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Human Monoclonal Antibody Detects a Cell Surface Antigen Expressed on Hematopoietic Malignant Cells of Lymphoid Lineage

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An antigen with a molecular weight of 150 kilodaltons expressed on certain leukemia and lymphoma cells was recognized by a human monoclonal antibody (3H12), which had been established by the fusion of lymphocytes from a small cell lung cancer patient with a mouse myeloma cell line (P3U1). Peripheral blood mononuclear cells from 3 out of 4 cases with lymphoid crisis of chronic myelogenous leukemia (CML) were positively stained by 3H12, while cells from 5 cases with myeloid crisis of CML did not react to this antibody. The antibody did not show any reactivity to cells from the chronic phase of CML, other types of leukemias or normal hematopoietic cells. We further examined 29 cell lines of hematopoietic origin and found that 2 undifferentiated cells (BV-173 and K-562) reacted to the 3H12 antibody. In addition, we found that 3 out of 6 Burkitt lymphoma cells (DAUDI, RAJI and HR1K) reacted to 3H12. Taken together, these results suggest that the antigen recognized by 3H12 is a differentiation-associated antigen expressed on immature lymphoid cells, and could potentially be a reliable cell lineage marker.

Key words: Human monoclonal antibody — CML — Blast crisis — Lineage marker

Monoclonal antibodies against hematopoietic cells are helpful to analyze the immune system at the molecular level and to improve the differential diagnosis of hematopoietic malignancy. The antigens recognized by these antibodies come to be expressed during the process of cell differentiation, and may possess certain functions such as mediating cell-cell interaction and signal transduction.[1)]

Chronic myelogenous leukemia (CML)[6] is characterized by a prolonged but irreversible cell proliferation of granulocyte lineage in the chronic phase, leading eventually to the drastic clinical event of blast crisis. This crisis is classified as myeloid or lymphoid blast crisis according to the cellular phenotype with clonal proliferation. It is important to diagnose the blast transformation and the blast cell type at the earliest period because knowledge of the blast cell type will help to determine the choice of chemotherapy.[2,3)] There are several clinical and hematological indications which suggest the blast crisis of CML.[4)] However, convenient detection methods for the blast cell type are not available. One of the candidate markers is terminal deoxynucleotidyl transferase (TdT), but the percentage of TdT-positive cells in lymphoblastic cells of CML varies case by case.[5,6)] Moreover, we often encounter cases in which morphological discrimination of the blast cell type is difficult. One of the approaches is to detect antigens expressed on restricted lineages of cells with monoclonal antibody. The use of a human antibody would be advantageous from the standpoint of therapeutic trials.

In this study we describe the further characterization of the human monoclonal antibody 3H12, which was previously reported as being specific for small cell lung carcinomas (SCLC).[7)] This antibody reacts to leukemic blast cells of lymphoid origin from CML patients and undifferentiated cell lines, suggesting that the 3H12 antibody recognizes a new cell surface marker in the lymphoid lineage.

MATERIALS AND METHODS

Cell culture All human hematopoietic cell lines tested for reactivity with the 3H12 antibody were maintained at the Fujisaki Cell Center, Hayashibara Biochemical Lab., Inc., Okayama. They were maintained with either RPMI-1640 (GIBCO Laboratories, Grand Island, NY) or Dulbecco's modified Eagle's medium (GIBCO Laboratories) supplemented with 5% fetal calf serum (FCS) (GIBCO Laboratories), 50 units/ml penicillin and 100 μg/ml streptomycin.

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[6] The abbreviations used are: CML, chronic myelogenous leukemia; TdT, terminal deoxynucleotidyl transferase; SCLC, small cell lung carcinomas; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; CD, Cluster of Differentiation; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; EB, Epstein-Barr; CALLA, common acute lymphoblastic leukemia antigen.

Cells from patients and volunteers Peripheral blood cells were obtained by venipuncture from volunteers and mononuclear cells of bone marrow were supplied by a bone marrow transplantation donor. They were collected after Ficoll-Paque gradient centrifugation (Pharmacia Biotechnology, Uppsala)[8] and used for cell surface staining.

