High Terminal Deoxynucleotidyl Transferase Activity in Acute Myelogenous Leukemia

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SUMMARY

Although leukocytes from all 13 acute lymphoblastic leukemia patients examined had high terminal deoxynucleotidyl transferase (terminal transferase) activity (20 to 100 units/mg of cellular DNA, where 1 unit equals 1 nmole of nucleotide polymerized in 1 hr) and those from 21 acute myelogenous leukemia patients had low terminal transferase activity (0.2 to 2 units/mg of cellular DNA), the bone marrow and peripheral blood leukocytes from 2 patients with acute myelogenous leukemia, diagnosed on the basis of clinical features and the morphology, cytochemistry, and cytogentic features of the leukemic cells, had terminal transferase activity (39 to 52 units/mg of cellular DNA) equivalent to that found in leukemic lymphoblasts. These results bring under question the specificity of high terminal transferase activity outside of the thymus as a marker for leukemic lymphoblasts and, secondarily, the derivation of acute lymphoblastic leukemia cells in all cases from thymocytes. Perhaps malignant transformation in a pleuripotent stem cell with derepression of the genome for terminal transferase could account for high terminal transferase activity observed in certain leukemic cells.

INTRODUCTION

Terminal transferase3 catalyzes the polymerization of deoxyribonucleotide on the 3'-hydroxyl ends of oligo- or poly-deoxyribonucleotide initiators in the absence of a template (1). Although low activity of this enzyme can be found in phytohemagglutinin-stimulated normal lymphocytes, thymus-independent cell lines of normal and malignant origin (7–9), normal bone marrow (3, 5), and leukocytes from patients with chronic lymphocytic leukemia, CML, and AML (3, 6–8), high activity of terminal transferase has thus far been found only in thymus (3, 5, 7), ALL cells (2, 5–8), and thymus-dependent (T-) cell lines of ALL origin (6, 7, 9). The only other situations where high terminal transferase activity has been reported include a case of acute myelomonocytic leukemia where ALL was not ruled out (3) and certain rare cases of CML in blastic phase (6), in which the blast cells resembled lymphoblasts in appearance. These findings have suggested that high activity of terminal transferase outside of the thymus could serve as a marker for leukemic lymphoblasts (5, 8). In this study we report high activity of this enzyme in leukocytes from 2 patients with AML whose blasts were characterized as myeloblasts on the basis of morphological and cytochemical features (4).

MATERIALS AND METHODS

Patient H. H. The patient (H. H.) was a 70-year-old male who had splenomegaly and hepatomegaly but no evidence of thymic enlargement. Initial laboratory values were: hemoglobin, 8.6 g/100 ml; leukocyte count, 71,900/cu mm with a differential of 31 segmented cells, 2 stab cells, 3 promyelocytes, 7 lymphocytes, and 57 blasts; platelets, 72,500/cu mm. There were 9 nucleated RBC/100 peripheral blood leukocytes. A bone marrow aspirate showed a differential of promyelocytes, 12.4; myelocytes, 1.2; metamyelocytes, 1.8; stab cells, 2; segmented cells, 4.8; erythroid precursors, 12; lymphocytes, 4.2; and blasts, 61. Wright’s Giemsa-stained blasts in both peripheral blood and bone marrow (Fig. 1, A and B) showed abundant light blue cytoplasm with some granulation and nuclei containing several prominent nucleoli. Cytochemical studies on bone marrow using standard procedures (4) showed 86% of the marrow cells positive for peroxidase (Fig. 1C), 82% positive for Sudan black (Fig. 1D), 10% of marrow cells (erythroid precursors) positive for nonspecific esterase, and 34% positive for chloroacetate esterase. Blasts and erythroid precursors were negative for periodic acid-Schiff stain, whereas differentiated granulocytes were positive. Alkaline phosphatase reaction on the few mature granulocytes present in bone marrow was negative. Cytogenetic studies on the bone marrow cells showed a normal chromosome constitution, thus ruling out the possibility of blastic transformation of Philadelphia chromosome-positive CML. The patient was started on remission induction chemotherapy but died 1 week later due to congestive heart failure.

