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The Journal of
The American Society of Hematology

VOL. 51, NO. 2 FEBRUARY 1978

Terminal Deoxyribonucleotidyl Transferase Activity in B-Cell Acute Lymphocytic Leukemia

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Moderately high levels of activity of the enzyme terminal deoxyribonucleotidyl transferase (TdT) were found in the leukemic cells of a patient with acute lymphocytic leukemia. The proliferating cells were B lymphocytes bearing IgG antibody, and the disease was associated with an IgG monoclonal spike and a mediastinal mass. The observations in this case suggest that TdT is related more to the immaturity and proliferation of certain lymphoid stem cells than to their progress toward B- or T-cell differentiation.

THE ENZYME terminal deoxynucleotidyl transferase (TdT), first identified in calf thymus,[1] has subsequently been found in vertebrate thymus of several animals.[2] The properties that distinguish this transferase from the DNA polymerases have been described.[3,4] TdT activity has been detected in the blast cells of many children with acute lymphoblastic leukemia (ALL)[5,6] and some patients with chronic myelogenous leukemia (CML) in blast crisis.[6-8] The thymus derived cell (T-cell) lines have TdT activity several times higher than the B-cell lines examined.[9] It has been suggested that the enzyme is a marker for immature cells proceeding along a pathway to T-lymphocyte differentiation.[7,10]

In this paper we describe ALL associated with a mediastinal mass in a 16-yr-old white male. This case is one of B-cell leukemia associated with the presence of an IgG monoclonal gammopathy. The peripheral blood leukocytes contain levels of TdT reported in cases of null-cell or T-cell ALL. This report suggests the association of TdT with immature and proliferating blast cells, rather than any specific differentiating pathway to B- or T-cell status.

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Submitted October 22, 1976; accepted August 30, 1977.

Supported in part by USPHS Grant AI-11785 from the National Institutes of Health and Grant CA-08341 from the National Cancer Institute.

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MATERIALS AND METHODS

Case Report

The patient, A.G., was a 16-yr-old white male who presented with a cough of 3 mo duration, increasing dysnpea on exertion, and left shoulder pain. Physical examination was unremarkable except for pallor. Hemoglobin was 9.5 g/dl, hematocrit 29%, platelet count 124,000/cu mm, and white cell count 17,500/cu mm, with 42% blasts, 6% segmented cells, and 52% lymphocytes. Bone marrow aspirate contained over 80% blasts with a high nuclear-cytoplasmic ratio resembling those seen in the peripheral blood. There were no granules in the cytoplasm and they stained negatively for peroxidase and the periodic acid-Schiff reaction.

A diagnosis of ALL was made. The blood urea nitrogen was 17 mg/dl and uric acid 4.2 mg/dl. Chest x-ray demonstrated a large mediastinal mass. He was treated with 2 units of packed cells and 600 rads to the mediastinum. Prednisone 70 mg/day orally and vincristine 2 mg/wk intravenously were administered for 4 wk, after which the marrow demonstrated complete remission. The hemoglobin was then 12.7 g/dl, hematocrit 36%, platelet count 330,000/cu mm, and white cell count 5400/cu mm, with 75% segmented cells, 3% monocytes, and 22% lymphocytes. The chest x-ray appeared normal. Consolidation therapy was commenced with L-asparaginase 70,000 U/day intravenously for 10 days. Intrathecal methotrexate was administered in doses of 22 mg/wk for four doses and 2400 rads of whole brain radiation were given as prophylaxis. Thereafter, maintenance therapy consisted of 6-mercaptopurine, 175 mg/day orally and methotrexate 30 mg orally every week. Eighteen months after therapy had commenced, his bone marrow was still in complete remission and he had experienced a testicular relapse.

Immunologic Studies

At the time of initial diagnosis, peripheral blood lymphocytes were examined. B- and T-cell markers were sought in peripheral lymphocytes using standard techniques that identify T cells by their ability to rosette sheep erythrocytes (E)[11] and B cells by the surface-membrane immunoglobulin (SMIg) they carry.[12] For the latter studies, fluorescent polyvalent and monovalent anti-human immunoglobulin sera were used to identify surface-membrane markers of different classes. In addition, the patient's lymphocytes were stimulated with the mitogen phytohemagglutinin (PHA) and pooled mitomycin-treated allogeneic lymphocytes. DNA synthesis was measured by thymidine incorporation and compared to similar measurements using normal cells. All these techniques have previously been described in detail.[13] The above studies were repeated after chemotherapy had induced an apparently full remission.

