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Methods and Clinical Relevance of Terminal Deoxynucleotidyl Transferase Determination in Leukemic Cells*

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A. Summary

Terminal deoxynucleotidyl transferase (TdT) is a unique DNA polymerase which is only found in immature cells of lymphoid lineage (pre-T/pre-B). Because of this restricted distribution of TdT, biochemical and immunofluorescence techniques have been employed to determine the distribution of TdT phenotypes in human leukemias and lymphomas, showing high levels of TdT in ~95% of acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LBL), ~50% of patients with acute undifferentiated leukemia (AUL), ~10% of patients with acute nonlymphoblastic leukemia (ANLL), and ~30% of patients with chronic myeloid leukemia (CML) and other myeloproliferative (MPS) or myelodysplastic (MDS) syndromes in blast crisis. High levels of TdT activity are associated with a clinical response to remission inducing therapy with vincristine and prednisone in a high proportion of patients (50%–90%), irrespective of clinical and morphologic diagnosis. Preliminary studies furthermore suggest that TdT might serve as a sensitive indicator of subclinical disease in ALL in complete remission.

B. Introduction

Terminal deoxynucleotidyl transferase is a unique DNA polymerase which adds deoyribonucleotides onto an appropriate primer molecule in the absence of any directing template polynucleotide (Bollum 1979). Under physiologic conditions TdT is restricted to thymocytes and to a subpopulation of bone marrow lymphocytes which exhibit characteristics of immature T (Incefy et al. 1980; Silverstone et al. 1976) or B cells (Janossy et al. 1979, 1980; Vogler et al. 1978). All other cell types, including myeloid, erythroid, and putative pluripotent stem cells, do not exhibit detectable TdT activity (Mertelsmann et al. 1979a). Because of the restricted distribution to early developmental stages of lymphocytes, which loose TdT activity during final maturation into circulating cells, it has been speculated that TdT might play a role in the generation of immunologic diversity (Bollum 1979). Figure 1 depicts a schematic model of human hematopoiesis and the cell types to which TdT appears restricted under physiologic conditions.

Since the first report by Kung et al. (1978) about high levels of TdT in ALL, this enzyme has been widely employed in the phenotypic analysis of hemopoietic neoplasias (Bollum 1979; Donlon et al. 1977; Gordon et al. 1978; Janossy et al. 1980; Kung et al. 1978; Mertelsmann et al. 1978a,b, 1979a,b, 1981; Modak et al. 1980; Sarin et al. 1976; Vogler et al. 1978). This report reviews current technologies for demonstration of TdT and its significance in the clinical and pathophysiologic evaluation of human leukemias and lymphomas.

C. Techniques for Demonstration of TdT

Several biochemical methods have been developed to quantitate TdT activity in human tissues, yielding qualitatively similar results. In order to be able to study routinely obtained

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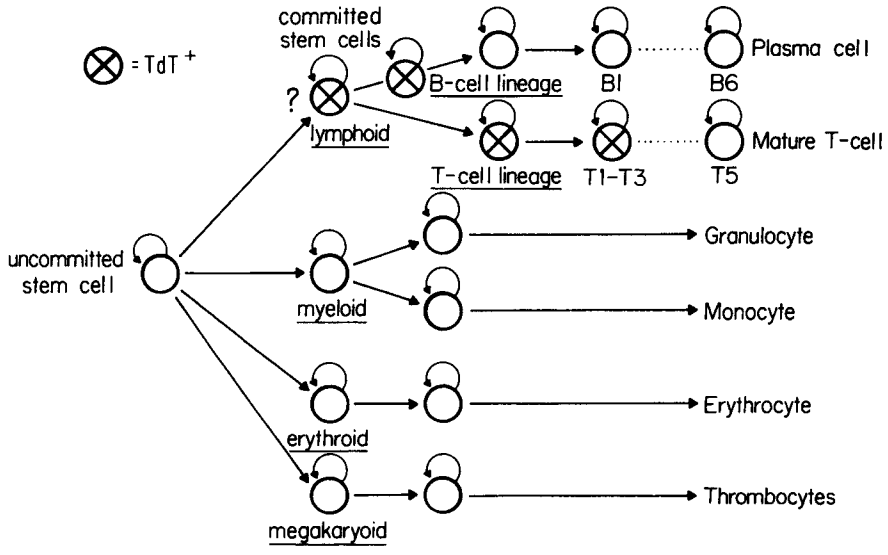

Fig. 1. Schematic model of human hematopoiesis

blood (~5 ml) and marrow specimen (~1 ml), we have recently developed a biochemical microassay which is highly sensitive and specific for TdT in human blood cells (Modak et al. 1980).

