse marrow with 20 percent blasts being TdT positive, six percent positive for common acute lymphocyte leukemic antigen (CALLA), and chromosomal abnormalities in 37 percent of blasts including 5p translocation to chromosome 15 and deletions of 6q and 12p.

Discussion

It has now been amply demonstrated that TdT arises in the early cells of the lymphoid series. With few exceptions, leukemic cells which are TdT positive have other lymphocyte markers.

In some patients, particularly children, with undifferentiated acute leukemia, the finding of TdT activity in the blasts may allow reclassification as ALL and appropriate treatment. Vogler described patients in whom the blast cells contained TdT in the nucleus and intracytoplasmic IgM, suggesting they were pre B cells. Other studies have demonstrated that typical B Cell ALL is negative for TdT. Lymphocytes from patients with infectious mononucleosis and mitogen stimulated lymphocytes are also negative. Reactive lymph nodes and non lymphoblastic lymphomas are usually TdT negative. Some confusion exists about the cell of origin of rare cases of acute undifferentiated leukemia where morphology and special stains (peroxidase) indicate acute myelocytic leukemia (AML),
yet TdT activity is found in greater than 10 percent of the blasts.

Two such cases were found in one series of 40 patients with AML. In one case, auer rods were present. Similarly, Grogan reported three patients with morphologic features of AML with azurophilic granules and punctate nonspecific esterase activity who had positive blasts for TdT. Only one of the three attained a complete remission. The patient was a 14-year-old girl who showed poor response to daunorubicin and ARAC, but developed complete remission after vincristine, prednisone, and Adriamycin.

One of the other two patients was a 38-year-old female who died 40 days after the start of vincristine, prednisone, and L-asparaginase. She had a hypoplastic marrow and died of a fungal septicemia. The last patient was a 33-year-old male who had Ph chromosome positive AML and showed a partial response to Adriamycin, vincristine, ARAC, prednisone and later hydroxyurea and 6-mercaptopurine. He did not attain remission and died 11 months after diagnosis.

These were the only three cases observed with myeloid features out of 45 patients with ALL observed at two major clinics.

Therapeutic implications exist because TdT not only identifies blasts as probably lymphoid in nature, but also because TdT positive cells have been shown to be sensitive to steroids while TdT negative stem cells appear to be resistant. The TdT positive cells have been observed to regenerate rapidly from pluripotential stem cells in rats. Thus, it seems likely that TdT positive leukemias may respond to steroid treatment even when the morphology appears myeloid.

Janossy reported that even blast crisis in CML responded to steroids and vincristine if the blasts showed a large proportion with ALL markers. Thus, TdT was an important aid in establishing whether or not the blasts were lymphoid. There was a positive response by 14/15 patients, while 21/25 patients who were CALLA negative, and TdT negative failed to respond to prednisone and vincristine. In his series, two patients had myeloblasts morphologically, but had ALL and TdT markers; however, four patients had lymphoblasts but were ALL and TdT negative. Response to therapy seemed to correlate with markers rather than with morphology, but the numbers were small. These results are consonant with those of Marks and McCaffrey but disparate with Srivastava et al.

Ross et al reported that loss of TdT activity in one patient appeared to herald the emergence of resistance to chemotherapy and suggested TdT might be a marker for chemotherapy sensitivity. While these observations may reflect the difference between the lymphoid blast cells and myeloid blast cells, Bertazzoni et al recently reported that a substantial series of patients with Ph positive for CML morphologically and TdT positive blasts showed a better prognosis (on the average, six months longer life) than similar patients with TdT negative blasts. The TdT positive patients were treated with vincristine and prednisone while the TdT negative patients were treated with 6 thioguanine and ARAC. Bertazzoni and coworkers suggest, as did Bradstock et al that TdT could also be expressed in non lymphoid cells. This may indicate a common precursor cell for CML cells and some types of lymphocytes.

Indeed, TdT has become an important marker in the leukemias. It is helpful when the proportion of positive cells is high, because this most often signifies lymphocytic origin of the cells and a sensitivity to vincristine and prednisone. While further work is required to clarify the importance of TdT positive cells in myelogenous leukemia, it seems clear that the presence of such cells, particularly in chronic myelogenous leukemia...
blastic phase indicates a better prognosis than in TdT negative cases.

References