Enzyme Assays

Tritium-labeled deoxyguanosine triphosphate (dGTP) and deoxythymidine triphosphate (dTTP) (New England Nuclear), nonradioactive nucleotides, and oligo deoxyribonucleotides (P.L. Biochemicals) were used. Molt-4 cells were kindly provided by Dr. M. S. Coleman, University of Kentucky. Purified TdT from calf thymus was a gift from Dr. P. S. Sarin, National Institutes of Health, Bethesda, Md.

Heparinized whole blood was allowed to stand and the buffy coat was removed and centrifuged at 1000 *g* for 5 min. The cells were counted, lysed by treating them with 0.5% Triton X-100 and 0.8M KCl, and centrifuged at 100,000 *g* for 1 hr. The resulting supernatant was used for the assay of enzyme activity. TdT activity was assayed in a standard reaction mixture that contained 50 m*M* Tris-HCl (pH 8.0), 2 m*M* dithiothreitol (DTT), 0.6 m*M* $MnCl_2$, 2 μg of $(dA)_{\sim 15}$, 200 μM of ^{3}H-dGTP (300 cpm/pmole), and enzyme in a total volume of 50 μl. The reaction was terminated after 1 hr of incubation at 37°C by adding 50 μg of hydrolyzed yeast RNA and 3 ml of 10% trichloroacetic acid containing 0.02 *M* sodium pyrophosphate. Acid-insoluble radioactivity was collected on glass fiber filters (Whatman GF/A) and counted in a liquid scintillation counter. DNA polymerase β activity was assayed with 50 m*M* Tris-HCl (pH 8.5), 8 m*M* $MgCl_2$, 2 m*M* DTT, 60 m*M* NaCl, 80 μM each of dATP, dGTP, and deoxycytosine triphosphate (dCTP), 20 μM of ^{3}H-dTTP (1200 cpm/pmole), 10 μg of activated salmon sperm DNA, and enzyme in a total volume of 50 μl. Other conditions were as described above.

Assays were done on peripheral blood cells of our patient before therapy was commenced and 1 yr after he had been in complete remission.

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Table 1. Specificity of TdT Assay

Components of Reaction Mixture	Initiator	Addition	^{3}H-dGMP Incorporated (pmole)	
			TdT	DNA Polymerase β
Complete	(dA)~15		8.3	0.12
Complete	Activated DNA		5.83	19.42
Complete	None		0.26	
Complete	(dA)~15	NEM	0.25	0.11
Complete	(dA)~15	NEM + ethanol	0.15	0.11

DNA polymerase β purified from L1210 cells[11] was used. In assays where activated DNA was used as initiator, the (dA)~15 was replaced with 10 μg activated salmon sperm DNA. Other conditions of the TdT assay are described in Materials and Methods. A similar amount of the DNA polymerase β preparation, when assayed for the DNA polymerase activity, incorporated 180 pmoles of ^{3}H-dTMP.[15]

RESULTS

Distinguishing of TdT Activity From DNA Polymerase β

Since we did not purify and characterize TdT in detail, it was necessary to distinguish it from DNA polymerase β, one of the cellular DNA polymerases that in crude extracts may be confused with it.[14] To distinguish activity, the ability of a DNA polymerase β, purified from L1210 cells,[15] to incorporate ^{3}H-dGMP under the conditions used to assay the TdT activity was compared. The results shown in Table 1 indicate that the DNA polymerase β incorporated very little ^{3}H-dGMP under these conditions. Furthermore, *N*-ethyl maleimide (10 m*M*) and ethanol (10%) specifically inhibited the TdT activity but not the DNA polymerase β activity. The TdT activity of the ALL leukocytes was more with (dA)~15 as initiator than with the activated DNA, whereas the DNA polymerase β preferred the activated DNA. The incorporation of dGMP in the TdT assay was linear for up to 2 hr.

