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A Monoclonal Antibody to Common Acute Lymphoblastic Leukemia Antigen (CALLA) and Its Expression on Several Human Tumor Cell Lines

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We describe a newly-made murine monoclonal antibody to the common acute lymphoblastic leukemia antigen (CALLA), named SHB-10. The antigen detected by SHB-10 has a molecular weight of about 105 kDa. This antibody is very similar to that of conventional anti-CD10 Ab on indirect flowcytometric analysis using lymphoid malignant cell lines and peripheral lymphocytes of acute lymphoblastic leukemia (ALL) patients. The binding of anti-CD10 to Daudi cell and peripheral lymphocytes of ALL patients is blocked by SHB-10. Thus this monoclonal antibody is thought to detect the CALLA. The distribution of antigen detected by SHB-10 on several cell lines of neuroectodermal tumor and lymphoid malignancy was analysed and a slight difference in their cell surface expression is observed when compared with that by conventional anti-CD10.
Further biochemical analysis is now under way for a better characterization of this antigen.

Key Words: *Common acute lymphoblastic leukemia antigen (CALLA), monoclonal antibody*

INTRODUCTION

The common acute lymphoblastic leukemia antigen (CALLA: CD10) is a membrane glycoprotein with a molecular weight of 95,000-100,000 dalton (Jongeneel et al., 1989). The CALLA is expressed in the majority of acute lymphoblastic leukemia (ALL) as well as in certain other malignancies derived from immature lymphoid populations such as lymphoblastoid, Burkitt's and nodular poorly differentiated lymphocytic lymphomas, and in chronic myelogenous leukemias in lymphoid blast crisis (Greaves et al., 1975; Ritz et al., 1980; Greaves et al., 1983). In contrast, CALLA is not found in leukemic cells of myeloid lineage or in lymphoid malignant cells with more mature phenotypes (Greaves et al., 1983). While CALLA is not expressed on normal T and B lymphocytes and on monocytes, it is found on lymphoid precursors in fetal liver and bone marrow, and on normal granulocytes (Hokland et al., 1983; Cossmann et al., 1983; Braun et al., 1983). Many characteristics of CALLA have been investigated. The gene, which encodes the 100 kDa type II transmembrane glycoprotein, appears to be a single copy locus of greater than 45 kb which is nonrearranged in malignancies expressing cell surface CALLA. Cell hybrid analysis indicated that the CALLA-related DNA sequences are found on human chromosome 3. In situ hybridization studies revealed the regional location of the CALLA gene to be 3q21-27 (Baker et al., 1989). The restricted expression of CALLA in hemopoietic malignancies has made it both an extremely useful diagnostic marker and the target of numerous therapeutic strategies (Ritz et al., 1987).

In this study we describe a monoclonal antibody produced in this laboratory which recognizes CALLA, and the expression pattern of CALLA detected by this monoclonal antibody on several human tumor cell lines and on normal tissue.

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MATERIALS AND METHODS

Production of a monoclonal antibody against Burkitt's lymphoma cell line.

Two 6-week-old BALB/c mice were immunized i.p. with 10^7 Daudi cells on days 1,10, and 20. Spleen cells were harvested on day 23 and fused with mouse myeloma cell line SP2/0-Ag14 using polyethylene glycol as previously described (Koehler and Milstein, 1975). The hybrids were cultured in flat-bottom microculture trays and incubated at 37°C in an atmosphere of humidified air containing 5% CO_2. After 10 days, culture supernatants were harvested and tested for reactivity to Daudi cells using flowcytometry. Cells from one microculture well were cloned by limiting dilution, and the culture supernatants of the clones were retested for antibody production. Mass culture was done and culture supernatant was used for all tests. For comparison, anti-CD10 antibodies were purchased from Dako (clone: SS2/36) and Becton-Dickinson (clone: W8E7). The isotype of SS2/36 is IgG1 and that of W8E7 is IgG2a.

