CONCISE REPORT

Expression of Common Acute Lymphoblastic Leukemia Antigen (CALLA) by Lymphomas of B-Cell and T-Cell Lineage

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Previous studies have demonstrated that the common acute lymphoblastic leukemia antigen (CALLA) is expressed by leukemic cells from approximately 80% of patients with non-T-cell ALL and 30%–50% of patients with chronic myelocytic leukemia in blast crisis. A small number of normal bone marrow and fetal liver cells also express CALLA, but the functional role of this molecule is unknown. In the present study, we have used a monoclonal antibody (J5) specific for CALLA to study the expression of this antigen in non-Hodgkin's lymphomas. Within the B-cell lymphomas, it was found that CALLA was expressed by almost all Burkitt's and nodular poorly differentiated lymphocytic lymphomas. Within the T-cell lymphomas, CALLA was expressed in 40% of patients with lymphoblastic lymphoma. Three of 3 Burkitt's lymphoma cell lines and three of eight T-lymphoblast cell lines were also found to express CALLA. Normal spleen, lymph node, and thymus cells were not reactive with J5 antibody. These findings indicate that expression of CALLA is not limited to relatively undifferentiated leukemic lymphoblasts but also occurs in more differentiated lymphoid malignancies. However, normal differentiated lymphoid cells in lymph node, spleen, and thymus, which have a phenotype similar to that of lymphoma cells, do not appear to express CALLA.

THE COMMON acute lymphoblastic leukemia antigen (CALLA) has become a standard marker used in the identification and classification of patients with acute lymphoblastic leukemia (ALL). This antigen was first identified by conventional rabbit heteroantisera,[1] but more recently, a murine monoclonal antibody (J5) specific for CALLA has been generated.[2] CALLA has been shown to be a cell surface glycoprotein of approximately 100,000 daltons[2,3] which is expressed by leukemia cells from approximately 80% of patients with non-T ALL and 30%–50% of patients with chronic myelocytic leukemia (CML) in blast crisis.[4-6] In addition, the presence of CALLA on tumor cells in both children[7,8] and adults[9] with ALL and in patients with CML blast crisis[10] appears to correlate with a better prognosis. CALLA is also expressed by a small number of normal bone marrow cells,[11,12] but the precise functional role of this cell surface glycoprotein is unknown. In the present report, we have investigated the expression of CALLA by various types of lymphomas of both B-cell and T-cell lineage.

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MATERIALS AND METHODS

Cells

Leukemia and lymphoma cells were obtained from patients at the time of initial presentation or at relapse. In each instance, the diagnosis was established independently on the basis of standard morphological and histochemical parameters. Leukemic cells were isolated from heparinized peripheral blood or bone marrow by Ficoll-Hypaque density sedimentation. Lymphoma cells were obtained from bone marrow, spleen, or lymph nodes that were replaced by tumor cells. Single cell suspensions were obtained using previously described techniques[13] and cytocentrifuge smears were used to confirm that tumor cells comprised at least 75% of each population. In each case, tumor cells were cryopreserved in vapor phase of liquid nitrogen prior to analysis by indirect immunofluorescence. In some instances, tissues were snap-frozen in OCT compound and stored at −70°C for subsequent analysis by immunoperoxidase. Fetal cells were obtained following therapeutic abortions and normal bone marrow was obtained from adult volunteers. The KG-1 cell line was provided by Dr. David Golde. All other cell lines were provided by Drs. Herbert Lazarus or Jun Minowada.

Monoclonal Reagents

Each population of tumor cells was analyzed with a series of monoclonal antibodies that have been previously described. J5[2] is an IgG_{2a} antibody that is specific for CALLA. Because binding of J5 to some lymphoma cells was weak and difficult to distinguish from nonspecific Fc binding, a J5 $F(ab')_2$ reagent was used to type all lymphoma samples. This was purified from immune ascites after ammonium sulfate precipitation and pepsin digestion.[14] B1 antibody[15] has previously been shown to be specific for normal B cells with the exception of terminally differentiated plasma cells and is also reactive with malignant cells derived from the B lineage.[13] T-cell surface antigens were identified by a series of monoclonal antibodies described previously.[16] Other monoclonal reagents that were used were anti-Ia (I-2),[17] and anti-Ig (μ, γ, κ, λ) (prepared by V. Raso and L. Nadler).

