Quantitation of Human Thymus/Leukemia-Associated Antigen by Radioimmunoassay in Different Forms of Leukemia

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Using a radioimmunoassay, increased levels of a human thymus/leukemia-associated antigen (HThy-L) have been detected in leukemic cells and plasma from most patients with E-rosette-positive acute lymphoblastic leukemia (ALL) and a number of patients with E-rosette-negative ALL, acute myeloblastic leukemia (AML), acute monomyelocytic leukemia (AMML), and acute undifferentiated leukemia (AVL). Low levels of HThy-L have been demonstrated in white cells from patients with chronic myelocytic leukemia (stable phase) and in mononuclear cells from patients with chronic lymphatic leukemia. The relationship between HThy-L and differentiation of hematopoietic cells is discussed.

HUMAN THYMUS/LEUKEMIA associated antigen (HThy-L) appears to be linked to differentiation of T lymphocytes and other hematopoietic cells.[1] In normal tissues, large quantities of this antigen have only been detected in thymocytes. In immunodiffusion, increased quantities of HThy-L have been demonstrated in blast cells from a small group of patients with acute lymphoblastic leukemia (ALL) and fewer with acute myeloblastic leukemia (AML). Furthermore, this antigen was demonstrated in the sera of four patients with T-cell ALL prior to therapy.[2] The low sensitivity and semiquantitative nature of immunodiffusion has limited its clinical application. Recently, we have isolated and purified HThy-L from thymus tissue[3] and developed a radioimmunoassay (RIA) for its quantitation.[4] This method was several thousand times more sensitive than immunodiffusion and has been applied to the quantitation of HThy-L in lymphoid cells from various sources: large quantities were detected in normal thymocytes[4] as well as in all T-lymphoblastoid cell lines studied, regardless of the presence or absence of receptors for sheep erythrocytes (E).[5] The data obtained supported the suggestion that HThy-L may represent a differentiation antigen of hematopoietic cells. The goal of the present study was to quantitate HThy-L in peripheral blood cells and plasma from patients with different forms of leukemia, and from nonleukemic patients, and healthy subjects (Table 1), and evaluate the significance of this antigen as a marker of leukemic cells.

MATERIALS AND METHODS

Patients

Leukemia was diagnosed on the basis of both morphological and cytochemical examination of peripheral blood and bone marrow.[6] Clinical data concerning patients with different forms of leukemia

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Table 1. HThy-L in Healthy Subjects and Patients With Nonleukemic Diseases

	HThy-L in Cell Extracts*		HThy-L in Plasma	
	No.	μg/mg	No.	ng/ml
Healthy subjects	13†	0.17 ± 0.13‡	8§	<20
Pregnancy	2	<0.3	5	ND
Cardiac defects	5	<0.1	5	16 ± 9
Renal disease	5	<0.1	5	<20
Mononucleosis	1	0.5	9	22 ± 11
Immunodeficiency	4‖	0.21 ± 0.29	4¶	11 ± 13
Osteogenic sarcoma	1	0.2	1	26
Mediastinal tumor	1	0.2	1	23
Other diseases	6**	0.11 ± 0.14	13††	10 ± 11

ND, not detectable.

*Peripheral blood mononuclear cells.

†Nine adults and four children.

‡Mean ± SD.

§Four adults and four children.

‖Severe combined immune deficiency disease (SCID), agammaglobulinemia, and common variable immunodeficiency.

¶SCID, C3 deficiency, and Di George syndrome.

**Trauma, anemia, myasthenia gravis, gastritis, and Stevens-Johnson disease.

††The same diseases as listed in ** plus tonsillectomy and adenoidectomy, respiratory diseases, osteochondritis, and hemolytic disease of the newborn.

are summarized in Tables 2 and 3. ALL was classified as either E-rosette-positive ALL (E^+-ALL) or E-rosette-negative ALL (E^--ALL) depending on their capacity to bind sheep red blood cells. All patients with acute leukemias were untreated. Patients with chronic leukemia were either untreated or in relapse, but were without treatment for at least 2 mo.