TdT Levels

The peripheral leukocytes of the patient obtained before therapy incorporated 261 nmoles of ^{3}H-dGMP/hr/10^9 cells. Table 2 compares this measurement with other results obtained in our laboratory. One year after complete remission was obtained, the bone marrow cells contained no TdT activity.

T- and B-Cell Characterization

The differences in the immunologic markers before treatment and after the achievement of complete remission are shown in Table 3. Seventy-four per cent

Table 2. Levels of TdT Activity

Source of Cell	Incorporation of ^{3}H-dGMP (nmoles/hr/10^9 cells*)
A.G. cells	261
Molt-4	440
Rat thymus gland	220
Normal peripheral leukocytes	0.3
T cells from normal peripheral leukocytes	0.2

TdT activity was assayed as described in Materials and Methods.
*10^9 cells represent 1 g cells.

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Table 3. Comparison of Immunologic Markers Before and After Treatment

Subjects	WBC	PMN	Lymphocytes Blasts	Lymphocytes Normal	SMIg+	Monovalent Studies* IgG+	IgM+	IgA+	E	Stimulation Indices† Null	PHA	ML
A.G. before treatment	17,500	1050 (6%)	7350 (42%)	9100 (52%)	12,173 (74%)	9860 (81%)	1947 (10%)	365 (3%)	658 (4%)	3619 (22%)	2X	4X
Normal‡ subjects	7,600	3876 (51%)	0	2508 (33%)	376 (15%)	12 (3%)	316 (84%)	8 (2%)	1756 (70%)	376 (15%)	>35X	>20X
A.G. after treatment	5,400	4050 (75%)	0	1188 (22%)	213 (18%)	NT	NT	NT	724 (61%)	249 (21%)	57X	44X

Abbreviations: WBC, total leukocyte count; PMN, segmented cells; $SMIg^+$, cells with readily detectable surface membrane-bound immunoglobulins; E, cells that spontaneously rosette with sheep red blood cells; Null, unidentifiable lymphocytes; ML, mixed lymphocytes; NT, not tested.

*Monovalent studies in which lymphocytes were labeled with fluoresceinated cell-specific anti-heavy chain antiserum.

†Degree of stimulation induced by phytohemagglutin or allogeneic cells: (cpm from stimulated cells)/(cpm from unstimulated cells).

‡Mean of 20 normal subjects (SD ≈ 30%).

of the patient's peripheral blood lymphocytes carried SMIg and did not form rosettes with E. These cells were considered B lymphocytes and the number (13,125 cells/cu mm) represented an absolute increase (normal, 17%-24%, 600-1200 cells/cu mm).[12] Four per cent of the peripheral blood lymphocytes (700 cells/cu mm) rosetted E and were considered T cells. This value represented both a relative and absolute decrease in T lymphocytes (normal, 55%-65%, 2600-3300 cells/cu mm).[11] Eighty-one per cent of the cells identified as B lymphocytes by a polyvalent antiserum carried surface membrane IgG, 16% carried IgM, and 3% carried IgA. No IgE- or IgD-bearing cells were identified. Serum electrophoresis demonstrated an M-spike, which on immunoelectrophoresis was found to be monovalent IgG. This finding, together with the high number of B cells carrying SMIgG alone, strongly suggested that this patient had a B-cell leukemia and that the monovalent spike might represent shed monovalent receptor material.

A stimulation index of less than 2 (normal, >35) was found when peripheral blood lymphocytes were stimulated with PHA at varying doses. Similarly, poor responses were found in the mixed lymphocytic reaction (stimulation index of 4; normal, >20).

Following remission the above abnormalities were corrected. B cells now comprised 18% (972 cells/cu mm) and T cells 61% (3294 cells/cu mm) of the patient's peripheral blood lymphocytes. Dose-response curves to PHA revealed that high and medium doses of PHA (10 and 2 μg/dl, respectively) gave stimulation indices of 44 and 57, while stimulation with a low dose of PHA (0.5 μg/dl) gave only fourfold stimulation, a result which is abnormally low. The stimulation index for the mixed lymphocytic reaction using the same pooled, frozen mitomycin-treated allogeneic cells was 44.

