Evidence for Distinct Lymphocytic and Monocytic Populations in a Patient With Terminal Transferase-positive Acute Leukemia


Two distinct cell populations with lymphoblastic and monocytic characteristics were separated and characterized by multiple cell markers in a patient with terminal transferase-positive acute leukemia. The clinical course and sequential cell marker studies were consistent with the interpretation of a defect at the level of a common stem cell giving rise to a terminal transferase-positive lymphoblastic cell population at diagnosis and, following initial therapy, a terminal transferase-negative monocytic population.

Terminal deoxynucleotidyl transferase (TdT), a DNA polymerase with unique biochemical properties, has attracted interest as a possible diagnostic marker enzyme for normal and neoplastic cells of T cell lineage. In all vertebrate species tested under physiologic conditions this enzyme appears to be restricted to the thymus and a small subpopulation of bone marrow cells shown to be thymopoietin responsive.

High activities of TdT were later found in virtually all patients with acute lymphoblastic leukemia (ALL), in approximately 30% of patients with chronic myeloid leukemia (CML) in blast phase, and in some patients with leukemic diffuse poorly differentiated lymphocytic lymphoma, as well as in cell lines of T cell lineage derived from a variety of human and animal lymphoproliferative disorders. In general, no or very low levels of TdT activity were observed in normal phytohemagglutinin-stimulated lymphocytes, in B cell-related tissues and neoplasms, and in leukocytes from patients with acute myeloid leukemia (AML), acute myelomonocytic leukemia (AMML), and chronic phase CML. N-alkaline phosphatase has recently been shown to be a unique marker enzyme for lymphoproliferative disorders of T and B cell lineage, allowing a biochemical classification of the leukemias into lymphoid and nonlymphoid categories.

This report describes a patient with an acute monocytic leukemia by morphology, cytochemical findings, and high serum and urinary lysozyme concentrations who also had high levels of TdT in peripheral blood and bone marrow cells as well as N-alkaline phosphatase activity in peripheral blood plasma at the time of diagnosis. Cell separation studies were carried out, and the cells
were characterized by multiple functional, biochemical, and surface markers at diagnosis and then sequentially during remission-inducing therapy in order to analyze the significance of high levels of TdT in some patients with "non-lymphoid" leukemias.4,13,14

MATERIALS AND METHODS

Patient. The patient was a 33-yr-old (Asian) Indian female who presented with generalized adenopathy, fevers and chills, and pregnancy of 6 mo duration. Physical examination showed enlarged tonsils, gingival hypertrophy, an ulcerated infection in the right submandibular area, bilateral cervical, axillary, and inguinal lymphadenopathy, and splenomegaly (4 cm below the left costal margin). Hemoglobin was 10.1 g/dl, platelets 68,000/cu mm, with 4% polymorphonuclear granulocytes, 32% monocytes, 23% lymphocytes, and 41% blast cells. The bone marrow smear showed a hypercellular marrow with 2.5% myeloid cells, 9% lymphocytes, 2.5% erythroid cells, 10% monocytes, 2% plasma cells, 15.5% intermediate lymphocytes, and 58.5% blast cells. Approximately two-thirds of the blast cells in the bone marrow were lymphoid in appearance (round nucleus, coarse chromatin, prominent nucleolus, high nuclear/cytoplasmic ratio) while the rest had predominantly monocytoid features (larger blasts, more basophilic cytoplasm, nuclear indentations). The majority of the blast cells showed trace to 2+ very fine PAS-positive granules. Sixty percent of bone marrow cells and 49% of peripheral blood cells were positive for the α-naphthyl esterase stain, while all of the blast cells were negative for Sudan black, ASD esterase, and peroxidase stains. Serum lysozyme level was 75 μg/ml (normal ≤8), urine lysozyme 336 μg/ml (normal ≤2). Cytogenetic analysis of the bone marrow showed a normal chromosome pattern. A lymph node biopsy from the right cervical region showed a diffuse replacement by immature mononuclear cells consistent with a diagnosis of acute leukemia. There was no evidence of mediastinal enlargement.