Methods

Mononuclear cells were isolated from peripheral blood by Ficoll-Hypaque gradient centrifugation;[7] leukocyte-rich cell suspensions were obtained by plasmagel sedimentation (Laboratoire Roger Bellon, Neuilly, France). The E-rosette assay was performed as described elsewhere.[8] Extracts of cells were prepared as described previously,[1] and protein concentrations were assessed according to Lowry et al.[9] Purification and iodination of HThy-L and preparation of rabbit antiserum to purified preparations of this antigen have been described.[3,4] The competitive RIA for HThy-L was performed as previously described.[4] Briefly, varying concentrations of cell extracts were incubated with rabbit anti-HThy-L antiserum followed by the addition of iodinated HThy-L and normal rabbit serum, and coprecipitation of bound labeled antigen with sheep antiserum against rabbit gammaglobulin. A standard curve was established in each experiment using known quantities of "cold" unlabeled HThy-L. The quantity of HThy-L in replicate test samples was extrapolated from the standard curve at 50% inhibition. The concentrations of HThy-L (micrograms of protein) were expressed per milligram of soluble extracted protein (μg/mg) or nanograms of HThy-L per milliliter of plasma (ng/ml). Serum and plasma were equally reactive in the radioimmunoassay.

RESULTS

Low levels of HThy-L were detected in extracts of peripheral blood mononuclear cells (≤ 0.5 μg/mg) and plasma (< 35 ng/ml) from healthy children and adults as well as from patients with nonleukemic diseases (Table 1). Results of our studies in a series of patients with both forms of ALL are presented in Table 2. Samples from 7 patients with E^+-ALL were tested: concentrations of HThy-L ranged from 1 to 13 μg/mg in cell extracts and from 42 to 250 ng/ml in the plasma of 6 patients. Only one patient (S.J.) had normal quantities of antigen in both cell extracts and

Table 2. HThy-L in Acute Lymphoblastic Leukemia

Patient	Age (yr)	Sex	Mediastinal Involvement	WBC/cu mm × 10^3	Blast Cells (%)	E-Rosettes (%)	HThy-L in Cell Extracts (μg/mg)	HThy-L in Plasma (ng/ml)
E^+-ALL								
H.G.	6	F	+*	321	96	60	5.3	>200
S.M.	14	M	+	194	90	54	1.5	>200
B.P.	4	F	–	29	80	25	3.8	NT
M.J.	5	M	–	16	80	34	1.0	42
R.D.	2	M	–	175	95	28	4.1	250
S.J.	18	M	–	150	70	67	0.1	13
N.M.	9	M	–	157	100	4	13.0	217
E^--ALL								
R.D.	9 (mo)	F	–	208	96	0	3.3	>200
D.S.	2	M	–	38	85	1	1.3	55
M.D.	5	M	–	13	47	0	1.2	265
P.N.	14	F	–	40	86	12	1.5	193
R.M.	3	F	–	62	89	5	2.6	18
T.G.	13	M	–	90	90	1	3.9	35
H.D.	6	F	+	75	99	0	10.3	1735
T.V.	4	F	–	4	20	25	1.9	539
G.L.	11 (mo)	F	–	730	100	0	1.2	217
M.W.	36	F	–	67	74	0	1.0	170
P.E.	5	F	–	96	100	0	0.3	NT
J.T.	17	M	–	84	50	0	0.8	NT
F.C.	3	F	–	15	97	0	0.6	NT
B.S.	15	M	–	154	94	3	0.1	>200
H.A.	15	M	–	12	73	0	0.7	34
B.D.	9	M	–	6	6	4	ND	NT
M.D.	4	M	–	9	32	18	0.4	ND
S.S.	2	M	–	20	45	10	0.7	ND
B.R.	1	M	–	27	5	23	ND	154
K.Y.	10	F	–	21	79	2	0.5	476
Q.R.	12	M	–	7	37	1	0.3	12
H.	4	F	–	24	89	0	ND	NT

NT, not tested.
ND, not detectable.
* +, Enlarged thymus.

plasma. In these patients, 30%–70% of their lymphoblasts formed E-rosettes at 4°C. In one patient (N.M.), only 4% of the cells formed E-rosettes, but 100% of the cells were lymphoblasts, and this patient was included in the group of E^+-ALL.