DISCUSSION

Our case appears to represent a B-cell ALL. The majority of cells carried IgG SMIg. We realize that this IgG may not represent receptor material for antigen but rather IgG bound to the B-cell receptor for the Fc portion of IgG. Recent reports[16,17] suggest that T cells may carry receptors for the Fc portion

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of IgG, but this receptor is difficult to demonstrate,[18] and IgG is not detected on rosette-forming cells by our antisera.

It could be argued that the cells with SMIg were not blast cells, but were a residual normal population of B cells. The cells separated on Ficoll-Hypaque were over 90% lymphocytes, using the lack of ability to phagocytose latex beads and to stain with myeloperoxidase as indicators of this cell type being non-phagocytic in nature. The blast cells could not be distinguished from the rest of the lymphocyte population after Ficoll-Hypaque as there was some distortion of the architecture in normal cells after sedimentation. However, less than 3% of the pelleted cells were morphologically lymphocytes, so that blast cells clearly did not pass through the gradient. There was considerable evidence that the proliferating cells were not T cells and were not all null cells. First, there were more SMIg-positive cells in the peripheral blood than blasts, normal lymphocytes, or null cells (Table 3). Second, there was a gross increase in SMIg-positive cells before treatment, but little change in the absolute number of T cells before and after treatment.

The possibility that a receptor for the Fc portion of the IgG could have been on cells labeled as SMIg must be considered. Our laboratory has done labeling experiments with IgG-coated red cells and fluoresceinated anti-IgG and has not been able to show that our antisera can bind to the receptors for the Fc portion of IgG. However, even if this were so, these cells would have receptors for the Fc portion of IgG and would not be, by definition, null cells. They were clearly not T cells, and thus it is most likely that they were B lymphocytes.

Eighty-one per cent of the SMIg-positive cells bore IgG, and an IgG kappa spike was found in the serum, thus confirming the likelihood of the relationship between the protein and the SMIg on the cells. Although it could be argued that the immunoglobulin found in the serum could represent antibody to tumor-specific leukemic antigens on proliferating cells, this would seem highly unlikely, as a heterogeneous antibody response to these antigens would be expected and not a monoclonal one. It is much more likely that a proliferating monoclonal group of cells was producing the antibody. The possibility that the disease was a lymphoma present in the bone marrow must be borne in mind.

B-cell ALL is uncommon, occurring in about 2% of cases.[19] One case of B-cell ALL has been described in which there was an associated mediastinal mass,[20] a finding usually associated with T-cell ALL.[21,22] In B-cell development, various cell membrane markers are added at varying stages of maturation,[23] and this may be true for T cells as well. Thus, most null-cell ALL could represent malignant proliferation within an immature thymocyte population as yet devoid of the capacity to bind E to form rosettes.[24] This suggestion would account for the observation of high levels of activity of TdT in such null cells and the association of this enzyme with immature thymocytes.

The story becomes complicated in light of recent studies showing TdT activity in human bone marrow lymphocytes.[25,26] The occasional association of high TdT activity in adults with chronic granulocytic leukemia in blastic transformation points to a common stem cell origin for TdT-containing cells.[6-8] A recent observation of high TdT activity in the blast cells of two patients with acute myelocytic leukemia[27] makes this hypothesis even more interesting.

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The TdT activity found in the leukocytes of this case of ALL represents an intermediate level of enzyme activity (Table 2). Since at the time of the measurement of TdT, only 4% of the cells were T cells, it could be assumed that most of the activity was in the B-cell population. Mediastinal masses in ALL are presumed to be of thymic origin, so it is of interest that such a mass was present in our case of B-cell ALL. Its association with B-cell proliferation further emphasizes the need to understand the basic cellular biology of leukemia if the oncogenic process is to be understood and therapeutic approaches are to be improved.

Addendum. Two years after the initial diagnosis, the patient underwent a bone marrow transplantation from his HLA-identical sister for bone marrow relapse. However, he developed pseudomonas septicemia that was resistant to antibiotics and died. Postmortem examination was not done.

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