Cell preparations and cell surface marker analysis. Mononuclear cells were separated from heparinized peripheral blood or bone marrow by Ficoll-Hypaque flotation.15 Adherent cells were separated by passage through nylon wool columns. After incubation at 37°C for 60 min in 20% fetal calf serum, the nonadherent cells were eluted from the column by washing with Hanks' balanced salt solution (HBSS) at room temperature. The adherent cells were recovered by repeated washings with calcium- and magnesium-free HBSS and agitation of the nylon mesh with a glass rod.

A fluorescein-conjugated polyvalent rabbit antiserum to human immunoglobulin was obtained from Hyland Laboratories, Costa Mesa, Calif. The determination of surface immunoglobulins was performed as previously described.10 Indirect binding of aggregated IgG was carried out according to Dickler and Kunkel.16 Spontaneous rosette formation with sheep erythrocytes (SRBC-rosette) was performed as described.10 For identification of phagocytic cells, cell suspensions were incubated for 1 hr at 37°C in 20% autologous plasma in HBSS with 0.8-μm polysterene particles. Viability of cells, as determined by Trypan blue dye exclusion, always exceeded 90% of the total.

Colony formation in soft agar. Technique and analysis of colony formation in soft agar was as described.17 Abnormal ratios of colony and cluster formation and the presence of nondividing single persisting cells are diagnostic of AML and AMML, while no colony growth is typical of ALL.17,18

N-alkaline phosphatase assay. For determination of N-alkaline phosphatase (N-A.Pase), heparinized whole blood was centrifuged to remove cells and the cell-free plasma assayed for N-A.Pase by the method of Neumann et al.14 Briefly, 0.1 ml plasma was added to 0.9 ml 0.5 M Tris buffer (pH 9.0) containing 1 mM p-nitrophenyl phosphate, and absorbance at 400 nm was measured every 3 min at room temperature. V0 (μmoles nitrophenyl phosphate hydrolyzed/min/ml plasma) was determined from the linear portion of the enzyme reaction. Similarly, 0.1 ml plasma was added to 0.9 ml buffer containing 1 mM cysteamine-S-phosphate and 0.4 mM dithionitrobenzoate, and V′ (μmoles cysteamine phosphate hydrolyzed/min/ml plasma) was determined from the linear increase in absorbance at 412 nm after correcting for spontaneous hydrolysis using water instead of plasma. N-A.Pase indicative of lymphoproliferative disorders is indicated by V′/V₀ < 0.30.
Terminal transferase assay. For determination of TdT activity, a modification of the procedure described by McCaffrey et al.\textsuperscript{2} was used. Separated mononuclear cells were sonicated in 0.15 M K\textsubscript{2}CO\textsubscript{3} TEGD (50 mM Tris-HCl pH 7.8, 0.1 mM K-EDTA, 20\textsuperscript{o} glycerin, 1 mM dithiothreitol, 1 mg/ml bovine serum albumin) in closed tubes using the cup horn of the Heat Systems-Ultrasonics sonicator (Model 185, potentiometer setting 90\%, 6 x 5 sec with 10-sec intervals). The high-speed-centrifugation supernatant (1 hr, 100,000 g) was applied to a 6 x 50 mm phosphocellulose column (Whatman P-11), equilibrated with 0.15 M K\textsubscript{2}CO\textsubscript{3} TEGD; the column was subsequently washed with 20 ml of this buffer (10 ml/hr). TdT was eluted in a single peak between 0.3 and 0.4 M K\textsubscript{2}CO\textsubscript{3} using a 3.0-ml gradient from 0.15-0.5 M K\textsubscript{2}CO\textsubscript{3} in TEGD. Then 25 \mu l of each 1-ml fraction was assayed for TdT activity as described by Bhalla et al.\textsuperscript{19} using (\textsuperscript{3}H)-dGTP (deoxyguanosine triphosphate) as substrate at a concentration of 25 \mu M (1000 cpm/pmole) in the presence of oligo (dA\textsubscript{12-18}) as primer.

