TERMINAL DEOXYRIBONUCLEOTIDYL TRANSFERASE ACTIVITY IN ACUTE UNDIFFERENTIATED LEUKEMIA

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SUMMARY

High levels of terminal deoxyribonucleotidyl transferase (TdT) were found in and partially purified from leukocytes obtained from a patient with acute undifferentiated leukemia. The majority of cells lacked lymphoid cell surface markers, suggesting that the disease may have originated from a popoulation of primitive lymphoid progenitor cells. Phosphocellulose chromatography resolved two peaks of TdT activity, and further studies revealed that a) the two forms of TdT possessed identical molecular weights, b) oligonucleotide primer and substrate preferences for the two enzyme forms were identical to those of TdT from calf thymus, and c) DNA polymerase γ was detected as a contaminant in TdT fractions.

The identification of terminal deoxyribonucleotidyl transferase (TdT) activity (1) in leukocytes from childhood patients with acute lymphoblastic leukemia (ALL) was first reported by McCaffrey et al (2). Further studies confirmed this observation, and also indicated that up to 50% of patients with chronic myelogenous leukemia (CML) in "blast crisis" contain TdT in peripheral leukocytes (3-5); however, this finding is probably due to the presence of lymphoid elements in such cell populations (5). Low levels of TdT activity have been reported in leukocytes from patients with acute myelogenous leukemia (6), although some controversy exists on this point (5). In this communication, we report the detection of high levels of TdT activity in leukocytes obtained from an adult patient with acute undifferentiated leukemia. Cell surface marker studies revealed that the vast majority of the leukemic cell population lacked lymphoid cell markers. This report represents the first identification of TdT activity in such cells, and suggests that, in the absence of other markers, TdT may be used to identify those leukemias having a clonal origin in a primitive lymphoid progenitor cell. We have partially purified TdT activity from these cells and have resolved two fractions by phosphocellulose chromatography. The comparative biochemical properties of the two TdT fractions, with respect to reaction requirements, primer preference, and molecular weight, are also reported in this communication.

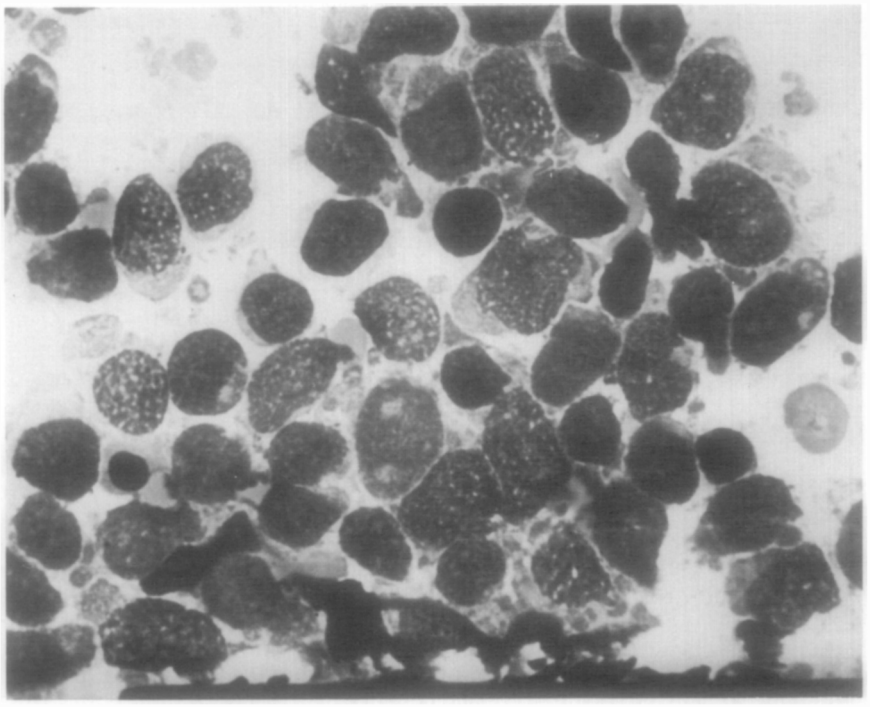

Figure 1. Field showing undifferentiated leukemic blasts from peripheral blood of the acute undifferentiated leukemia patient which were used in this study.

MATERIALS AND METHODS

Source and Nature of Leukemic Cells. The patient was a 26 year-old male who presented with a peripheral blood leukocyte count of 128,000/mm^3 with 86% blast forms. Figure 1 shows a representative field from a blood smear indicating the undifferentiated nature of these cells. The large blast forms contained no granules, no Auer rods, and were peroxidase negative. The diagnosis reached from these studies was one of undifferentiated leukemia. The white blood cells were collected by leukophoresis using an IBM cell separator and the buffy coat was made 10% in glycerol (v/v) and stored at -70°C. Cells from patients with ALL, CML, or acute myelogenous leukemia (AML) were obtained as peripheral blood or leukophoresed samples and stored in the same manner.