Patient I. P. The patient (I. P.) was a 67-year-old female who had no organomegaly. Initial laboratory values were: hemoglobin, 11.2 g/100 ml; leukocyte count, 130,000 cu
mm with a differential of 4 segmented cells, 4 stab cells, 1 monocyte, 4 promyelocytes, 5 myelocytes, 10 lymphocytes, and 72 blasts; platelets, 102,500/cu mm. A bone marrow aspirate showed a differential of promyelocytes, 5;4; myelocytes, 4;2; metamyelocytes, 0;2; stab cells, 1;4; segmented, 2;2; erythroid precursors, 2; lymphocytes, 3;8; and blasts, 81. Wright’s Geimsa-stained blasts in both peripheral blood and bone marrow had the same appearance as those from Patient H. H. (Fig. 1). Cytochemical studies on bone marrow showed 26% of the marrow cells positive for peroxidase and Sudan black, 6% of the marrow cells (erythroid precursors and histiocytes) positive for nonspecific esterase, and 24% positive for chloroacetate esterase. Blasts were negative for periodic acid-Schiff stain. Cytogenetic studies on the bone marrow by direct examination and following culture showed 46 chromosomes with pseudodiploid mode; Philadelphia chromosome was not detected. The patient was started on adriamycin and arabinosyl cytosine but died 1 month later without achieving complete remission. Only bone marrow from this patient was available for study.

To bone marrow aspirate or leukocyte-rich blood (obtained by leukapheresis) 0.1 volume of 6% dextran was added, and the RBC were allowed to settle for 2 hr at 37°. Leukocytes from leukocyte-rich plasma were recovered by centrifugation at 800 × g for 10 min and washed 6 times with phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 8 mM Na2HPO4, 1.5 mM KH2PO4, 0.5 mM MgCl₂, and 0.7 mM CaCl₂), pH 7.2, prior to enzyme extraction.

The procedures for the extraction of cells with Buffer A (25 mM Tris-sulfate, pH 8.3; 1 mM MgSO₄; 6 mM NaCl; 4 mM dithiothreitol; and 0.1 mM EDTA), separation of soluble (30,000 × g supernatant) and chromatin fractions, and the solubilization of terminal transferase from chromatin (extraction of chromatin with 1 M NaCl followed by dialysis of the extract to 0.15 M NaCl to precipitate DNA-histone and leave terminal transferase in the supernatant) have been described (7, 8). Similarly, the procedures for the precipitation of terminal transferase and DNA polymerases from soluble and solubilized chromatin fractions by 70% saturation with ammonium sulfate and their fractionation on 10 to 30% glycerol gradients prepared in Buffer A containing only 0.1 M NaCl [which separates 3 to 4 S terminal transferase from 7 S DNA polymerase α (10) and γ and 3 to 4 S DNA polymerase β which aggregate to dimeric forms], have also been described (7, 8).

Quantitative estimation of terminal transferase activity in the samples was made by summing the activity on the glycerol gradients for both soluble and chromatin fractions. Estimates of DNA polymerase activity on the whole-cell homogenates (representing DNA polymerase α and β activity) and on 30,000 × g supernatant from it (representing essentially DNA polymerase α) were also made. Terminal transferase or DNA polymerase activity in the sample was expressed as units/mg of cellular DNA, where 1 unit equals 1 nmole of nucleotide polymerized in 1 hr. DNA in the sample was determined as described elsewhere (8).

Additional characterization of terminal transferase from peripheral blood leukocytes from H. H. was carried out by column chromatography and by examination of some of its properties. Thus, the terminal transferase containing fractions from glycerol gradients for the chromatin [which represent > 80% of the total terminal transferase activity under our extraction conditions (7, 8)] was pooled, dialyzed against starting buffer, and chromatographed sequentially on DEAE-Sephadex A-25 (9) and phosphocellulose (7) columns.