Labeling procedures and autoradiography. Approximately 0.5 ml bone marrow was added to a 5-ml glass centrifuge tube containing 0.6 ml McCoy’s 5A medium (modified), 1000 U heparin, 2 \mu Ci tritiated thymidine (specific activity 6 Ci/mmole). The sample was incubated at room temperature for 1 hr, and the mononuclear cells were then separated on a Ficoll-Hypaque gradient. Ten smears were prepared by a centrifuge (Shandon), dried with a hair dryer, and fixed in methanol for 15 min; the autoradiographs were then processed as described previously.\textsuperscript{20}

RESULTS

Figure 1 shows a graphic presentation of this patient’s clinical course and cell marker characteristics. Cell marker analysis performed at diagnosis revealed that 58\% of the mononuclear cells in the peripheral blood and 88\% in the bone marrow were “null,” since they lacked any identifiable surface markers. Similarly, 85\% of the cells in suspension prepared from the initial lymph node biopsy did not show any of the cell markers studied.

While serum and urinary lysozyme levels indicated a monocytic leukemia, colony formation in soft agar (0 colonies, 0 clusters), N-A Pase activity ($V_s/V_0 < 0.3$), and TdT levels clearly indicated a lymphoproliferative disorder of probable T cell lineage. Terminal transferase from bone marrow and peripheral blood eluted at the typical position from the phosphocellulose column, required an oligodeoxynucleotide primer for activity, and was more than 80\% inhibited by 50 \mu M ATP.\textsuperscript{19} In all of these properties, the TdT from this patient resembled TdT from ALL cells, lymphoblastic acute phase CML, and calf thymus.\textsuperscript{7} After fractionation of bone marrow and peripheral blood cells on nylon fiber columns, TdT was found only in the nonadherent, nonphagocytic cell population.
The labeling index of the leukemic population measured 10%, and the majority of the labeled cells were large "blasts," consistent with what is generally found in acute leukemias.21

Table 1 shows a comparison of the cell marker findings in this patient with the findings in patients with ALL and with AMML studied during the same period of time.

Because it was not known whether the leukemia was lymphoblastic or monocytic, it was decided to treat the patient initially with vincristine and prednisone, reasoning that these drugs would be less toxic than those used in the treatment of nonlymphoblastic leukemia and perhaps allow the fetus to come to term. After beginning prednisone and after the first injection of vincristine, there was a sharp drop in white blood cell count from 25,000 to 7,000/cu mm (Fig. 1). The spleen was no longer palpable, and the enlarged lymph nodes showed a marked reduction in size. At this point TdT was no longer detectable in peripheral blood and N-APase was within normal limits. However, following the second and third injections of vincristine and while continuing prednisone, the white blood cell count rose steadily. TdT again became detectable in the peripheral blood, although at a lower than the initial level. At this point, 0 colonies and 9 clusters per 10³ bone marrow cells and a high number of single persisting cells were observed in the agar marrow cultures, a finding characteristic of myelomonocytic leukemias. At the same time, the number of phagocytic cells in the bone marrow and peripheral blood gradually increased (Fig. 1), with no change in other cell markers (E-rosettes, surface immunoglobulins).

Therapy was then changed to 2,2'-anhydroarabinosyl-5-fluorocytosine (AAFC) and 6-thioguanine (6-TG), which are the initial drugs in the L-12 protocol currently used at Memorial Hospital for acute nonlymphoblastic leukemias.22 AAFC and 6-TG were given twice daily for 11 days, following which a profound neutropenia occurred; 95% of the peripheral blood leukocytes were phagocytic and TdT was no longer detectable. Three weeks later the

