Clinical Significance of TdT, Cell Surface Markers and CFU-C in 297 Patients with Hematopoietic Neoplasias*

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1. Introduction

Considerable progress has been achieved in recent years in the therapy of human leukemias and lymphomas. However, the less than 100 percent complete remission rates and the comparatively small number of long-term remissions in hematopoietic neoplasias of adults have stimulated efforts to detect additional clinical and cellular prognostic factors. Characterization of the cell phenotype in these disorders has proved to be a most promising approach in this context, based on the hypothesis that the neoplastic cell type is the most important determinant of clinical behavior [1–3,5,10,11,13,14,24]. Furthermore, cell marker analysis might contribute to our understanding of normal cellular differentiation assuming that most neoplastic cell types represent malignant proliferations "frozen" at distinct stages of the normal developmental sequence of the different cell lineages. Colony formation of myeloid committed stem cells in agar (CFU-c, 19), terminal deoxynucleotidyl transferase activity (TdT [4,6,7,12,15–18,22]) and cell surface marker analysis [1–3,5,8–11,13,14,24] have previously been shown to be of diagnostic and prognostic significance in human hematopoietic neoplasias. The study leading to the results described here, has been designed to evaluate a possibly increased discriminatory potential of multiple cell marker analysis in the diagnostic and prognostic evaluation of patients with hematopoietic neoplasias.

2. Materials and Methods

Studies on tissues from a total of 297 patients, predominantly from the adult Leukemia-Lymphoma Service of Memorial Hospital, have been evaluated. Diagnoses of leukemia were made on peripheral blood and bone marrow smears and in most cases confirmed by cytochemical stains. TdT determinations [18], CFU-c assays [19] and cell surface marker analysis [11] were carried out as described previously. Lymphomas were diagnosed on lymph node biopsies and classified according to Rappaport [20,21].

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3. Results and Discussion

3.1. *TdT Distribution in Normal Tissues*

Highest levels of TdT activity were detected in normal thymocytes and low activities in bone marrow mononuclear cells as has been described by others [6]. TdT activity containing cells from bone marrow could be enriched by SRBC-rosetting (×2), dessity separation (×5) and sedimentation at 1×g (×100). The latter procedure consistently produced two separate populations of TdT activity exhibiting cells at sedimentation velocities of 3.5 and 4.5 mm/h, with the 3.5 mm/h peak expressing high levels of the HTLA marker [23] after induction with thymopoietin (Fig. 1). Enriched human CFU-c and mouse pluripotent stem cell (CFU-s) fractions (N. Williams, R. Mertelsmann, unpublished) consistently exhibited no detectable levels of TdT activity.

