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Childhood Lymphoblastic Lymphoma, a Cancer of Thymus-derived Lymphocytes[1]

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SUMMARY

Populations of tumor cells obtained from children with lymphoblastic lymphoma were compared with tumor cells from children with acute lymphoblastic leukemia for thymus- or bone marrow-derived lymphocyte characteristics. Thymus derived lymphocytes were identified by their ability to bind sheep erythrocytes as rosette-forming cells. Bone marrow-derived lymphocytes were identified either by the presence of complement receptors or by the presence of immunoglobulins on their surface.

Similar comparison was made between the thymus or bone marrow-derived lymphocyte properties of lymphocyte cell lines established from children with lymphoblastic lymphoma and those established from patients with other lymphoproliferative diseases. The results obtained support the notion that childhood lymphoblastic lymphoma is a cancer of thymus-derived lymphocytes and is clearly different in origin from acute lymphoblastic leukemia.

INTRODUCTION

Recent studies have established that human T-cells[3] form nonimmune rosettes with EL (14, 28), whereas B-cells have CRL (21) or surface Ig (19).

By use of these markers, B-cells have been found to characterize the tumor cells of some patients with chronic lymphocytic leukemia (12, 20, 21, 23, 26) and adult lymphosarcoma (20). In like manner, tumors of patients with Sezary's syndrome (7), Sternberg sarcoma (25), prolymphocytic leukemia (8), chronic lymphocytic leukemia (15), and the atypical lymphocytes found in patients with infectious mononucleosis (24) have been reported to be T-cells.

In this study, tumor cells from 4 children with lymphoblastic lymphoma and 8 children with acute lymphoblastic leukemia were examined for T- and B-cell markers. For comparison, similar studies were done with established cell lines derived from subjects with various lymphoproliferative diseases. These included, most importantly, 3 lines originating from 2 patients having childhood lymphoblastic lymphoma. The results obtained from patients and cell lines indicate that lymphoblastic lymphoma is a cancer of thymus-derived lymphocytes. Acute lymphoblastic leukemia tumor cells, on the other hand, appear to be undifferentiated cells.

MATERIALS AND METHODS

Patients

Normal Adults

Eight normal adults were bled to establish control parameters for each technique.

Children with Lymphoblastic Lymphoma

K. R., a 10-year-old girl, presented with mediastinal and abdominal masses. At thoracotomy, a lymphoblastic lymphoma of the thymus was excised. Subsequently, both ovaries were found to be massively infiltrated with lymphoblasts and were removed. Cells were obtained for study at the time of initial diagnosis from both thymic and ovarian tumors.

W. W., an 11-year-old boy, presented with enlarged cervical glands. An initial bone marrow examination was normal. One month later, he developed hepatosplenomegaly and lymphoblasts were seen in both peripheral blood and bone marrow. No mediastinal mass was ever seen on chest X-ray. Tumor cells were obtained from peripheral blood at the time of initial diagnosis.

N. B., a 17-year-old girl, was found to have a mediastinal mass and inguinal lymphadenopathy. Lymphoblastic lymphoma was diagnosed on the basis of a lymph node biopsy. Despite chemotherapy and X-irradiation, tumor dissemination to bone marrow and central nervous system occurred. Cells were obtained from CSF 9 months after the onset of illness and, 10 months later, from peripheral blood.

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[3] The abbreviations used are: T-cell, thymus-derived lymphocyte; B-cell, bone marrow-derived lymphocyte; E, sheep erythrocytes; EL, sheep erythrocyte-binding cell; CRL, complement receptor cell; Ig, immunoglobulin; CSF, cerebrospinal fluid; RPMI, Roswell Park Memorial Institute; HBSS, Hanks' basal salt solution.

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T. L., a 4-year-old boy, presented with cervical adenopathy and a mediastinal mass in November 1972. A cervical lymph node biopsy showed lymphoblastic lymphoma. One month later, lymphoblasts were found in bone marrow and peripheral blood. In May 1973, tumor cells were obtained from his CSF.

Children with Acute Lymphoblastic Leukemia

Eight patients (C. R., J. P., M. B., D. N., J. R., T. S., S. W., P. J.), 4 female and 4 males, ranging in age from 3 to 14 years, were diagnosed as having acute lymphoblastic leukemia. Peripheral blood or CSF specimens had 80 or 90% lymphoblasts, respectively, as determined by morphological criteria. Cells were obtained from Patient J. P. at the time of initial diagnosis. Cells from the other 7 patients were obtained during relapses after initiation of chemotherapy.