Cell Surface Markers. E rosette formation was assayed using the method of Bentwich et al (8). Cells bearing surface immunoglobulins were detected by immunofluorescence as previously described by Siegal et al (9). The presence of the high-affinity Fc receptor on cell surfaces was determined by the method of Froland and Natvig (10).

DNA Polymerase Assays. TdT activity was assayed using a total volume of 0.1 ml containing the following components: 50mM Tris-HCl, pH 7.8, 10mM dithiothreitol (DTT), 0.6mM $MnCl_2$, 0.01% (wt/vol) bovine serum albumin, 0.5 µg $(dA)_{12-18}$, and 10µM (^{3}H)-dGTP at a final specific activity of 1000 cpm per picomole. Primers, template-primer combinations, and substrate deoxynucleoside triphosphates were purchased from P-L Biochemicals, Inc. (^{3}H)-deoxynucleo-

side triphosphates were purchased from New England Nuclear, Inc., and Amersham-Searle, Inc. One unit of TdT activity is defined as that amount catalyzing the incorporation of 1 nmole of (^{3}H)-dGMP into trichloroacetic acid insoluble material per hour at 37°. Specific activity is defined as the number of enzyme units per 10^8 nucleated cells. Background levels of incorporation averaged 25 cpm. DNA polymerase activity other than TdT was assayed using $(rA)_n.(dT)_{10}$ as template primer. The reaction mixture used, in a 0.1 ml volume, was identical to that described above except for the use of 0.5mM $MnCl_2$, 1mM DTT, 0.1M KCl, 0.5 µg $(rA)_n.(dT)_{10}$ and 20µM (^{3}H)-dTTP as substrate. TdT assays were incubated at 37°C for 30 mins., while $(rA)_n.(dT)_{10}$-directed synthesis was carried out at 27°C for 30 mins. Reactions were begun by the addition of reaction mixture to enzyme fractions, and trichloroacetic acid-insoluble radioactivity was collected on glass fiber filters and measured in a liquid scintillation counter as previously described (11).

Molecular Weight Estimation. Velocity sedimentation was performed in 5 ml preformed 10-30% glycerol gradients in 0.05M Tris-HCl buffer (pH 7.8) containing 1mM DTT and 0.4M KCl. Column fractions were diluted in the same buffer used to prepare the gradients, layered over the gradient, and centrifuged for 18 hours at 45,000 rpm at 4°C in the SW50.1 rotor. Fractions were collected from the bottom of the tube.

RESULTS

TdT in Leukemic Cells. Crude extracts of leukemic cells were obtained by detergent-treatment of freeze-thawed samples as previously described (5). Extracts were centrifuged at 150,000 x g for one hour at 4°, and the supernatant applied at a flow rate of 18 mls/hour to a 0.9 cm X 10 cm. phosphocellulose column which had been previously equilibrated with buffer containing 0.05M Tris-HCl, pH 7.8, 1mM EDTA, 0.1mM DTT, 20% glycerol (v/v), and 0.1% (wt/vol) bovine serum albumin. Enzyme activity was eluted with a 0-1M KCl linear gradient in the same buffer. Figure 2 shows representative elution profiles of TdT activity in column runs using cells derived from a patient with acute undifferentiated leukemia and from adult patients with ALL, CML or AML. Two peaks of TdT activity were resolved in those cases yielding positive results, the first (peak I), eluting at 0.28-0.30M KCl, and the second (peak II) eluting at 0.4-0.45M KCl. Re-chromatography of peaks I and II on phosphocellulose using 0-0.5M KCl gradients pinpointed their elution at 0.28M KCl and 0.4M KCl, respectively. Incorporation of substrate was found to be completely primer- and divalent cation-dependent. Cells obtained from the patient with acute undifferentiated leukemia contained extremely high levels of TdT activity (2.4 units per 10^8 cells). This value was 5 to 10 times that which we found to be present in circulating cells obtained from adult patients with ALL. We have not observed levels of TdT in adult ALL cells above 0.5 units per 10^8 cells. When an equivalent amount of calf thymus was processed in an identical manner, a single peak of activity was observed eluting at 0.34M KCl (data not shown). Levels of TdT activity in calf thymus varied from 1.5-2.8 units per 10^8 cells