The reaction mixture for the assay of terminal transferase contained 50 mM Tris-HCl, pH 7.5; 100 mM KCl; 5 mM dithiothreitol; 0.5 mM MnCl₂; bovine serum albumin, 200 μg/ml; (dA)₁₂₋₁₈, 50 μg/ml; 10 μM [³H]dGTP (specific activity, 14 Ci/mmmole); 90 μM cold dGTP, and the enzyme in a final volume of 0.1 to 0.2 ml. The reaction mixture for the assay of DNA polymerase contained 50 mM Tris-HCl, pH 8.3; 6 mM magnesium acetate; 20 mM dithiothreitol; activated calf thymus DNA, 60 μg/ml (7); dATP, dCTP, dTTP, and [³H]dGTP (specific activity, 14 Ci/mmmole), 100 μM each; and enzyme in a final volume of 0.1 to 0.2 ml. After 30 min incubation at 37°, 50 μg of yeast RNA and 1 ml of 10% trichloroacetic acid containing 3% sodium pyrophosphate were added. The precipitates were collected on nitrocellulose membrane filters, washed with 5% trichloroacetic acid, dried, and counted in a scintillation counter (7). Dithiothreitol, (dA)₁₂₋₁₈, [³H]dGTP, and other nucleotides were purchased from Sigma Chemical Co., St. Louis, Mo., P-L Biochemicals, Milwaukee, Wis., Schwarz/Mann, Orangeburg, N. Y., and Worthington Corp., Freehold, N. J., respectively.

RESULTS AND DISCUSSION

Terminal transferase from leukocytes of Patient H. H. on glycerol gradient centrifugation (Chart 1) gave a sedimentation coefficient similar to that of ovalbumin marker (3.6 S). In addition, its chromatographic behavior on DEAE-Sephadex A-25 and phosphocellulose columns was the same as reported for terminal transferase from other human cells (6, 7, 9). As in ALL cells and T-cell lines of ALL origin (7-9), about 80% of the terminal transferase activity in bone marrow or peripheral blood leukocytes from Patient H. H. and bone marrow from Patient I. P. was found in the chromatin fraction, whereas the rest was present in the soluble fraction.

Terminal transferase from glycerol gradients or from chromatography fractions required an oligodeoxynucleotide initiator for activity (oligodeoxyadenylate > oligodeoxycytidylate > oligodeoxyguanylate > oligodeoxythymidylylate) and it could use any one of the deoxymibonucleoside triphosphates as the substrate (dGTP > dATP > dCTP > dTTP) (data not shown). With [³H]dGTP as the substrate and activated DNA (7) as the initiator, 90 to 95% reduction in [³H]dGMP incorporation was noted on adding unlabeled dATP, dCTP, and dTTP to the terminal transferase assay mixture. The terminal transferase also preferred Mn²⁺ (0.5 mM) over Mg²⁺ (5 mM), showed no inhibition by 0.25 M NaCl, but was strongly inhibited by 10 mM N-ethylmaleimide or 5 mM sodium pyrophosphate, and was completely destroyed on heating for 5 min at 50° (data not shown). In all the above properties, the terminal transferase from AML cells of Patient H. H. resembled the enzyme from ALL cells (7, 9). Lastly, the antibodies to thymus terminal transferase prepared in our laboratory, which inhibited (> 80%) terminal

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terminal transferase obtained after phosphocellulose chromatography was 263 units/mg of protein. Chart 1. Glycerol gradient centrifugation, DEAE-Sephadex A-25 chromatography, and phosphocellulose chromatography profiles of chromatin fraction terminal transferase from an AML patient (H. H.). Aliquots (0.5 ml) of the enzyme preparation (equivalent to 0.56 mg cellular DNA) were layered onto 4.6 ml of linear 10 to 30% (w/v) glycerol gradient prepared in Buffer A containing 0.1 M NaCl. The gradients were centrifuged at 40,000 rpm for 16 hr at 2°C in a Spinco SW50.1 rotor, and 12-drop fractions were collected by puncturing the bottom of the tube. Terminal transferase peak fractions from 2 glycerol gradients were pooled, dialyzed against Buffer B (50 mM Tris-HCl, pH 7.5; 20 mM KCl; 1 mM dithiothreitol, and 20% glycerol) and then loaded on a column (1.1 x 10 cm) of DEAE-Sephadex A-25 equilibrated with Buffer B. After a brief wash with Buffer B, the column was eluted with a linear gradient formed with 25 ml of Buffer B and 25 ml of Buffer B containing 0.2 M KCl. Fractions for DEAE-Sephadex A-25 column containing terminal transferase were dialyzed against Buffer A containing 10% glycerol (Buffer C) and loaded on a phosphocellulose column (1.1 x 8 cm) equilibrated with Buffer C. After a brief wash with Buffer C, the column was eluted with a linear gradient formed with 50 ml of Buffer C and 50 ml of Buffer C containing 0.7 M NaCl. Aliquots (30, 30, and 100 μl, respectively) from glycerol gradient, DEAE-Sephadex A-25 and phosphocellulose column fractions were assayed for terminal transferase activity as described under "Materials and Methods." The specific activity of terminal transferase obtained after phosphocellulose chromatography was 263 units/mg of protein.

