Marrow Terminal Deoxynucleotidyl Transferase Activity in Adult Acute Leukemia

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Terminal deoxynucleotidyl transferase (TdT), a unique DNA polymerase that does not require a template, is found in thymocytes and marrow T-lymphocytes normally [1,2]. Shortly after these discoveries, McCaffrey et al. [3] described the presence of this enzyme in the leukemic cells of patients with acute lymphoblastic leukemia (ALL) and Sarin et al. [4] discovered that some patients with chronic myelocytic leukemia in blast crisis have TdT activity in leukemic cells. Currently, the data from a number of laboratories indicates that most patients with ALL have TdT-positive blasts and most patients with acute nonlymphocytic leukemia (ANLL) have TdT-negative blasts [5,6].

We have measured TdT in the bone marrow of 40 adult patients with acute leukemia prior to any therapy, 30 with ANLL and 10 with ALL by the method of Sarin and Gallo [4] as previously described. One unit of TdT activity equals one (n) ^{3}HdGMP incorporation/hr/10^{9} marrow cells. In this report, TdT results are correlated with the Wright's stained morphology of the bone marrow, Periodic acid-Schiff (PAS) reaction, Sudan black reaction and response to therapy.

Results

Marrow TdT results prior to therapy in adult patients with ALL are given in Table 1. All 10 patients had significant elevations of TdT ranging from 3,1 to 313,6 units, with a mean of 120 units. These results are consistent with those of others [5,6]. In 6 of 8 patients studied the PAS reaction was positive, and 5 PAS-positive patients achieved a complete remission with chemotherapy which always included vincristine and dexamethasone. Of 2 PAS negative patients, 1 achieved complete remission and the other did not.

Twenty-four of 30 ANLL patients had TdT activity $< 0,5$ units in bone marrow cells prior to therapy and 6 patients had levels ranging from 1,6 units to 26,3 units. The PAS reaction was studied in 15 TdT negative patients ($< 0,5$ units) and not done in 9. The reaction was positive in 5% to 75% of the marrow blast cells in 8 of the 15 patients studied and negative in 7. Of the 7 PAS negative patients who were also TdT negative, 4 achieved complete remission, one failed therapy, and 2 are currently undergoing treatment. Six of the 8 PAS positive TdT negative patients achieved com-

Table 1. Marrow terminal deoxynucleotidyl transferase in adult ALL

Patient	TdT[a]	PAS	SB	Response to Therapy
1.	292	+ +	–	CR
2.	3.1	+	–	CR
3.	124	–	–	CR
4.	83.5	+ +	–	CR
5.	30	ND	ND	CR
6.	13.7	+	ND	NR[b]
7.	220	–	–	NR[b]
8.	313.6	ND	ND	NR[b]
9.	105	+ + +	–	CR
10.	15.4	+	–	CR

[a] entries are (n) moles incorporation of ^{3}HdGMP/hr/10^9 cells

[b] died of infection while showing significant response to treatment TdT = terminal deoxynucleotidyl transferase, PAS = Periodic acid-Schiff stain, SB = Sudan Black stain, CR = Complete remission, NR = no useful response, ND = Not done

plete remission and 2 failed therapy. Initial therapy consisted of an anthracycline antibiotic and cytosine arabinoside for each patient.

The 6 TdT positive ANLL patients (> 0,5 units), patients 5, 11, 12, 20, 23, and 26 in Table 2 were similarly studied. Four of the 6 were PAS positive, and 2 were negative including the ANLL patient with the highest TdT activity (patient 26). These patients are described in more detail below.