Cell Lines. Frozen aliquots of established cell lines were thawed and grown out as suspension cultures in 250-ml flasks containing RPMI-1640 medium (Flow Laboratories, Rockville, Md.) supplemented with 20% fetal bovine serum. Fresh medium was added every 2 or 3 days, and the cell density was maintained between 0.5 and 3 $\times$ 10^6 cells/ml. Suspension cultures were maintained in log growth in this manner for 2 months for replicate sampling of cells.

Cell Lines Derived from Childhood Lymphoblastic Lymphoma Patients

Lines CCRF-CEM, CCRF-SB, and CCRF-H-SB-2 were kindly provided by Dr. George E. Foley, Childrens Cancer Research Foundation, Boston. CCRF-CEM was derived directly from peripheral blood of a 3-year-old girl with leukemia secondary to lymphosarcoma. CCRF-SB and CCRF-H-SB-2 were both derived from the same patient, a boy with leukemia secondary to lymphosarcoma. CCRF-SB was initiated by placing the boy's cells directly into suspension culture. Cells from the same patient were first inoculated into newborn hamsters as a serially transplantable tumor. The suspension culture CCRF-H-SB-2 was initiated from the tumor carried in hamsters. Isolation and characterization of these cell lines has been extensively described (1-4, 11).

Cell Lines Derived from Other Lymphoproliferative Diseases

Lines H-37 (J. L. Riggs, unpublished observation), SK-L1 (9), HS445T (5), and Raji (22) from patients with adult lymphocytic lymphoma, acute monomyelogenous leukemia, Hodgkin's disease, and Burkitt's lymphoma, respectively, were kindly provided by Dr. Walter Nelson-Rees, Naval Biomedical Research Laboratories, Berkeley, Calif. Line CCRF-RKB (10), from a patient with infectious mononucleosis, was provided by Dr. Foley, and line RPMI 6666 (18), from a patient with Hodgkin's disease, was kindly sent to us by Dr. George E. Moore, Roswell Park Memorial Institute, Buffalo, N. Y.

Cell Line from a Normal Adult

Line RPMI 7666 (17), derived from the buffy coat of peripheral blood from a normal adult male, was also supplied by Dr. Moore.

Preparation of Cells

Lymphocytes were obtained from heparinized venous blood with a Ficoll-Hypaque gradient (6) and then suspended at 10^6 cells/ml in HBSS (Grand Island Biological Co., Grand Island, N. Y.) for T-cell characterization. Monocytes were removed prior to B-cell characterization, using iron particle ingestion followed by magnet separation as described by Pincus *et al.* (21).

Cells were obtained from thymic and ovarian tumors minced in 0.15 M NaCl, washed twice, and suspended to 10^6 cells/ml in HBSS.

Unwashed cells in CSF were used at concentrations of from 0.7 to 1.0 $\times$ 10^6 cells/ml or diluted with HBSS to 10^6 cells/ml. Washing these cells in HBSS did not alter the results of the tests.

The presence of tumor cells was confirmed in all cell suspensions by morphological examination after Wright staining. Cell viability exceeded 95% as measured by trypan blue dye exclusion.

Cells from established cell lines growing in suspension cultures were washed 3 times in HBSS and diluted to 10^6 cells/ml.

Detection of EL

Determination of the percentage of cells that bind sheep erythrocytes, a T-cell characteristic, was done as previously described (14) by mixing 0.25 ml of a 10^6-cells/ml suspension with 0.25 ml of 0.5% sheep erythrocytes in HBSS.

Detection of CRL

Cells that bind erythrocytes coated with antibody and complement, a B-cell characteristic, were enumerated as follows: 0.25 ml of a 0.5% suspension of complement and antibody-coated erythrocytes, prepared as previously described (21) with human serum as the source of complement, were mixed with 0.25 ml of a 10^6/ml cell suspension, incubated at 37° for 15 min, centrifuged at 200 $\times$ g for 6 min, and then reincubated at 37° until read. After vigorous mixing for 15 sec with a vortex mixer, they were examined for rosettes. As a control to detect possible contaminating EL rosette formation, suspensions containing cells mixed with sheep erythrocytes were treated identically. Under these conditions no EL rosettes formed.