Determination of antibody isotype

The isotype of SHB-10 was determined by enzyme immunoassay using mouse monoclonal subisotyping kit EK-5050 (Hyclone, Utah, USA). Isotyping was performed with rabbit anti-murine isotype-specific antisera (IgG1, IgG2a, IgG2b, IgG3, IgM, Kappa, Lambda) followed by peroxidase-labeled goat anti-rabbit IgG as the secondary antibody. With the addition of o-phenylene diamine and hydrogen peroxide (H_2O_2) substrate, positive samples turned an intense yellow.

Materials

Fresh peripheral blood from normal volunteers and from ALL patients was obtained for flowcytometric analysis. The Daudi and Raji (Burkitt's lymphoma cell lines), Jurkat (T-ALL cell line), U937 (human histiocytic lymphoma cell line), Molt4 (T cell acute leukemia cell line), H-9 (human T cell line), K562 (chronic myelogenous leukemia cell line), and CCRF-CEM (Acute lymphocytic leukemia) grown in RPMI1640 medium supplemented with 10-20% fetal calf serum were used. Other human tumor cell lines that are thought to be of neuroectodermal origin: SK-N-MC (neuroblastoma), SK-N-SH (neuroblastoma), IMR-32 (neuroblastoma), A375 (melanoma), A172 (glioblastoma), T98G (glioblastoma), were grown under the same conditions. All of the above cell lines used were purchased from ATCC (American type culture collection, Rockville, Maryland). 10^6 cells were collected for every flowcytometric analysis.

Immunohistochemical study

Fresh non-neoplastic tonsils and lymph nodes were obtained from the surgical and autopsy specimens of Seoul National University Hospital for cell suspension and frozen section. Snap-frozen sections of fresh non-neoplastic tonsils and lymph nodes were stained first SHB-10 (supernatant from without dilution), then by purified biotinylated goat anti-mouse IgG followed by avidin-horseradish peroxidase conjugate. The reaction pattern was analysed based on the serial hematoxylin-eosin stained sections. Paraffin-embedded tonsil sections were also used for immunohistochemical study after deparaffinization.

Immunoblotting

Daudi cells were lysed with 1% NP-40 in 50 mM Tris-HCl, pH 7.4, 50 mM EDTA, and 1 mM PMSF. The lysates were mixed gently by slowly inverting the tube and were incubated at 4°C for 30 minutes and centrifuged at 13,000 g for 15 minutes for removal of nuclei. The supernatant was used for SDS-PAGE under the nonreduced conditions, according to the method of Laemmli (Laemmli, 1970). The acrylamide concentration of the separation gels was 10% and appropriate molecular weight markers were used. The electrophoretic transfer of proteins to nitrocellulose was done at 45 V for 16 hours (Towbin et al., 1979). After protein transfer, the nonspecific binding sites of the nitrocellulose were blocked by a solution of 5% skimmed milk. The antigens were detected by incubating the paper with monoclonal antibody, SHB-10. After the nitrocellulose was washed with 1 × PBS-Tween 20 (0.05%), it was incubated for 1 hour at room temperature with peroxidase-conjugated goat anti-mouse IgG (Zymed, California, USA) diluted 1:500 in blocking solution. After washing with 1 × PBS-Tween 20 (0.05%), the bound peroxidase was incubated with 1 × PBS-Tween 20 (0.05%) containing 1.26 mmol 3,3'-diaminobenzidine tetrahydrochloride and 0.005% H_2O_2 per liter. The staining reaction was stopped by rinsing the nitrocellulose with distilled water.

Flowcytometry and blocking study

Fresh non-neoplastic lymphocyte of peripheral blood, peripheral blood from ALL patients, cell suspension of tonsils and lymph nodes, and various human tumor cell lines, were prepared and analyzed on a fluorescence-activated cell sorter (FACScan, Becton Dickinson, Mountainview, Calif.). Indirect immunofluores-

cence study was performed by incubating 10^6 cells with supernatant of primary antibody or controlled immunoglobulin in phosphate-buffered saline containing bovine serum albumin (0.2%) and sodium azide (0.05%) for 1 hour at 4°C. After three washes, 100 μl of the fluorescein-conjugated goat anti-mouse IgG $F(ab)'_2$ (Cappel, Belgium) was added and incubated for 30 min at 4°C. Fluorescence was assayed on a fluorescence-activated cell scan (FACS), at 500 mW of laser power (argon laser), 488 nm wavelength, 630 V photomultiplier tube sensitivity, and at a fluorescence gain setting of 4 on a scale of 16. The data was recorded on a Hewlett-Packard computer, 1023-channel histograms were generated and the percentage of fluorescent cells as well as the mean fluorescence intensity (mean channel number) were calculated.