Bollum (1979) and Kung et al. (1978) reported the successful preparation of specific antisera against homogeneous calf thymus TdT, which recently also have become commercially available, including a monoclonal anti-calf thymus TdT antibody (BRL Biochemicals, Bethesda, Md). Using anti-TdT antibody and indirect immunofluorescence, TdT in bone marrow cells appears predominantly in the nucleus, while thymocytes reveal predominantly cytoplasmic fluorescence (Bollum 1979). However, more sophisticated fixation techniques preserving cellular ultrastructure are required for the definitive subcellular localization of TdT (Steinmann et al. 1981). Newer techniques for analysis of TdT distribution in human blood cells include application of flow cytometry (unpublished work) and of metabolic labeling techniques for quantitation of newly synthesized TdT molecules in a given cell suspension (unpublished work). All techniques for demonstration of TdT have yielded almost identical results regarding the distribution of TdT in hemopoietic tissues and neoplasias. All observations will therefore be reviewed together, without further reference to the particular techniques employed.

Table 1. TDT activities in acute leukemias and in lymphomas. Total No. studied: 1000

Clinical diagnosis	% TdT+
Acute leukemias	
ALL	
non-T, non-B	94
T	85
pre-B	80
B	0
ANLL	9
AUL	55
Lymphomas	
LBL	96
Burkitt's	0
DPDL	0
DHL, DML	8
NPDL, NML, NHL	0
DWDL (CLL)	0
Hodgkin's	0
IBLA	0

D. Distribution of TdT in Human Leukemias and Lymphomas

I. Acute Leukemias

In acute leukemias (Table 1) the highest levels of TdT activity are found in ~95% of "non-T, non-B" and T cell ALL. ALL of the TdT negative [TdT(−)] T cell type probably represents leukemic or lymphomatous proliferation of a more mature T cell, while the exact phenotype and physiologic equivalent of TdT-negative non-T, non-B ALL remains to be determined (Mertelsmann et al. 1979a). Recently, a previously unrecognized ALL phenotype exhibiting intracytoplasmic μ chains

("pre-B" phenotype) has been described which was found to be associated with high levels of TdT in ~80% (Janossy et al. 1980; Vogler et al. 1978). The absolute level of TdT in ALL lymphoblasts has not been found to correlate with clinical features or prognosis. Most leukemias exhibiting high levels of TdT activity appear to be sensitive to vincristine and prednisone, including the TdT(+) "non-B, non-T", "T", and the "pre-B" phenotypes (Janossy et al. 1980; Vogler et al. 1978).

Elevated levels of TdT have also been observed in some patients with ANLL (Bollum 1979; Gordon et al. 1978; Mertelsmann et al. 1978a, 1979a). In our own cases with 10% overall incidence of TdT(+) phenotypes in 300 cases of ANLL (unpublished), more extensive phenotypic analysis revealed evidence of involvement of more than one cell lineage in the leukemic process in approximately one-half of these TdT(+) ANLL cases, leading to the concept of bi- or polyphenotypic leukemias (Mertelsmann et al. 1978a, 1979a) (Fig. 2). In other TdT(+) cases mostly carrying a diagnosis of AUL with equivocal morphology and cytochemistry only the TdT assay allowed cell type identification (Gordon et al. 1978). The exact phenotype can sometimes not be determined even when employing multiple marker techniques including TdT assays and could represent "nonlymphoid, nonmyeloid" stem cells, e.g., early megakaryoblasts and erythroblasts, which require even more sophisticated techniques for diagnosis (Mertelsmann et al. 1979a,b). Recently, we have been able to show correlation between elevated TdT activity and production of T cell growth factor (TCGF, Interleukin 2), suggesting a specific common regulatory abnormality in all TdT(+) human leukemias irrespective of clinical diagnosis (Mertelsmann et al. 1981).