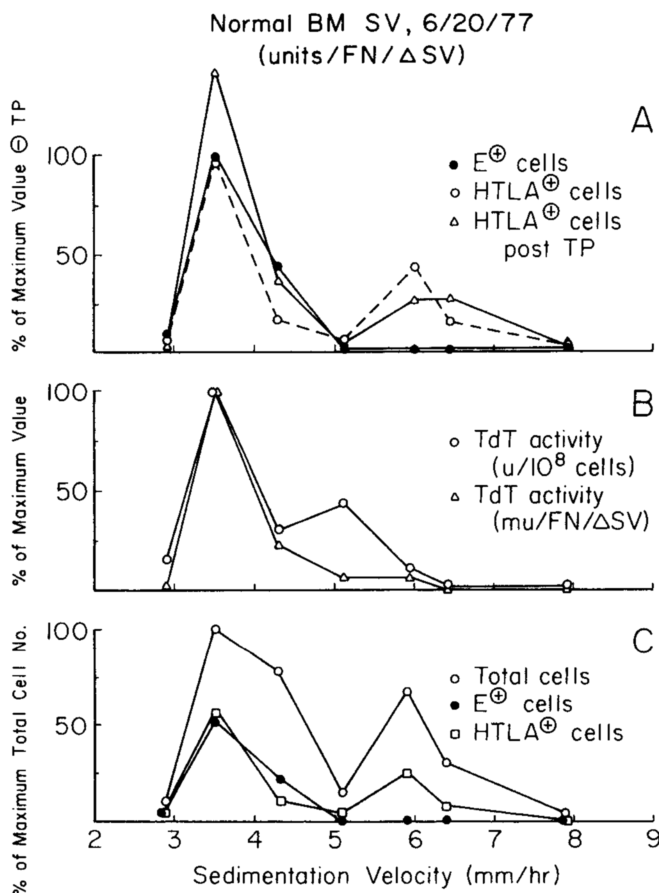

Fig. 1. Separation of normal human bone marrow mononuclear cells by sedimentation velocity at 1×g. Results are expressed in appropriate units per fraction per sedimentation velocity increment.

A. E^+ cells = cells forming rosettes with sheep erythrocytes; $HTLA^+$ cells = cells exhibiting the HTLA marker [23] before and after incubation in the presence of thymopoietin (post TP).

B. TdT activity expressed as specific activity per 10^8 cells and as absolute milliunits of TdT activity recovered per fraction and sedimentation velocity increment.

C. Total number of nucleated cells recovered and distribution of cells forming rosettes with sheep erythrocytes and of cells exhibiting the HTLA marker

3.2. *TdT, CFU-c and Cell Surface Markers in the Differential Diagnosis of Leukemias and Lymphomas*

Analysis of cell marker patterns, clinical diagnoses and TdT activities of 182 patients with acute leukemias (Table 1) and of lymphomas [16] demonstrated highest TdT activities in 70 cases of T and null cell acute lymphoblastic leukemia (ALL). These cases showed absent or low CFU-c formation with normal colony to cluster ratio, characteristic of lymphoid leukemias [19]. Twelve of 32 cases of acute phase chronic myeloid leukemia (CML) also exhibited high TdT activities and lymphoid cell marker characteristics (CML-LB) as did 2 cases of leukemic diffuse histiocytic lymphoma (DHL), 1 acute undifferentiated leukemia (AUL), 1 patient each with polycythemia vera and with a refractory anemia (myelodysplastic syndrome, MDS) who developed an acute leukemia (AL), and 5 cases with a morphological diagnosis of acute myeloid leukemia. Four cases with a morphological diagnosis of acute myelomonocytic leukemia (AMML) and cell marker data consistent with both, ALL and AML, probably represent a 2 "lineage" AL, as has been demonstrated by us in one of these patients [17]. Similar observations have been made by others in acute phase CML with simultaneous or subsequent demonstration of differ-

Table 1. TdT. cell surface markers and CFU-c in the differential diagnosis of acute leukemias[a])

Surface	TdT	CFU-c pattern	Clinical diagnosis	Cases studied n	TdT Specific activity PB mean (μ/10⁸ cells)	TdT Specific activity BM mean (μ/10⁸ cells)
null	+	L	ALL	30	12.8	10.9
			LBL. leukemic	21	3.57	6.59
			CML. LB	12	23.6	32.7
			MDS. LB	1	—	15.7
			DHL. leukemic	2	—	10.6
			AUL	1	46.7	—
			AML	5	5.19	5.82
mono	+	AML	AMML. 2 clones?	4	—	3.48
T	+	L	ALL	14	14.2	17.8
			LBL. leukemic	5	11.4	—
T	–	L	ALL. LBL	2	<.05	<.06
null/mono	–	AML	AML. AMML. AMOL. EL	57	<.05	<.05
null		AML	AUL	3	<.01	<.01
null		AML	CML. MB	20	<.05	<.05
mono		AML	DHL	1	<.01	<.01
B	–	L	ALL	1	<.01	—
B	(+)	L	ALL	2	.14	—
null (pre-B?)	(+)	L	CML. LB	1	.26	.15