Indirect Immunofluorescence Assay

Single cell suspensions of both tumor cells and normal populations were analyzed with specific reagents utilizing a standard

indirect immunofluorescence assay. Briefly, 10^6 cells were incubated with specific antibody for 30 min at 4°C. After 2 washes to remove excess antibody, cells were incubated with fluorescein conjugated goat anti-mouse Fab (GM-FITC), washed twice again, and analyzed on the Fluorescence Activated Cell Sorter (FACS-I) (Becton Dickinson, Mountain View, Calif.). Background fluorescence was determined by substituting nonspecific ascites for specific monoclonal antibody. Either 1 or 4 × 10^4 cells were analyzed in each sample.

Immunoperoxidase Procedures

A four-step immunoperoxidase technique was used to analyze frozen tissue sections. This technique has been previously reported in detail.[18]

RESULTS

Expression of CALLA by Hematopoietic Cell Lines

Table 1 summarizes the reactivity of 24 different cell lines with monoclonal antibodies specific for CALLA (J5), B1 antigen, and Ia antigen (I-2). These human cell lines represent a spectrum of transformed or malignant cells from a variety of hematopoietic cell lineages, and the expression of these antigens correlates closely with that found in uncultured cells. Ia antigen, which is found on normal B cells, monocytes, and myeloid precursor cells, is also expressed on B-lymphoblastoid cells, Burkitt's lymphoma lines, and some myeloid cell lines. B1 antigen, which is found only on B cells, is expressed by B-lymphoblastoid cells and Burkitt's lymphoma lines. In addition, Nalm-1 cells, which were derived from a patient with CML blast crisis, expression B1 antigen. These cells have previously been shown to have a pre-B-cell lymphoid phenotype and express CALLA as well as cytoplasmic immunoglobulin.[19] As expected, CALLA is expressed by Laz 221 cells,[20] which were derived from a patient with non-T ALL. However, CALLA is also found on three of eight T-lymphoblast cell lines and all three Burkitt's lymphoma cell lines that were tested. J5 antibody did not react with EB virus transformed B-cell lines or myeloid cell lines.

Table 1. Expression of CALLA by Hematopoietic Cell Lines

	CALLA	B1	Ia
ALL			
Laz 221	+ +*	–	+ + +
B lymphoblastoid			
7 lines†	–	+ + +	+ + +
T lymphoblast			
CEM	–/+‡	–	–
HSB2	–	–	–
Molt 4	–	–	–
HPB-ALL	+	–	–
HPB-MLT	+	–	–
45	–	–	–
DND-41	+	NT§	–
JM	–	NT	–
Burkitt's lymphoma			
Daudi	+ +	+ + +	+ + +
Raji	+ +	+ + +	+ +
Ramos	+ +	+ + +	–
Myeloid			
KG-1	–	–	+ +
HL-60	–	–	–
U937	–	–	+
CML blast crisis			
Nalm-1	+ +	+	+ + +
K562	–	–	–

*Expression of specific antigens was determined by indirect immunofluorescence assay and FACS analysis. Intensity of fluorescence was graded: – no fluorescence above background; + weak fluorescence; + + moderate fluorescence; + + + strong fluorescence.

†Cell lines were established by in vitro transformation with EB virus from 7 different individuals. The phenotype of each of these cell lines was identical with respect to the antigens listed above.

‡Some subcultured lines from CEM cells have weak reactivity with J5 antibody, while others are completely unreactive.

§Not tested.

Expression of CALLA in Leukemias and Lymphomas

As shown in Table 2, in acute and chronic leukemias, CALLA is primarily expressed by tumor cells from patients with non-T-cell ALL and CML in blast crisis. A small percentage of patients with T-ALL also exhibit weak reactivity with J5 antibody. Stable phase CML, AML, and CLL cells were not reactive with J5 antibody. When lymphomas of various histologic types

Table 2. Reactivity of Monoclonal Anti-CALLA (J5) with Leukemias and Lymphomas

	Number Tested	Number Reactive	Percent Positive
Leukemias			
ALL			
Null cell	166	132	80
T cell	33	3	9
AML	99	0	0
CLL			
B cell	20	0	0
T cell	5	0	0
CML			
Stable phase	18	0	0
Blast crisis	22	10	45
Lymphomas			
B cell			
Diffuse-histiocytic	15	2	13
Diffuse-poorly differentiated lymphocytic	16	2	13
Nodular-poorly differentiated lymphocytic	4	4	100
Burkitt's	6	5	83
Myeloma	3	0	0
T cell			
Lymphoblastic lymphoma	12	5	42
Sezary	1	0	0
Total	420		