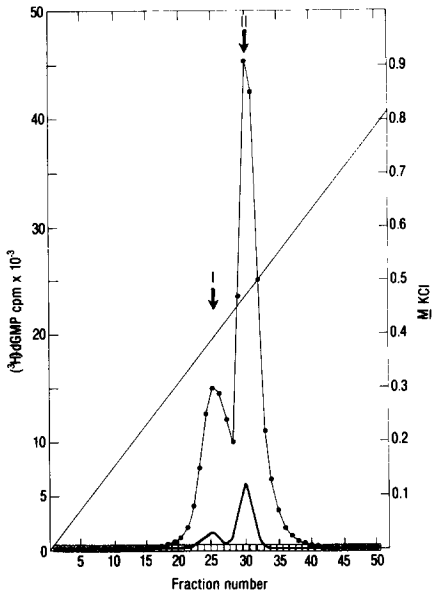

Figure 2. Comparison of TdT activities obtained from 1 x 10^9 cells obtained from: (●——●), a patient with acute undifferentiated leukemia, (——) representative results from 2 adult ALL patients, and (□—□) one patient with CML and one patient with AML. Cells were extracted and columns equilibrated and run as described in Materials and Methods. Twenty µl of each column eluate fraction (fraction volume = 1 ml) was assayed.

(thymocytes). We were unable to detect TdT activity by the above procedure in cells obtained from a patient with AML and from one patient with CML in "blast crisis." Thus the level of TdT found in cells from a patient with undifferentiated leukemia was equivalent to that found in calf thymus and is also within the range of activity previously reported for normal human thymocytes (2,5). Cell surface marker studies were carried out on the cells from the patient with acute undifferentiated leukemia. The cells were negative for E rosette formation (8) and cells bearing surface immunoglobulins (9) were also found to be absent. Fourteen percent of the cellular population possessed Fc receptors (10). These results were interpreted as suggesting that the vast majority of cells from this patient carried no B- or T-cell lymphoid surface markers, and that the acute leukemia was of an unknown type (Dr. F. P. Siegal, personal communication).

Properties of TdT from Peaks I and II. The biochemical characteristics of TdT from acute undifferentiated leukemic cells were studied and compared with those of the calf thymus enzyme. Both forms of the enzyme from leukemic cells were

TABLE 1

Substrate and Primer Preference of Human Leukemic Cell and Calf Thymus Terminal Transferases

Primer	Enzyme Source	Substrate dTTP	dATP	dCTP	dGTP
$(dA)_{12-18}$	PC I	7.2	4	38	100
	PC II	6	4	27	100
	CT	6	3	29	100
$(dC)_{12-18}$	PC I	2	2	9.5	41
	PC II	2	1.5	8	42
	CT	2	1.5	10	44
$(dG)_{12-18}$	PC I	2.5	3	6	20
	PC II	2.5	3.2	5	17
	CT	3	2.5	6	19
$(dT)_{12-18}$	PC I	<1	<1	1	20
	PC II	<1	<1	1	10
	CT	1	1	1.5	18

Assays were carried out as described in Materials and Methods, with the various appropriate primers and tritiated substrates substituting for $(dA)_{12-18}$ and (^3H)-dGTP. PC I and PC II correspond to TdT activities from acute undifferentiated leukemia cells eluting at 0.28M KCl and 0.4M KCl, respectively, from phosphocellulose columns. CT designates calf thymus TdT activity eluting as a single peak at 0.34M KCl from similar columns. All activities were normalized to the values observed for $(dA)_{12-18}$ with dGTP as substrate, which was taken as 100 percent activity. Actual picomole incorporation for 100% activities were: PC I = 30; PC II = 60; CT = 60.

inhibited by 5mM N-ethylmaleimide and showed identical reaction requirements. The possibility that TdT in each of the two peaks obtained from phosphocellulose column runs might demonstrate different primer or deoxynucleoside triphosphate preferences was examined. The experiment shown in Table 1 clearly

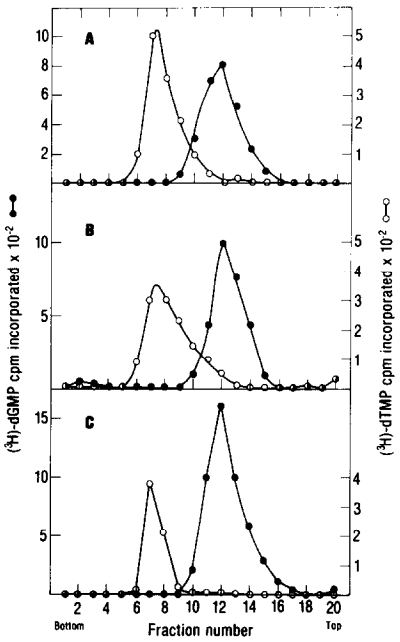

Figure 3. Glycerol gradient centrifugation of (A) PC I 0.28M KCl TdT peak fraction; (B) PC II 0.4M KCl peak TdT transferase fraction; (C) 0.34M KCl calf thymus TdT peak fraction from phosphocellulose column runs. Centrifugation was carried out as described in Materials and Methods. Twenty-five μl of each gradient fraction was assayed for TdT (●——●) and $(rA)_n.(dT)_{10}$-directed DNA synthesis (○——○) as described in Materials and Methods.