Detection of Surface Ig

Cells were examined for surface Ig, another B-cell characteristic, by a direct immunofluorescence method. Aliquots (0.2 ml) of a washed cell suspension containing 1 to

2×10^7 cells/ml diluent consisting of HBSS with 10% fetal calf serum were incubated for 30 min at 4° with 0.05 ml fluorescein-conjugated antisera for the human heavy chains of IgG, IgM, and IgA (Hyland Laboratories, Costa Mesa, Calif.). Cells were then washed 3 times in diluent and examined for surface fluorescence using a Zeiss fluorescence microscope with an HBO-200 W/4 mercury vapor lamp, excitation filter Interference FITC, heat absorption filter KG1, and red suppression filter BG38. The percentage of cells positive for surface Ig was calculated by adding the separately determined percentages of cells staining for surface IgG, IgM, and IgA.

RESULTS

Tumor cells from 4 children with lymphoblastic lymphoma and 8 children with acute lymphoblastic leukemia, as well as lymphocytes from 8 normal adults, were examined for T- or B-cell characteristics.

As shown in Table 1, suspensions of normal peripheral blood lymphocytes contain a mixed population of cells. Sixty % of the cells form rosettes with E, a T-cell characteristic. The B-cell markers of CRL and surface Ig are found on 17 and 27% of lymphocytes, respectively. These values are consistent with those previously reported for peripheral blood (14, 15, 19, 21).

In contrast, suspensions of tumor cells from each of the 4 patients with lymphoblastic lymphoma contain predominantly T-cells since most of the cells from rosettes with EL. Only an occasional cell with CRL is found. The 1 lymphoma tumor cell suspension examined by immunofluorescence was obtained from peripheral blood of Patient N. B. Only 2% of the cells stained positively for surface Ig, additional evidence that the lymphoma tumor cells lack B-cell markers. Also shown in Table 1 are the significantly different results found with tumor cells from children with acute lymphoblastic leukemia. Almost all of these cells lack properties of either T- or B-cells. They do not bind E nor are CRL or surface Ig, where tested, demonstrable. By the criteria of T- and B-cell markers, tumor cells from these cases appear to be undifferentiated.

For further substantiation of the finding that lymphoblastic lymphoma tumor cells are T-cells, similar studies were carried out on cell lines established from children with lymphoblastic lymphoma and from subjects with various other lymphoproliferative disorders. Such cell lines afford both ease of manipulation and the opportunity for repetitive testing. As shown in Table 2, only lines CCRF-CEM and CCRF-H-SB-2, both of which originate from children with lymphoblastic lymphoma, have T-cell characteristics as defined by predominance of EL and absence of reaction for B-cell markers.

Interestingly, CCRF-SB, a cell line derived from the same patient as CCRF-HSB-2, has B-cell characteristics. Most of the cells stain for surface Ig and a small number are CRL, while none of the cells form rosettes with E. The data support the findings of Adams *et al.* (3) that these 2 cell lines are distinctly different despite their origin from the same patient.

B-cell characteristics also are found for cell lines derived from 6 patients with other lymphoproliferative diseases and from 1 normal subject. They all contain a significant proportion of cells with Ig. Two of the lines, Raji and H-37, also have considerable numbers of cells with CRL. None of the cells in any of these lines have the T-cell property of capacity to bind EL.

Table 1

Comparison of T- and B-cell characteristics of tumor cells from patients with either lymphoblastic lymphoma or acute lymphoblastic leukemia

The data are percentages of cells with the T-cell marker EL and with the B-cell markers CRL and surface Ig.

Subject	Tumor	Cell source	% EL	% CRL	% cells with surface Ig
8 normals		Peripheral blood	60 ± 8[a]	17 ± 4	27 ± 5
K. R.	Lymphoma	Thymic tumor	66	0.5	—[b]
K. R.		Ovarian tumor	73	6	—
W. W.	Lymphoma	Peripheral blood	85	0.5	—
N. B.	Lymphoma	CSF	90	0	—
N. B.		Peripheral blood	19	0	2
T. L.	Lymphoma	CSF	64	0	—
G. R.	Leukemia	Peripheral blood	2	9	2
J. P.	Leukemia	Peripheral blood	1	0	4
M. B.	Leukemia	CSF	0	0.5	0
D. N.	Leukemia	CSF	0.5	0	—
J. R.	Leukemia	CSF	1	1	—
T. S.	Leukemia	CSF	2	2	—
S. W.	Leukemia	CSF	0	0	—
P. J.	Leukemia	CSF	0.5	2	—

[a] Mean ± S.D.
[b] —, not done.