For the blocking study of epitope of CALLA by SHB-10, peripheral blood samples of ALL patients and Daudi cells were pre-incubated with a saturated dose of unlabeled SHB-10 and washed three times. After washing, direct immunofluorescence was performed with 10 μl of FITC-conjugated anti-CD10 antibody (SS2/36 or W8E7). Fluorescence was assayed as above.

RESULTS

Determination of antibody isotype and characterization of the SHB-10 Target Antigen

The isotype of SHB-10 was determined by enzyme immunoassay. The reaction pattern was compatable with that of the IgG1 subclass.

The molecular weight of the SHB-10 antigen was determined by immunoblotting, using Daudi cell lysate. The major precipitation band of the SHB-10 antigen corresponded to a molecular weight of approximately 105 kilodaltons (Fig. 1).

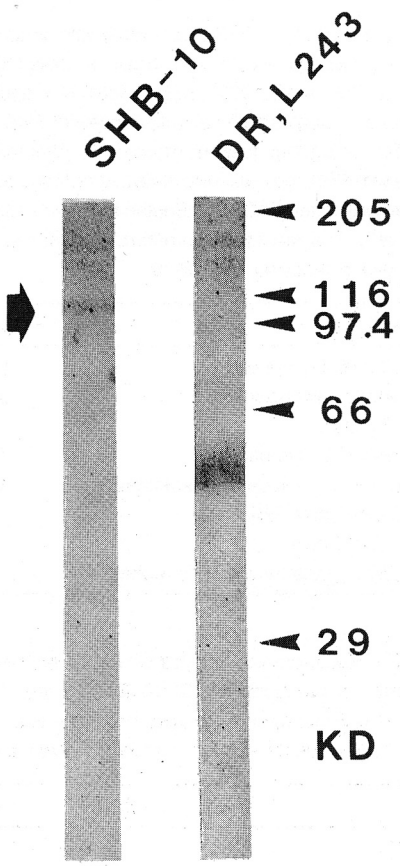

Fig. 1. Molecular characteristics of CALLA recognized by SHB-10 (arrow). Human Burkitt's lymphoma cell line, Daudi is used for lysis and SDS-polyacrylamide gel electrophoresis. The markers in descending order are myosin (m.w. 205kDa), ß-galactosidase (m.w. 116kDa), phosphorylase B (m.w. 97.4kDa), bovine albumin (m.w. 66kDa), and carbonic anhydrase (m.w. 29kDa) (Sigma, MW-SDS-200). DR, L243 mAb is used for positive control.

Flowcytometric analysis of various cell lines, cell suspension of tissue, and peripheral blood

To investigate the distribution of the SHB-10 determinant, several tumor cell lines, cell suspensions of normal lymphoid tissue, and neoplastic (ALL) and non-neoplastic peripheral blood lymphocytes were stained with SHB-10 and analysed on FACS. The results of typical FACS analysis with the various cells are given in table 1 and 2. It should be noted that Daudi and Raji cells showed strong reactivity to SHB-10 while other lymphoid cell lines did not. Some solid tumor cell lines such as SK-N-SH (neuroblastoma) and A172 (glioblastoma) showed reactivity to SHB-10 in varying degrees. The conventional anti-CD10 antibody (SS2/36) showed broader reactivity to several tumor cells than that of SHB-10. There were some differences between the expression patterns of anti-CD10 (SS2/36) and SHB-10. The reactivity of SHB-10 to SK-N-SH and A172 was less intense than that of conventional anti-CD10 (SS2/36). The reaction pattern of SHB-10 seemed to be more specific, showing reactivity to limited human cell lines as compared with conventional anti-CD10. Cell suspensions of tonsils and lymph nodes from non-neoplastic patients and peripheral blood from normal volunteers were stained and analysed with SHB-10 and conventional anti-CD10 (SS2/36). These cells showed no reactivity to either SHB-10 or con-