found to be equally inhibitory to terminal transferase from AML cells of Patient H. H.

High terminal transferase activity in ALL cells (2, 5–8) and low (3, 6, 8) to undetectable (5) activity in AML cells have been found in this and other laboratories. This has led to the possibility that high terminal transferase activity outside of thymus may be a marker for leukemic lymphoblasts (5, 8). Although we have always found high terminal transferase activity in bone marrow or peripheral blood leukocytes from ALL patients (20 to 100 units/mg cellular DNA), in other laboratories, we also have found high levels in an occasional patient whose blasts cells were morphologically and cytochemically not classifiable as either clear-cut ALL or AML. However, Patient H. H. who clearly had AML (Fig. 1), showed high terminal transferase activity both in bone marrow (39 units/mg cellular DNA) and peripheral blood (47 units/mg cellular DNA) which was of the same order of magnitude as found in ALL cells. Similarly, the bone marrow from AML Patient I. P. also had high terminal transferase activity (52 units/mg cellular DNA).

The DNA polymerase activities (units/mg DNA) in the whole-cell homogenate (representing DNA polymerase α and β) of peripheral blood and bone marrow of Patients H. H. and bone marrow of I. P. were, respectively, 58, 83, and 71 units, and those found in 30,000 g supernatant (essentially representing DNA polymerase α) in the above samples were, respectively, 54, 77, and 66 units. The difference between the 2 values gives the approximate amount of DNA polymerase β activity in the above samples. The values for terminal transferase and DNA polymerase in cells from Patients H. H. and I. P. are comparable to activity of these enzymes found in ALL cells (8) or T-cell lines of ALL origin (9). DNA polymerase activity in cells from Patients H. H. and I. P. is, however, also comparable to that found in cells from those AML patients who have low terminal transferase activity. Thus far, we have examined bone marrow and peripheral blood leukocytes from 23 cases of AML, and, except for Patients H. H. and P. I., the rest had low terminal transferase activity (0.5 to 2 units/mg cellular DNA). The only other exception has been an acute myelomonocytic leukemia patient (A. G.) (where bone marrow was only available) who also had high terminal transferase activity (28 units/mg cellular DNA). Whereas the frequency of AML having high terminal transferase activity would be known only by examination of a larger patient population, the results presented here demonstrate the occurrence of hematologically diagnosed AML with low and high terminal transferase activity. The presence of high terminal transferase activity in leukocytes from AML patients also brings under question the specificity of high terminal transferase activity outside of thymus as a marker for leukemic lymphoblasts and, secondarily, the derivation of ALL cells in all cases from thymocytes. The apparent conversion of CML to ALL reported by Sarin et al. (6) and 1 such case found in this laboratory, and the conversion of ALL to AML in another of our patients (unpublished data), together with the high terminal transferase activity observed in ALL and certain cases of AML, suggest the possibility that malignant transformation in a pluripotent stem cell with derepression of the genome for terminal transferase could account for all of the above phenomena.

transferase from calf thymus, Molt-4, and ALL cells but not DNA polymerase α, β, and γ from these cells,* were also

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REFERENCES


Fig. 1. Wright's Giemsa staining of peripheral blood (A) and bone marrow (B) cells and the cytochemical staining of bone marrow cells for peroxidase (C) and Sudan black (D) from Patient H. H.
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