Patient 5: This 45 year old lady was referred with a diagnosis of acute myelocytic leukemia on the basis of Wright's stain bone marrow morphology. Her white blood cell count was 130000/mm^3 with 92% blasts. The platelet count was 67000/mm^3. The spleen was palpable on physical examination and she had bilateral inguinal lymphadenopathy. Her blast cells were agranular, had abundant cytoplasm, and large amphophilic well defined nucleoli. The serum muramidase was 96 μg/ml, the PAS reaction was positive with fine granules staining in 68% of the marrow blast cells. The Sudan black reaction was positive in 80% of marrow cells. The correct diagnosis was felt to be acute myelomonocytic leukemia. The pretreatment bone marrow TdT determination was 7,9 units. The patient received 2 courses of daunorubicin and cytosine arabinoside without benefit. She then was treated with radiotherapy for a small bowel obstruction secondary to leukemic infiltration to which she responded. Subsequently she was treated with vincristine, methotrexate, L-asparaginase, and dexamethasone. Marrow TdT was still elevated, 1,8 units, just prior to initiation of this therapy. There was no response and the spleen increased in size. She was then treated with azacytidine and pyrazofuran to which she responded with a brief complete remission of less than 2 months. She has received additional drugs without benefit although she remains alive 9 months after her diagnosis.

Patient 11: This 48 year old man was referred with a diagnosis of acute myelomonocytic leukemia with a WBC of 34700/mm^3 with 62% blasts. The platelet count was 154000/mm^3. The marrow had 90% blasts with granules

Table 2. Marrow terminal deoxynucleotidyl transferase in adult ANLL

Patient	TdT[a]	PAS	SB	Organomegaly	Response to Therapy	Miscellaneous
1.	<0.5	–	+	No	CR	
2.	<0.5	–	–	No	CR	
3.	<0.5	+	+	No	CR	
4.	<0.5	–	+	No	CR	
5.	7.9	+ +	+	S+N+	NR	Normal Karyotype
6.	<0.5	+ +	+	No	CR	
7.	<0.5	ND	+	No	CR	
8.	<0.5	ND	+	No	NR	
9.	<0.5	ND	+	S+	PR	
10.	<0.5	ND	ND	No	CR	
11.	1.9	–	+	No	CR	
12.	1.7	+	+	No	CR	Normal Karyotype
13.	<0.5	–	+	No	CR	Auer Rods
14.	<0.5	ND	ND	No	NR	
15.	<0.5	+	+	No	NR	
16.	<0.5	+	+	No	CR	
17.	<0.5	ND	ND	No	NR	
18.	<0.5	ND	+	S+	CR	
19.	<0.5	+	+	No	CR	
20.	24.1	+ +	+	No	CR	
21.	<0.5	+	+	No	CR	
22.	<0.5	–	–	No	NR	
23.	1.6	+ +	+	S+	See Text	Auer Rods Normal Karyotype
24.	<0.5	+	+	N+S+	NR	Auer Rods
25.	<0.5	ND	ND	No	NR	
26.	26.3	–	+	S+	See Text	Normal Karyotype
27.	<0.5	+ +	+	No	CR	
28.	<0.5	–	+	No	TE	
29.	<0.5	ND	ND	No	NR	Auer Rods
30.	<0.5	–	+	No	TE	

[a] See table 1. PAS, SB, ND, CR, NR = Same as table 1. S+ = splenomegaly, N+ = lymphadenopathy, TE = Too early to evaluate

and abundant cytoplasm. The PAS reaction was negative and virtually all marrow blasts showed a fine granularity with the Sudan black reaction. There was no organomegaly. Marrow TdT prior to treatment was 1,9 units and serum muramidase was 50 μg/ml. The patient was treated with adriamycin and cytosine arabinoside and had a complete remission which continues for 2+ months.

Patient 12: This 66 year old man with acute myelomonocytic leukemia presented with a WBC of 13800/mm^3 and 50% blasts. The platelet count was 17000/mm^3. There was no organomegaly or lymphadenopathy. The marrow contained virtually 100% blasts and most were agranular but 15% had fine granules on Wright's stain. The blasts had abundant cytoplasm and many had convoluted nuclei with large, amphophilic, well defined single nucleoli. The PAS reaction was positive in 37% of marrow blasts (fine

granules) and the Sudan black reaction was positive in 61%. Serum muramidase assay was not done. The patient had a normal karyotype and a pretreatment marrow TdT of 1,7 units. He achieved a 3 month complete remission with adriamycin and cytosine arabinoside and is currently under treatment for relapse.