Table 1. Reactivity of SHB-10 antibody with lymphoid cell lines assessed by flowcytometric analysis. Cells (10^6) from the Daudi, Raji, Jurkat, U937, K562, Molt4, H-9, and CCRF-CEM were incubated with saturating levels of SHB-10 and with FITC-conjugated goat anti-mouse IgG. After three time washes with PBS, the cells were analyzed on a fluorescence-activated cell sorter (FACScan, Becton Dickinson, Mountain-view, Calif.). The results are summarized in percentage of cells showing reactivity to SHB-10.

Cell line	Percent (%)
Daudi (Burkitt's lymphoma)	92.5
Raji (Burkitt's lymphoma)	83.2
Jurkat (T-ALL)	7.6
U937 (histiocytic lymphoma)	11.1
K562 (chronic myelogenous leukemia)	11.8
Molt4 (T cell acute leukemia)	3.0
H-9 (human T-cell)	8.5
CCRF-CEM (acute lymphocytic leukemia)	5.0

Table 2. Flowcytometric analysis of several human tumor cell lines by conventional anti-CD10 (Dako, clone: SS2/36) and SHB-10. Flowcytometric analysis was done as described in table 1. The results are summarized in percentages.

	SS2/36	SHB-10
SK-N-MC (Neuroblastoma)	—#	—
SK-N-SH (Neuroblastoma)	42.3%	18.0%
IMR-32 (Neuroblastoma)	32.3%	—
A375 (Melanoma)	—	—
A172 (Glioblastoma)	68.7%	51.8%
T98G (Glioblastoma)	NC*	—
U87-MG (Glioblastoma-Astrocytoma)	20.9%	—
Y79 (Retinoblastoma)	48.1%	—
RD-ES (Ewing sarcoma)	36.5%	—

: positive cells are less than 10%
* : not checked

ventional anti-CD10. Less than 10% of cell suspensions and peripheral lymphocytes showed reactivity to SHB-10 or to conventional anti-CD10, and the pattern was thought to be nonspecific (data not shown). Most lymphocytes of ALL patient were reactive to both SHB-10 and conventional anti-CD10. The fluorescent intensity of anti-CD10 and SHB-10 to lymphocytes of ALL patient was slightly different but the staining pattern was similar (Fig. 2).

Tissue distribution pattern of the SHB-10 reactive antigen

Immunohistochemical stain was done in cryostat and paraffin-embedded sections of non-neoplastic lymph nodes and tonsils to determine the tissue distribution of CALLA. In both cryostat and paraffin embedded sections of lymph nodes and tonsils, SHB-10 failed to recognize any cells (data not shown).

Blocking the reaction of anti-CD10 to CALLA-positive cells by SHB-10

The antigen detected by SHB-10 was similar to CD10 in molecular weight, and the flowcytometric patterns of SHB-10 and conventional anti-CD10 were similar, so we performed a blocking study to see if SHB-10 recognizes the same CALLA epitope recognized by conventional anti-CD10. Peripheral blood of acute lymphoblastic leukemia patient and Daudi cells were used for the blocking study. The cells were preincubated with a saturated dosage of SHB-10 before adding FITC-conjugated anti-CD10 (W8E7 in blocking study of lymphocyte of ALL patient and SS2/36 in that of Daudi cells). After pretreatment with SHB-10, the reactivity of conventional anti-CD10 to ALL cells and Daudi cells disappeared (Fig. 2.).