Table 2

T- and B-lymphocyte markers on cells in established cell lines derived from patients with lymphoproliferative diseases

The data are the percentages of cells with the T-cell mark EL and the B-cell markers CRL and surface Ig.

Disease	Cell line	% EL[a]	% CRL[a]	% cells with[a] surface Ig
Lymphoblastic lymphoma	CCRF-CEM	51	0	0
	CCRF-HSB-2	43	0	0
	CCRF-SB	0	2	90
Adult lymphocytic lymphoma	H37	0	56	36
Acute monomyelogenous leukemia	SK1-L1	0	0	15
Hodgkin's disease	RPMI-6666	0	0	100
Hodgkin's disease	HS445T	0	0	61
Burkitt's lymphoma	Raji	0	63	96
Infectious mononucleosis	CCRF-RKB	0	2	100
Normal	RPMI-7666	0	2	52

[a] Average of 3 determinations of each cell line.

DISCUSSION

Tumor cells from all 4 children with lymphoblastic lymphoma obtained directly from either thymic tumor, ovarian tumor, peripheral blood, or CSF, were predominantly T-cells. As described for normal T-cells (14), they formed rosettes with E, while they lacked CRL, and, in the 1 case so studied, surface Ig.

Additional support for the concept that lymphoblastic lymphoma is a T-cell cancer comes from the study of established cell lines. Of 10 cell lines, only 2, CCRF-CEM and CCRF-HS-B-2, had T-cell properties. Significantly, these lines were both derived from children with lymphoblastic lymphoma.

The characterization of cell lines CCRF-CEM and CCRF-H-SB-2 as T-cells is consistent with recent studies by Adams *et al.* (4). They found that these 2 lines, as well as 1 other line from a child with lymphoblastic lymphoma, may be passaged as serially transplantable tumors in newborn hamsters. The tumors in hamsters progress to acute leukemia and do not secrete Ig. In contrast, they found that cell line CCRF-SB as well as a series of lymphoblastoid lines derived from normal subjects and from patients with various lymphoproliferative diseases form tumors ("immunoblastomas") that do not progress to acute leukemia in the hamster but do secrete Ig. They postulated that the differences in the 2 distinct types of tumors may be related to their origins as T- or B-cells. The results obtained in this study support their hypothesis.

The results also confirm their observation that 2 different lines were established from the same patient, one (CCRF-HSB-2) with T-cell characteristics and the other (CCRF-SB) with B-cell characteristics. CCRF-H-SB-2 is probably derived from malignant T-lymphoblasts. Since all lymphocyte cell lines obtained from normal subjects studied to date have B-cell characteristics (16), CCRF-SB is probably derived from a normal coexisting B-lymphocyte population.

Only 4 other lymphocyte cell lines have been previously described with T-cell properties (16). These lines were derived from a 19-year-old male reported to have acute lymphoblastic leukemia. It is possible, in light of the findings reported here, that this patient's leukemia was secondary to lymphosarcoma.

The T-cell characteristics of cells either obtained directly or grown in suspension culture from patients with lymphoblastic lymphoma provides compelling support for the concept that this is a cancer of T-cells. The origin of such lymphomas is most likely the thymus itself. In addition to the finding that tumor cells are T-cells, 3 of the 4 patients studied presented with thymic tumors in the early stages of the disease. The thymus is the target organ for neoplastic transformation in mice with radiation- and virus-induced lymphomas, and cells from these tumors are characterized by θ antigen, a mouse T-cell marker (13).

The frequent progression of lymphoblastic lymphoma to a leukemic state and similarities of the histological and cytological appearance in both tumors have raised doubts as to whether or not the 2 diseases are truly different (27). The results of this study indicate that lymphoblastic lymphoma is a T-cell cancer. In contrast, acute lymphoblastic leukemia appears to be a lymphoid tumor of undifferentiated stem cells with neither T- nor B-cell characteristics. Hence, these 2 diseases have different cellular origins and can be distinguished by the use of T- and B-cell markers. As additional lymphocyte markers are found, it is likely that further differences between the various lymphoid tumors will become apparent.

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