illustrates that peak I and peak II TdT possess virtually identical preferences for primer and substrate molecules, and that calf thymus TdT also exhibits these same preferences. For all three enzyme fractions, dGTP was the preferred substrate, and $(dA)_{12-18}$ the preferred primer molecule. The pattern of primer and substrate preference remained constant after re-chromatography of individual peak activities.

Although the biochemical characteristics of peak I and II TdT activities appeared identical, it was possible that differences in molecular weight or subunit structure might have been responsible for the separation of enzyme activities in phosphocellulose column eluate fractions. Molecular weight estimates of TdT activity from peak I and II were determined by velocity sedimentation studies with calf thymus TdT as a molecular weight marker (Fig. 3). The calf thymus enzyme from phosphocellulose column fractions and from homogeneous preparations sedimented through glycerol gradients in an identical manner with a sedimentation value of 3.7S (1). Both peak I and peak II leukemic cell TdT

activities sedimented at a rate identical with that of the calf thymus enzyme.

A DNA polymerase activity in phosphocellulose eluate fractions which could utilize $(rA)_n.(dT)_{10}$ as template-primer was found to overlap with TdT peaks I and II (data not shown) and could be separated from TdT on glycerol gradients. Assays of glycerol gradient fractions using this template-primer combination revealed a single peak of activity sedimenting at 5.6S, consistent with a molecular weight of approximately 110,000 daltons (Fig. 3). This enzyme preferred $(rA)_n.(dT)_{10}$ to activated DNA as a template, and was completely inhibited by 5mM N-ethylmaleimide. On the basis of these observations, this DNA polymerase activity was identified as DNA polymerase γ (12,13). DNA polymerase γ was also found to be present in calf thymus TdT preparations obtained in the same manner (Fig. 3C).

DISCUSSION

We have found extremely high levels of TdT in cells from an adult patient with acute undifferentiated leukemia. Previous studies have tended to stress the correlation of TdT activity with lymphoid cell populations, primarily from childhood ALL (2,5,14,15), although high levels of enzyme have been found in cell samples from some patients with CML in blast crisis (3-5). The levels of TdT present in undifferentiated leukemic cells were 5 to 10 times those observed in cells obtained by us from adult ALL patients, and equivalent to levels found in calf thymus. Equally high levels of TdT were previously reported in cells from a patient with a tentative diagnosis of acute myelomonocytic leukemia (6), but such findings are difficult to interpret due to the lack of cell surface marker studies and the presence of lymphoblasts in peripheral blood samples; acute lymphoblastic leukemia was not ruled out as a possible diagnosis.

The absence of cell surface markers in the undifferentiated leukemic cells used in this study suggests the absence of a lymphoid population which could be a source of TdT activity as previously reported (14). Our results indicate that those leukemic cells containing high levels of TdT but which lack morphological or cell surface markers characteristic of lymphoid cell differentiation may represent a population of multipotential lymphoid stem cells or primitive lymphoid cells. TdT may, therefore, prove to be an important marker for identifying such lymphoid cell populations which are refractory to diagnosis by the morphologic or immunologic procedures currently in use.

We have also described the separation of TdT activity from acute undifferentiated leukemic cells into two peaks by phosphocellulose column chromatography. This finding is in agreement with results obtained using ALL cells

and human thymocytes reported by McCaffrey et al (2,5,14). However, we have also investigated the possibility of differences between the two apparent forms of TdT activities with respect to primer and substrate preference and molecular weight estimations. In spite of their reproducible separation on phosphocellulose column chromatography, both forms of TdT demonstrated identical requirements, primer, and substrate preferences. They also possessed identical molecular weights as determined by velocity sedimentation studies. Calf thymus TdT exhibited properties which were essentially identical to those of the human enzymes.

A recent report (7) has indicated that the two forms of TdT differ in their distribution among mouse thymocyte subpopulations and that phosphocellulose peak II enzyme concentrations are greatly decreased by cortisone treatment, suggesting a functional difference in the two enzyme forms. While our studies indicate that the two forms of TdT from human leukemic cells possess similar biochemical properties, the possibility that they may differ functionally requires further investigation.

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