[a] for abbreviations see text

ent phenotypes [8,9]. No TdT activity was detected in 81 cases of leukemias exhibiting CFU-c and surface characteristics consistent with AML with clinical diagnoses of AML, acute monocytic leukemia (AMOL), AMML, erythroleukemia (EL), AUL, myeloblastic acute phase CML (CML-MB), and one case of "true" histiocytic lymphoma. No TdT activity was detected in 1 case each of T-cell ALL and lymphoblastic lymphoma (LBL) in leukemic phase, probably representing proliferations of more mature T cells. Two of 3 cases of B-cell ALL as well as 1 case of acute phase CML exhibited a lymphoid CFU-c pattern and low levels of TdT activity. Whether this represents low levels of TdT activity in early B-cells [25] or an admixture of cells containing high levels of TdT to a predominating TdT negative cell population is unknown at present.

No detectable TdT activity was observed in blood cells and, occasionally, low normal values in marrow cells from all patients studied with clinical and cell marker diagnoses of chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HCL), multiple myeloma (MM), Waldenström's macroglobulinemia (WMG), chronic phase CML, chronic myelomonocytic leukemia (CMMOL), and myelodysplastic syndrome. In several patients with CLL, HCL, CML, and myelodysplastic syndromes also lymph node, spleen and cells from involved body fluids were studied exhibiting very low to undetectable levels of TdT activity. One patient with CML who developed a TdT-positive acute phase, did not show any TdT activity in his peripheral blood cells 5 months prior to the acute phase. In the differential diagnosis of malignant lymphomas [16], determinations of TdT activity have been found most useful in confirming or ruling out a diagnosis of T or null cell LBL [18]. We have observed two cases of DHL exhibiting high levels of TdT activity [18] as has been reported by Donlon [4]. Most cases of so-called DHL represent B-cell proliferations, while "true" histiocytic lymphomas are rare (Table 2). Morphology does not appear to allow discrimination between these 3 different cellular types of DHL, which probably require different therapeutic approaches [10, 11]. Most leukemias and lymphomas of T-cell lineage are of immature T-cell type, exhibiting high levels of TdT activity and carrying clinical diagnoses of ALL or LBL. While we have seen rare examples of TdT negative ALL and LBL of mature T-cell type, mononuclear cells from patients with Mycosis fungoides or Sezary's syndrome consistently do not exhibit TdT activity in blood, marrow or other involved tissues. In addition, 1 case of diffuse poorly differentiated lymphoma (DPDL) and DHL exhibiting a TdT-negative T-cell phenotype have been observed. Sixty-two cases of B-cell lymphoma with morphological diagnoses of diffuse or nodular histiocytic (DHL, NHL), mixed (DML, NML) or poorly differentiated lymphocytic (DPDL, NPDL), diffuse well differentiated lymphocytic lymphoma (DWDL), Burkitt's lymphoma (BL) and Hodgkin's disease (HD) were negative for TdT activity (Table 2). Two cases of ALL of Burkitt's type exhibited low levels of TdT activity in peripheral blood mononuclear cells, while 2 additional patients revealed undetectable levels in blood cells and on lymph node biopsies. Whether this indicates low TdT activity in some cases of Burkitt's lymphoma or an admixture of a TdT positive progenitor cell remains to be analyzed.

Table 2. Cell phenotypes observed in 297 patients with hematopoietic tumors[a]

CFU-c pattern	Cell surface	TdT	Clinical diagnoses (n)
"lymphoid"	"null"	+	ALL (30). LBL (21). CML-LB (12). MPS-LB (2). MDS-LB (17). DHL (2). AUL (1)
	"null"	+	AMML (5). *misleading morphology?*
	T	+	ALL (14). LBL (5)
	T	–	ALL (1). LBL (1). DPDL (1). DHL (1). Sezary's Syndrome (2)
	"null"	(+)	CML-LB (1). Burkitt's lymphoma (2)
	B	–	DHL/NHL/DML/NML (17). DPDL/NPDL (27). CLL/DWDL (14). PLL (1). MM (4). WMG (1). BL (2)
	"hairy"	–	HCL (8)
	?	–	HD (6)
myeloid	"null"	–	AML/AMOL/AMML (57). CML-MB (20). AUL (3). DHL (1). CML (21). CMMOL (1). MDS (8)
	"null"	+	AMML (4). *2 lineages involved?*