Twenty-two patients with E^--ALL have been studied: increased quantities (≥ 1.0 μg/mg) of HThy-L were detected in peripheral blood mononuclear cells from 10 of these patients. Although cell extracts and plasma were not always available for parallel testing, elevated levels of HThy-L in cell extracts usually corresponded to elevated plasma levels. Only two patients had an increased quantity of HThy-L in cell extracts but low amounts of antigen in plasma, and conversely, three patients had low quantities of HThy-L in cell extracts and increased quantities in plasma. Among those cases where both cell and plasma levels could be tested, only three showed normal values in both. In addition, patients with higher white cell counts tended to have higher HThy-L levels in their cells.

Increased quantities of HThy-L in cell extracts were detected in five patients (of

Table 3. HThy-L in Nonlymphoid Acute Leukemias and Chronic Leukemias

Patient	Diagnosis	Age (yr)	No. and Sex	WBC/cu mm x 10^3	Blast Cells (%)	HThy-L in Cell Extracts (μg/mg)	HThy-L in Plasma (ng/ml)
C.T.	AML	1	F	120	90	1.6	ND
T.J.	AML	7	M	43	100	4.4	>200
S.A.	AML	57	F	15	90	2.7	113
B.P.	AML	14	M	100	94	0.05	>200
W.S.	AML	6	M	5.1	4	0.02	17
R.J.	AML	19	M	240	99	2.5	131
Mc.D.	AML	13	F	74	10	ND	12.9
K.B.	AML	57	F	43	65	0.9	NT
C.W.	AML	37	M	2.1	91	0.2	NT
R.C.	AML	21	F	44.5	69	2.0	NT
R.P.	AMML	2	M	53	95	0.3	ND
P.C.	AMML	63	F	39	95	1.9	NT
C.F.	AMML	47	M	84	79	6.5	62.1
M.D.	AMoL*	Newborn	M	9.3	91	0.4	40
D.G.	AUL	87	M	184	84	0.02	ND
V.Ph.	AUL	39	M	33	77	ND	NT
A.T.	AUL	20	M	46	97	1.4	230
I.J.	AUL	25	M	84	86	1.5	NT
S.T.	CML(b)†	18	M	65	93	0.6	NT
K.R.	CML(b)	30	M	88	87	2.6	NT
	CML(s)‡	>17	8M,10F	10–240	<10	0.05 ± 0.07	NT
	CLL	>17	6M, 4F	20–200	<5	0.16 ± 0.24	ND

ND, not detectable.

NT, not tested.

*AMoL, acute monocytic leukemia.

†CML(b), chronic myelocytic leukemia in blast crisis.

‡CML(s), chronic myelocytic leukemia in the stable phase of disease.

ten) with AML, two patients (of three) with acute monomyelocytic (AMML), and two patients (of four) with acute undifferentiated leukemia (AUL) (Table 3). As observed with ALL, there was no correlation between age, sex, or white cell counts and the level of HThy-L in samples from these patients. Increased quantities of HThy-L in extracts of cells from patients with nonlymphoid acute leukemias generally corresponded to increased quantities of antigen in plasma.

The majority of patients with CML studied were in a stable phase of their disease with normal or high white cell counts but less than 10% blast cells. Extracts of both mononuclear and buffy-coat cells from these patients, studied at the same time, contained low quantities of HThy-L. Two patients were in blast crisis with high white-cell counts and about 90% blast cells, but the lymphoid or myeloid origin of these blast cells was not distinguished. One of these patients had increased quantities of HThy-L in cell extracts.

In the ten patients with chronic lymphatic leukemia (CLL) levels of HThy-L were low both in extracts of peripheral mononuclear cells (0.16 ± 0.24 μg/mg) and plasma.

DISCUSSION

A radioimmunoassay has been used to quantitate HThy-L in healthy subjects and patients with different forms of leukemia. Elevated levels of antigen were found

in cells and/or plasma from a total of 30 of 47 patients with acute leukemia. Neither healthy subjects nor patients with nonleukemic diseases had increased quantities of HThy-L in peripheral white cells. Increased quantities of antigen in leukemic cells generally correlated with the percentage of peripheral blood blast cells. However, large quantities of antigen are not a common feature of all blast cells, since in several patients with large proportions of blast cells (90%–100%), only small amounts of HThy-L were present. That the expression of HThy-L is not a general feature of blastoid cells is also reflected by the demonstration of low antigen concentrations in mitogen-induced lymphoblasts[4] and most lymphoblastoid B-cell lines studied.[5]

Increased amounts of HThy-L were found in blast cells of both lymphoid and myeloid lineage. Competitive inhibition analysis in the RIA of samples from all sources, including the standard antigen preparation, suggested that the antigen demonstrated in these samples was at least antigenically identical to the HThy-L isolated from normal thymus tissue.