were examined for expression of CALLA, we found that tumor cells from almost all patients with either nodular poorly differentiated lymphocytic lymphoma (N-PDL) or Burkitt's lymphoma were reactive with J5 antibody. All of these tumors were clearly B-cell derived because they expressed surface immunoglobulin, monoclonal light chains, Ia antigen, and B1 antigen. Tumor cells from two patients with diffuse histiocytic lymphoma also expressed CALLA. Interestingly, both of these patients previously had N-PDL, which converted to a diffuse histiocytic morphology. Despite the change in morphology, these tumor cells maintained the expression of CALLA, which was more characteristic of the previous histologic pattern. Tumor cells from approximately 40% of patients with T-cell lymphoblastic lymphoma were also found to be reactive with J5 antibody. These tumor cells had been identified as T cells because they express antigens characteristic of normal thymocytes (T6) and differentiated T cells (T4, T8) as well as by the lack of expression of surface immunoglobulin, Ia antigen, and B1 antigen. In addition, tumor cells from two patients with diffuse poorly differentiated lymphocytic lymphoma were reactive with J5 antibody.

The expression of CALLA by lymphomas of both B-cell and T-cell derivation was also studied using a sensitive immunoperoxidase technique. As shown in Fig. 1 A and B, frozen tissues from additional patients with nodular lymphoma and lymphoblastic lymphoma demonstrated reactivity with J5 antibody using this method.

Expression of CALLA in Normal Hematopoietic Tissues

When normal adult hematopoietic tissues were examined for reactivity with J5 antibody by indirect immunofluorescence assay and FACS analysis, very few cells were found to express CALLA. In five samples of spleen and lymph node, 0.4% ± 0.8% of cells were found to be reactive with J5 $F(ab')_2$ antibody. In 5 thymuses removed at the time of cardiac surgery in children and infants, 0.7% ± 0.9% of cells were reactive with J5. In 7 adult bone marrow samples, 1.2% ± 1.0% of cells were J5 positive. In 14 children and adults receiving chemotherapy, "regenerating" bone marrow contained 3.0% ± 2.4% J5-positive cells. In fetal liver, between 14 and 24 wk gestation, 5.1% ± 1.9% of cells were found to express CALLA in 11 different samples. Thus, J5 reactivity in lymphoid organs was not distinguishable from nonspecific background binding, but small numbers of J5-positive cells were detectable in bone marrow samples and more significant reactivity was found in fetal liver.