DISCUSSION

The CALLA is a cell membrane glycoprotein unique to hemopoietic precursors and lymphoid malignant cells. The CALLA is a single glycosylated polypeptide with an approximate m.w. of 950,000-100,000 (gp 100) containing no intrachain disulfide linkage (Newman et al., 1981). The CALLA is not expressed on normal lymphocytes, but it is expressed in the majority of ALL as well as in other malignancies derived from immature lymphoid population (lymphoblastoid, Burkitt's and nodular poorly differentiated lymphocytic lymphomas, and chronic myelogenous leukemias in lymphoid blast crisis). It is expressed in malignant melanoma cell lines (Carrel et al., 1983). It is also expressed in non-neoplastic cells such as granulocytes and cultured human fibroblasts (Braun et al., 1983), and non-hemopoietic cells (renal tubular and glomerular cells, epithelial cells of the fetal small intestine and myoe-

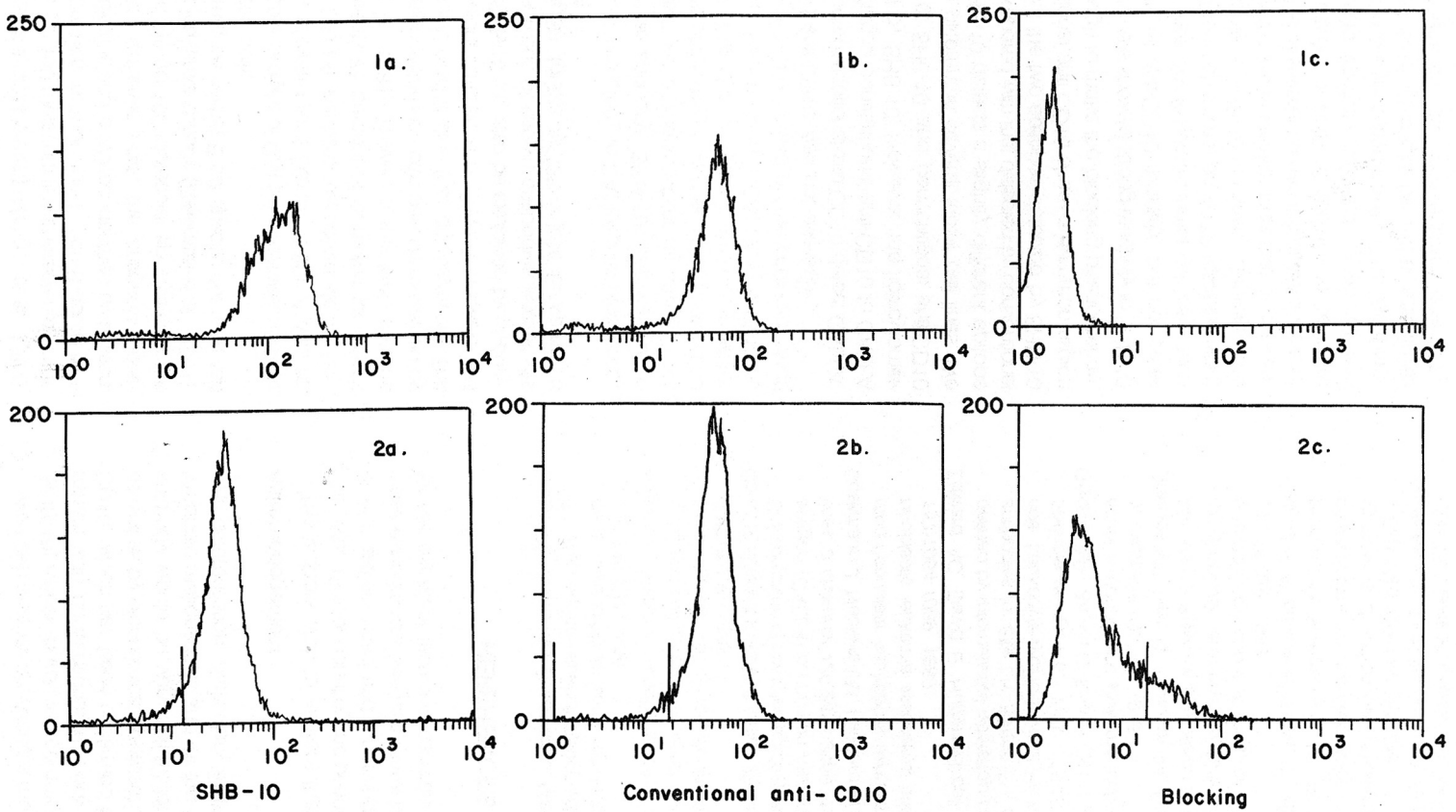

Fig. 2. Blocking study of CD10 by SHB-10 in lymphocytes of ALL patient (1a, 1b, and 1c) and Daudi cells (2a, 2b, and 2c). Lymphocytes of ALL patient and Daudi cells were preincubated with a saturated dose of nonconjugated SHB-10 before reaction with conventional anti-CD10. The cell clone of the conventional anti-CD10 monoclonal antibody used in lymphocytes of ALL patient is W8E7 and that used in Daudi cells is SS2/36. The results of flowcytometric analyses with SHB-10 (1a and 2a) were to similar to those with conventional anti-CD10 (1b and 2b). The binding of conventional anti-CD10 antibody to lymphocytes of ALL patient (1c) is completely blocked by pretreatment of SHB-10 and that to Daudi cells (2c) is mostly blocked.

pithelial cells of the adult breast) (Metigar et al., 1981). Although the gp 100 molecule is a characteristic marker of the common form of non-T, non-B acute lymphoblastic leukemia and is indeed identified by antisera raised against leukemic cells, the antigenic determinant(s) and the gp 100 molecular species itself are probably not leukemia specific (Newman et al., 1981). The CALLA may be neither B cell-specific nor restricted to hemopoietic cells.