The quantitative heterogeneity of HThy-L expression in leukemic cells was characteristic for each form of acute leukemia studied, although in E^+-ALL, only one of seven cases has normal levels of antigen. These T-cell leukemic lymphoblasts resembled normal thymocytes in a number of ways, including the presence of a surface human thymus/leukemia antigen (HTLA),[10] high terminal deoxynucleotidyl transferase (TdT) activity,[11] capacity to form E-rosettes at both 4°C and 37°C,[8,12] and significantly increased quantities of HThy-L.[1] In contrast, normal peripheral blood T cells are TdT-negative, have small quantities of HThy-L, and form E-rosettes predominantly at 4°C. Recently, several TdT-negative T-cell ALL have been described that seem to correspond to an advanced stage of T-cell differentiation.[11] ALL patients with a thymic phenotype are predominantly male, often with mediastinal involvement, high white cell counts, and large proportions of blast cells.[13] Previously, we demonstrated large quantities of HThy-L in lymphoblasts and plasma from these patients.[2] In this study, the group of T-cell (E^+) ALL was more heterogeneous with respect to presenting clinical features and quantitation of HThy-L; two patients were female and only two patients had mediastinal involvement. Leukemic cells from one of these patients had a low quantity of HThy-L consistent with the level of antigen in peripheral blood T cells. It is conceivable that this patient represented a more mature form of T-cell leukemia.

Increased quantities of HThy-L were detected in 59% of patients with E^--ALL. Lymphoblasts in most non-T, non-B-cell ALL carry Ia-like antigen and the common ALL antigen.[11] In several instances, the blast cells were shown to contain intracellular immunoglobulin,[14] and this presentation has been suggested to represent a form of ALL arrested at the pre-B-cell stage of differentiation. However, other non-T, non-B-cell ALL have been described where lymphoblasts carried HTLA and lacked Ia-like antigen;[15] these forms of ALL may represent an E-rosette negative variant of T-cell leukemia. This possibility is supported by findings that E-receptors are not a consistent feature of leukemic T cells.[5] The present studies of E^--ALL cells further emphasizes the heterogeneity of this apparently "non-T, non-B" form of ALL.

Under normal conditions, large quantities of HThy-L have been detected only in thymocytes,[1,4] suggesting a relationship between this antigen and intrathymic differentiation of T lymphocytes. However, the demonstration of increased quanti-

ties of HThy-L in a certain proportion of E^--ALL, AML, AMML, AUL, and CML in blast crisis also implies a relationship between this antigen and early stages of differentiation of hematopoietic cells regardless of their lineage.

We may speculate about the expression of this antigen in hematopoietic cells at different stages of maturation: a pluripotent stem cell may have small quantities of HThy-L. As maturation proceeds, hematopoietic cells may express larger quantities of antigen with a peak at the stage of differentiation corresponding to that of prethymic or intrathymic T lymphocytes. As the hematopoietic cells continue to differentiate, they express smaller quantities of HThy-L, with the lowest levels detected in mature cells. The quantities of HThy-L demonstrated in particular leukemic cells may depend on the stage of maturation at which these cells were arrested, regardless of their lineage. If so, this antigen may be used to estimate the degree of maturity of normal and leukemic cells.

HThy-L is not a specific feature of leukemic cells. However, excluding thymocytes, normal lymphocytes do not contain significant amounts of HThy-L, and increased quantities of antigen could only be detected in leukemic cells. It therefore seems that HThy-L provides a sensitive probe and a marker of peripheral blood leukemic cells in a certain proportion of patients with acute leukemia.

Increased quantities of HThy-L have also been detected in the plasma from patients with different forms of acute leukemia. Studies are now in progress to evaluate the significance of plasma levels of HThy-L, quantitated by RIA, as a means of monitoring the state of disease in patients with increased quantities of antigen in leukemic cells or plasma.

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ADDENDUM

HThy-L, isolated from human thymus tissue, has now been identified as a thymic isozyme of adenosine deaminase (Chechik BE, Schrader WP, Daddona PE: J Natl Cancer Inst [in press]).

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