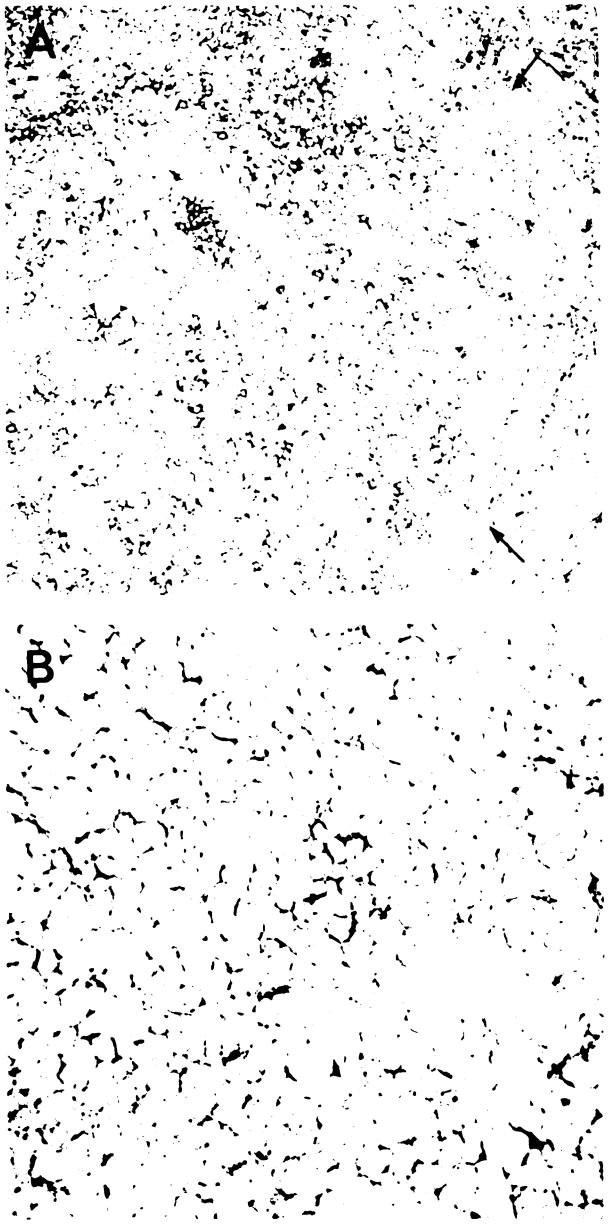

Fig. 1. Frozen sections were stained with J5 monoclonal antibody using an immunoperoxidase method. (A) A case of nodular lymphoma poorly differentiated lymphocytic type. The cells in the nodule show peripheral staining of variable intensity, whereas the cells outside the nodule (arrows) are either not stained or faintly stained (×160). (B) A case of lymphoblastic lymphoma. Most of the cells show peripheral staining (×400).

DISCUSSION

Previous studies have demonstrated that CALLA is primarily expressed by tumor cells from patients with non-T-cell ALL and with lymphoid blast crisis of CML. Within both of these distinct entities, CALLA-positive blast cells may have a pre-B-cell phenotype with presence of cytoplasmic heavy chain without expression of surface immunoglobulin.[21] Immunoglo-

bulin light chains are usually not detectable either in the cytoplasm or on the surface membrane of these cells, but B1 antigen, which is another marker of B-cell lineage, has been identified.[13]

The present study indicates that the expression of CALLA in hematopoietic tumors is not limited to leukemia cells but is also found in lymphomas of both B-cell and T-cell lineage and in various cultured cell lines derived from these tumors. Unlike CALLA-positive ALL cells, which most often do not exhibit characteristics of differentiated lymphoid cells, all of the CALLA-positive lymphomas clearly had characteristics of more mature lymphocytes. The nodular and Burkitt's lymphoma cells all expressed monoclonal surface immunoglobulin, Ia antigen, and B1 antigen in addition to CALLA. The lymphoblastic lymphoma cells that were CALLA positive also expressed T-cell antigens found on normal cortical thymocytes.[18,22]

Because of the observation that CALLA expression in lymphoid malignancies was not limited to relatively undifferentiated ALL cells but also included relatively differentiated B- and T-cell tumors, we investigated the expression of CALLA in the normal lymphoid organs from which these lymphomas arise. Although J5 antibody was reactive with almost all nodular and Burkitt's lymphomas, less than 1% of normal cells from lymph node, spleen, or thymus were found to be reactive with J5 antibody. The lack of reactivity of J5 antibody with normal lymph node cells and thymocytes, which was demonstrated with indirect immunofluorescence assay, was confirmed by examination of frozen tissues using an immunoperoxidase technique (data not shown).

It has previously been postulated that CALLA is a marker of early hematopoietic stem cells and that the expression of CALLA by ALL cells is simply a reflection of the derivation of these malignant cells from normal lymphoid precursor cells that also express CALLA.[11,12] The finding that some normal bone marrow and fetal liver cells have a similar phenotype to that of CALLA-positive ALL cells is indirect evidence that suggests that CALLA may be a marker of hematopoietic stem cells. Nevertheless, it has also been demonstrated that early myeloid precursor cells do not express CALLA.[23,24]

In the present study, we have demonstrated that CALLA is expressed by relatively differentiated lymphoid tumors but not by corresponding normal cells in lymph node, spleen, or thymus. Although these lymphoid tissues contain many lymphocytes whose surface phenotype approximates that of lymphoma cells, these normal cells do not express CALLA. The expression of CALLA in lymphomas may therefore be an aberration of normal differentiation in malignant cells, or alternatively, CALLA may not be a differentiation marker that is exclusively expressed by early hematopoietic precursor cells. In this regard, earlier studies have shown that CALLA is a member of a family of cell surface glycoproteins[25] and CALLA-related molecules are expressed on many differentiated as well as undifferentiated hematopoietic cells. CALLA itself may therefore be related to a specific transformational event or may perhaps have a functional role that has not yet been identified. Previous studies have demonstrated that the expression of CALLA on the cell surface of ALL cells will modulate in response to binding by J5 monoclonal antibody.[26] Antigenic modulation that results in the loss of CALLA from the cell membrane is a specific loss of this antigen and does not result in other cellular alterations that would suggest either differentiation or "dedifferentiation." The precise functional role of cell surface CALLA or whether all proteins identified by J5 antibody are identical remains unknown, but the present studies suggest that CALLA may not simply be a differentiation marker of early hematopoietic cells.

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