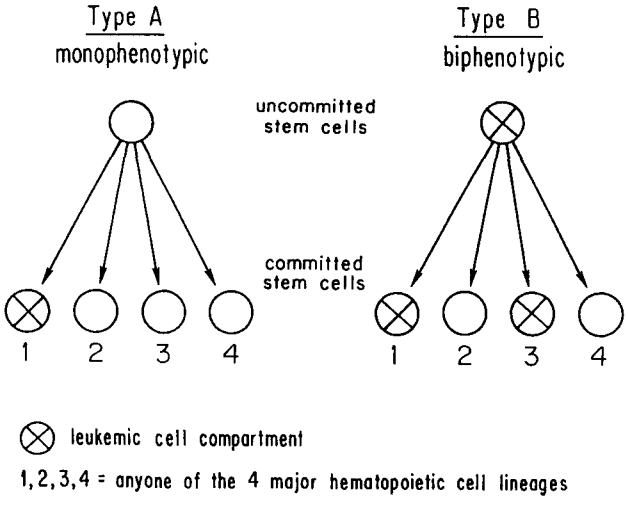

Fig. 2. Hypothetical pathogenetic types of human leukemia

II. Myelodysplastic and Myeloproliferative Syndromes

All myeloproliferative syndromes (MPS: P. vera, CML) and myelodysplastic syndromes (MDS: RAEB, CMMOL) were found to be TdT(−) during the chronic phase of their disease, while approximately 30% of blast phase CML and 15% of other MDS and MPS terminating in acute leukemia were found to exhibit high levels of TdT activity (Table 2). It is of clinical significance that the majority of TdT(+) acute leukemias preceded by MPS or MDS have also responded to therapy with vincristine and prednisone (data not shown, see Mertelsmann et al. 1979b).

The phenotype in MDS and MPS in blast phase appears to be labile with changes in the

Table 2. TdT activities in myeloproliferative (MPS), myelodysplastic (MDS), and lymphoproliferative (LPS) syndromes. Total No. studies: 300

Clinical diagnosis	%TdT+
MDS/MPS chronic phase	
CML	0
CMMOL	0
RAEB	0
Aplastic anemia	0
MDS/MPS acute phase	
CML	30
CMMOL	10
RAEB	10
P. vera	30
Aplastic anemia	10
LPS	
CLL	
- B	0
- T	10
Prolymphocytic leukemia	0
Hairy cell leukemia	0
LSA-leukemia	0
Mycosis f., Sezary's s.	5
Multiple myeloma	0
Waldenstroem's mg	0
Cold agglutinin s.	0

predominant cell type occurring either spontaneously or after therapy, while this appears to be more unusual in de novo ALL or ANLL (unpublished work).

III. Lymphoproliferative Disorders

Recent studies by Donlon et al. (1977), Kung et al. (1978), and ourselves (Mertelsmann et al. 1978b, 1979a) have demonstrated the clinical significance of TdT determinations in patients with non-Hodgkin's lymphoma (Table 1). In patients with lymphoblastic lymphomas of the T and "null" cell type high levels of TdT were observed as seen in ALL. Because a definitive histologic diagnosis is sometimes difficult to make in this group of patients we have often found TdT determinations providing important diagnostic information. Lymphoproliferative diseases of the mature T and B cell type have been found to be TdT(−) (Table 1). Although diffuse histiocytic lymphomas, which are diagnosed on the basis of the presence of large cells resembling histiocytes, generally represent B-cell proliferations, a few cases of "true" histiocytic lymphomas with cells exhibiting phagocytic properties and more recently lymphomas of the null cell type have been described which exhibited high levels of TdT in involved tissue (Gordon et al. 1978; Mertelsmann et al. 1978b). Although it is too early to determine the significance of these marker studies for the response to therapy and long-term prognosis in this group of patients, all patients with lymphoid neoplasias with high levels of TdT studied by us had a characteristic clinical course resembling that of ALL.

IV. TdT Determinations in the Monitoring of Disease Activity in TdT+ Neoplasia

We have reported significantly elevated TdT levels in ALL bone marrow in complete remission on and off therapy as compared to normal controls (Mertelsmann et al. 1978b). The TdT levels characteristically fluctuated, which could not be explained by technical problems or chemotherapy. A preliminary analysis of our own data suggests an increased risk of relapse in patients with persistently elevated TdT values in CR. The clinical and biologic significance of elevated TdT levels in ALL in patients in long-term remission off chemotherapy remains to be analyzed; if indicative of residual disease, this observation would be important for the understanding of the pathophysiology of leukemias and for the design of therapeutic strategies.

E. Conclusion

From our own studies and work by published others it appears that determination of TdT activity and definition of cell phenotypes in hematopoietic malignancies are useful tools for the classification of these disorders and have significant prognostic, clinical, and pathogenetic implications. Although several questions still remain unsolved, this comprehensive approach, when correlated with clinical presentation and conventional morphology, might help the physician by providing objective diagnostic criteria for prognostic subgroups unidentifiable by conventional methods. Furthermore, this approach will help to understand the underlying pathogenetic processes leading to the clinical syndromes of human leukemias and lymphomas.

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