| Table 1. Comparison of Cell Marker Findings in Patient T.M. and in Patients With “Null” Cell Acute Lymphoblastic Leukemia and Myelomonocytic Leukemia. |
|---|---|---|---|---|---|
| Assay | Site | Patient T.M. 7/30/76 | “Null” Cell ALL | Patient T.M. 8/18/76 | AMML |
| TdT (U/10⁶ cells*) | BM | 1.24 | 3.71 (0.237-22.5) | 10.893 | 0.009 (<0.004-0.015) | 2 |
| N-APase (V₁/V₀) | PB | 1.18 | 1.37 (0.427-5.04) | 10.127 | <0.004 (<0.001-0.024) | 10 |
| CFU-C | BM | 0.26 | 0.23 (0-0.36) | 0.34 | 0.46 (0.36-0.55) | 2 |
| Colonies | BM | 0 | 6 (0-26) | 11 | 0 | 0 | 8 |
| Clusters | BM | 0 | 29 (0-215) | 11 | 9 | 290 (0-1000) | 8 |
| SPC† | BM | – | +0% | 11 | +25% | 8 |
| E-rosettes | BM | 3 | 4.5 (1.5-8.0) | 4 | 3 | 31.0 (6.0-56.0) | 2 |
| (%) | PB | 17 | 7.2 (0.0-29.0) | 14 | 13 | 21.2 (1.0-87.0) | 9 |
| Surface Ig | BM | 7 | 2.2 (0.5-5.0) | 4 | 0 | 7.0 (0.0-14.0) | 2 |
| (%) | PB | 5 | 1.7 (0.0-5.0) | 14 | 5 | 28.9 (0.0-99.0) | 2 |
| Phagocytic cells | BM | 2 | 3.4 (0-15) | 14 | 27 | 17.2 (5-39) | 5 |
| (%) | PB | 17 | 3.5 (0-22) | 17 | 45 | 21.0 (7-24) | 8 |

BM, bone marrow; PB, peripheral blood.

*One unit = 1 nmole ³H-dGMP incorporated in 1 hr at 37°C.
†SPC, single persisting cells; +, present; -, absent, in per cent of patients studied.
patient achieved a complete remission, during which a premature but otherwise healthy child was delivered by cesarean section. At this writing (16 mo later) the patient is still in complete remission. No TdT activity or pathological N-APase ratios have been detected during followup. Bone marrow cultures in soft agar have shown a normal growth pattern since remission was achieved.

**DISCUSSION**

The specificity of TdT as a marker for leukemic lymphoblasts has recently been questioned because of the findings of high levels of TdT in occasional nonlymphoid leukemias as diagnosed by morphologic and cytochemical criteria. These observations were explained by a derepression of the genome for TdT or a “conservation” of TdT from a TdT-positive progenitor cell into some myeloid leukemic cells. We are unaware of any reports exploring an alternative possibility that two distinct leukemic cell lineages with differing characteristics might occur as a result of clonal evolution in some acute leukemias.

Clonal evolution has been well documented in CML during the accelerated phase and in some patients with CML with multiple acute transformations showing dissimilar morphology and membrane phenotype.

We would interpret our data of two distinct cell populations in this patient with the clinical and laboratory features of both acute lymphoblastic leukemia (morphology, TdT, CFU-C at diagnosis, N-APase) and acute monocytic leukemia (morphology, phagocytosis, adherence, lysozyme, CFU-C after vincristine and prednisone) as representing a phenomenon similar to that observed during clonal evolution in the accelerated phase of CML. A defect at the pluripotential stem cell level could lead to partial differentiation into more than one cell lineage, with a proliferative advantage for one lineage as a result of endogenous inhibitory or stimulatory factors and chemotherapy. A reactive “lymphoblastic” phenomenon or a parent-progeny relationship between the “lymphoid” and “monocytoid” cells of this patient cannot be totally excluded but appears unlikely because of the change in the soft agar growth pattern and the disappearance of TdT and N-APase during the second phase of this patient’s disease.

The rarity of similar observations in leukemic diseases can in part be explained by the rapidity with which clonal changes can occur, as witnessed in our patient. Even before institution of chemotherapy with vincristine and prednisone, TdT values in peripheral blood mononuclear cells fell from 1.18 to 0.63 U/10⁶ cells within 5 days, accompanied by an increase in phagocytic cells from 17% to 32%. Considering in addition the oscillating leukocyte counts prior to chemotherapy, a “natural” clonal evolution appears to have occurred that was subsequently enhanced by vincristine and prednisone.

Further studies employing multiple cell marker analysis will be necessary to investigate the significance of TdT in “nonlymphoid” leukemias and to evaluate the concept of clonal evolution as a general phenomenon in human acute leukemias. The results of these studies could be of great importance for the design of chemotherapeutic protocols.
REFERENCES

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