The molecular weight of CALLA is between 95kDa and 110kDa (Edward and David, 1986). It was reported that CALLA contains carbohydrate moiety, which represents 25 percent of its molecular weight (Newman et al., 1981). Therefore, it is likely that the molecular weight difference can best be explained by post-translational modification (i.e., glycosylation). The molecular weight difference of CALLA in Daudi cell detected by SHB-10 can also be explained by glycosylation. The isotypes of known antibodies against the CALLA are IgG1, IgG2a, IgG2b or IgM (Edward and David, 1986).

The anti-CD10 reactivity to CALLA was blocked by this monoclonal antibody, SHB-10. The CALLA is known to have two antigenic epitopes, and a "one-way" blocking phenomenon is observed (Edward and David, 1986). This phenomenon is also observed for T cell antigens. The blocking of CALLA to SHB-10 by conventional anti-CD10 could not be checked, since unconjugated anti-CD10 was not available.

SHB-10 and conventional anti-CD10 detect CALLA, and the binding of conventional anti-CD10 to CALLA was blocked by SHB-10. However, the flowcytometric patterns of SHB-10 and conventional anti-CD10 were slightly different in several tumor cell lines. We think that SHB-10 detects a slightly different epitope of CALLA compared with that detected by conventional anti-CD10, but the two epitopes detected by SHB-10 and conventional anti-CD10 share a common region which is enough to make the blocking phenomenon. Daudi and Raji cells showed strong reactivity to SHB-10 and conventional anti-CD10 similarly, but not other lymphoid cell lines. Some human tumor cell lines, such as SK-N-SH (neuroblastoma) an A172 (glioblastoma) showed reactivity to SHB-10 in varying degrees. The intensity of SHB-10 was weaker than that of conventional anti-CD10 in several human tumor cell lines and the reaction pattern of SHB-10 seemed to be more specific, showing reactivity to limited human cell lines as compared with conventional anti-CD10. We therefore think that SHB-10 might be more specific to lymphoid malignancy than conventional anti-CD10 (SS2/36).

We have recently produced a monoclonal antibody (SHB-10) which shared the common antigenic determinant with conventional antibody (SS2/36) against CD10, which has been confirmed by blocking study as well as by biochemical approach. We think that this antibody would be very helpful for the diagnosis of lymphoid malignancies and for the research of leukocyte differentiation, particularly in this country.

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