[a] for abbreviations see text

3.3. *Prognostic Significance of TdT in Leukemias and Lymphomas*

In AL patients with inconclusive morphology who received vincristine and prednisone because of some lymphoid features by morphology, and cytochemistry, 0/8 responses were seen in TdT negative cases as compared to 5/6 responses in TdT positive cases. One patient with probable 2-"lineage" AL achieved a complete remission (CR) on the ALL protocol (L-10M) while the second patient, after a partial remission (PR) on vincristine and prednisone achieved a CR on subsequent therapy with our protocol for acute non-lymphoblastic leukemia [17]. In acute phase CML, only 1 of 18 patients with myeloblastic CML by cell marker data and morphology, achieved a PR on chemotherapy in contrast to 4 CR and 1 PR out of 10 patients with lymphoblastic CML. Similar data of a controlled trial in acute phase CML have recently been reported by Marks et al. [15].

In 60 out of 62 patients with ALL or leukemic LBL, high levels of TdT were detected during active disease either before any chemotherapy was started or during remission induction. The 2 cases of TdT negative ALL, both of T-cell type, probably represent mature T-cell proliferations. It is of interest, that these two patients, as well as the patient with TdT negative DPDL of T-cell type, did not achieve a complete remission on vincristine and prednisone alone, but required cyclophosphamide and adriamycin. In 3 out of 6 patients with LBL without bone marrow involvement by morphological criteria, elevated TdT activity was observed in initial marrow samples. Two of these patients subsequently developed clinical marrow involvement suggesting an increased sensitivity of the TdT assay for detection of subclini-

cal marrow involvement as compared to cytological and histological techniques. During remission induction, lowest bone marrow TdT activities were observed in 7 patients who achieved long-term remissions (mean spec. act. 0.66 U/10^8 cells), while 6 patients who entered a CR of <1 year's duration showed a mean specific activity of 2.23 and 9 who did not enter CR of 5.25 U per 10^8 cells. However, there was a considerable overlap between specific activities from each group.

In continuous complete remission, 13 patients off chemotherapy exhibited a mean specific activity of 0.33 U/10^8 marrow cells, considerably higher than seen in normal controls (<0.1 U/10^8 cells). Patients with ALL in CR without relapse on chemotherapy showed a mean specific activity of 0.37 U/10^8 cells in contrast to patients who were studied during CR and relapsed subsequently, with a mean specific activity of 1.3 ($p < 0.05$). We have previously shown that marrow TdT activities in ALL in remission vary considerably over time, even in patients in CR off chemotherapy [18]. Further sequential studies are necessary in order to define criteria for impending relapse, because one single determination exhibiting a high or low level of TdT activity does not appear to allow prognostic conclusions.

Conclusions

1. TdT has been found to be a highly specific marker for immature cells of T-cell lineage in over 1500 samples from 297 patients with leukemias and lymphomas, and from normal controls. "True" exceptions must be rare.
2. Acute leukemias with cell marker and clinical features of ALL are not only seen in acute phase CML, but also in myeloproliferative and myelodysplastic syndromes terminating in an acute leukemia.
3. The majority of patients with leukemias and lymphomas exhibiting high levels of TdT activity will achieve complete remission on an ALL-type protocol irrespective of morphology.
4. The majority of patients with acute leukemias exhibiting low or undectable levels of TdT activity will not achieve complete remission on an ALL-type protocol.
5. Preliminary observations suggest that determination of TdT activity allows detection of subclinical bone marrow involvement in some cases of TdT positive lymphomas and that sequential analysis of TdT activities in marrow cells during remission induction and in complete remission might allow to predict early relapse in ALL.

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