Patient 20: This 57 year old female presented with acute myelomonocytic leukemia with a WBC of 1300/mm^3 and 70% blasts. The platelet count was 134000/mm^3. There was no organomegaly. The Wright's stained bone marrow smear showed 58% blasts with agranular abundant cytoplasm. Serum muramidase was 29 μg/ml and TdT was 24,1 units. The PAS reaction was strongly positive with 81% of the blasts showing fine granules or clumps of PAS-positive material. Essentially all the marrow blasts were Sudan black-positive. The patient was treated with daunorubicin and cytosine arabinoside and obtained a complete remission of 5 months duration. At relapse the marrow TdT was 1,9 units and she was retreated with daunorubicin and cytosine arabinoside again, but showed no response. Subsequently, she failed vincristine and prednisone therapy and she is currently under treatment with azacytidine and pyrazofurin.

Patient 23: This 37 year old woman presented with acute myelomonocytic leukemia with a WBC of 9600/mm^3 and 3% circulating blasts. The platelet count was 32000/mm^3. The marrow contained 63% blasts. These cells examined with Wright's stain showed convoluted nuclei and abundant cytoplasm. Fine granules were seen in 10% of blast cells, and 25% of marrow blasts contained Auer rods. The PAS reaction was strongly positive with 69% of marrow blasts containing clumped PAS positive material. The Sudan black reaction was positive in 72% of marrow blasts. Serum muramidase was not done. The marrow TdT activity was 1,6 units. The patient was treated with vincristine and prednisone and had no response. However, after 2 weekly courses of that therapy, the marrow TdT activity was $< 0{,}5$ units and only 18% of marrow blasts gave a positive PAS reaction. Subsequently she was treated with daunorubicin and cytosine arabinoside and had a good partial response with an M2 marrow after the third course which lasted for less than 2 months. She was then treated with azacytidine and pyrazofurin and died without response, 9 months after her diagnosis.

Patient 26: This 31 year old female had a refractory anemia of unknown etiology for 3 months before the diagnosis of acute myelomonocytic leukemia was made. At the time of diagnosis she had a WBC of 2900/mm^3, with 2% circulating blasts, and a platelet count of 113000/mm^3. Her spleen was palpable. The bone marrow contained essentially 100% blasts with abundant cytoplasm containing no granules. The nuclei of the blasts were round and contained large, single amphophilic nucleoli. The PAS reaction was negative and 43% of the marrow blasts gave a positive Sudan black reaction. The patient had a normal karyotype and a serum muramidase of 50 μg/ml. The marrow TdT was 26,3 units. The patient achieved a complete remission after 2 courses of adriamycin and cytosine arabinoside, and is currently receiving maintenance therapy.

Discussion

These data confirm the results of others [5,6] that patients with ALL usually have marrow TdT activity and patients with ANLL usually do not. This study indicates that, in a small series of adult ALL patients TdT activity was uniformly present in PAS positive as well as PAS negative marrows. Furthermore, results of treatment were independent of PAS results. Marrow TdT activity in ANLL patients was also unrelated to PAS positivity. These results are consistent with those of Hutton and Coleman [5]. It is interesting to note, however, that all 6 ANLL patients with marrow TdT activity had a diagnosis of acute myelomonocytic leukemia based on Wright's stain morphology and supported by elevated serum muramidase activity in 4 of 4 patients in which it was determined. Many [7,8] but not all [9], TdT-positive ANLL patients previously reported have had acute myelomonocytic leukemia. One of our TdT positive patients had Auer rods, which are universally accepted as evidence for ANLL as opposed to ALL, and Hutton and Coleman found TdT marrow activity in some patients with Auer rods also [5].

The overall complete response rate for ANLL patients in this study was 61%. Four of the TdT positive patients with ANLL achieved a complete remission (66%) and another achieved a brief good partial response. Thus, in this study, TdT presence or absence did not influence the response rate for patients with ANLL. TdT positivity did not indicate responsiveness to vincristine and prednisone in patient 23 who failed that therapy and subsequently had a good partial response to standard ANLL therapy. Patient 20 also failed vincristine and prednisone therapy for relapse. Thus, TdT assays prior to therapy appear to be of no clinical value in ANLL patients. The value of serial determinations in monitoring disease activity is currently under study. It does appear, however, that ANLL patients with acute myelomonocytic morphology are more likely to have marrow TdT activity than other ANLL patients.

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