Fluorescence-activated cell sorter (FACS) analysis Cells ($1-5\times10^5$) were incubated with 5 μg of biotinylated 3H12 antibody for 1 h at 4°C in staining buffer (phosphate-buffered saline [PBS]/10% FCS/0.1% NaN_3). They were washed three times with the staining buffer and then incubated with fluorescein isothiocyanate (FITC)-conjugated avidin. These cells were washed five times and reacted with propidium iodide (Sigma Chemical Co., St. Louis, MO) in order to exclude dead cells. They were then analyzed with a FACStar (Becton Dickinson, Mountain View, CA) equipped with a logarithmic amplifier.[9]

Cell surface staining and TdT assay The immunophenotypic profiles of all hematopoietic cell lines used were thoroughly characterized, and the cell lineages and differentiation stages were established as reported.[10, 11] Briefly, membrane and intracellular immunofluorescence tests were performed with a number of specific monoclonal and polyclonal antibody reagents. The majority of cell membrane antigens were defined by the Cluster of Differentiation (CD) classification according to the International Workshops for Human Leukocyte Differentiation Antigen.[12] Individual cell cultures from the stock culture were maintained in RPMI-1640/5% FCS and washed with saline, and the cell suspensions (approximately 1×10^5 cells in 15 μl) were stained with 15 μl of primary monoclonal antibody reagent for 30 min. The cells were then washed once with saline and stained again with 15 μl of FITC-conjugated goat anti-human immunoglobulin reagent (Cappel, Cochranville, PA) for 30 min. The cells were washed and resuspended with saline for FACS analysis or for fluorescence microscopy. For the TdT test, an indirect intracellular immunofluorescence test was carried out using a predetermined dilution of rabbit anti-calf thymus TdT antibody as the primary reagent (PL Laboratories, Inc., Milwaukee, WI) followed by FITC-conjugated goat anti-rabbit immunoglobulin reagent (Cappel) as the secondary reagent on methanol-fixed cells. Immunofluorescence microscopic analysis was performed to determine the positive cells. Based on multiple marker analysis, a particular marker profile of each hematopoietic cell line was established, allowing the assignment of each cell line to its respective hematopoietic differentiation lineage and stage.[10, 11]

Immunoprecipitation Cells (1×10^7) were metabolically labeled with [^{35}S]cysteine (37 MBq, ICN Radiochemicals, Irvine, CA) in 5 ml of cysteine-free RPMI-1640/10% FCS for 8 h. Radiolabeled cells were solubilized in 1% Nonidet P-40 (Sigma Chemicals Co.)/PBS at 4°C for 1 h. The labeled materials were preabsorbed with anti-human IgM-coupled Protein A Sepharose (Pharmacia Biotechnology) to remove nonspecifically bound substances before immunoprecipitation. Radiolabeled cell extracts were incubated with either 150 μg of 3H12 or with control human IgM (Cooper Biomedical Inc., Malvern, PA) for 2 h at 4°C. They were further reacted with goat anti-human IgM-conjugated Protein A Sepharose for 2 h at 4°C. After extensive washing with 1% Nonidet P-40/PBS, the precipitants were eluted with Laemmli's sample buffer[13] and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 5% acrylamide) under reducing conditions. The gels were dried and autoradiographed with Kodak XAR-5 film.

RESULTS AND DISCUSSION

Reactivity of 3H12 to normal and patients' blood cells We have established a stable mouse-human heterohybridoma, which produces human monoclonal antibody 3H12 recognizing a specific cell surface molecule on SCLC and certain malignant lymphoid cell lines. We further examined its reactivity to hematopoietic cells from peripheral blood cells of lymphoma patients by cell surface staining. The antibody reacted to lymphoid

Table I. Reactivity of 3H12 to Hematopoietic Cells from Various Malignancies and Normal Donors

Cell source	3H12-positive cases/total examined
CML[a]	
chronic phase	0/5
myeloid crisis	0/5
lymphoid crisis[b]	3/4
CLL	0/1
AML	0/4
ALL	0/4
Normal	
PBL	0/4
Monocyte	0/4
RBC	0/4
Platelets	0/4
Granulocytes	0/4
Bone marrow	0/1

a) The abbreviations used are: CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; PBL, peripheral blood lymphocytes; RBC, red blood cells.
b) Including one case of biphenotypic (lymphoid and myeloid) crisis.

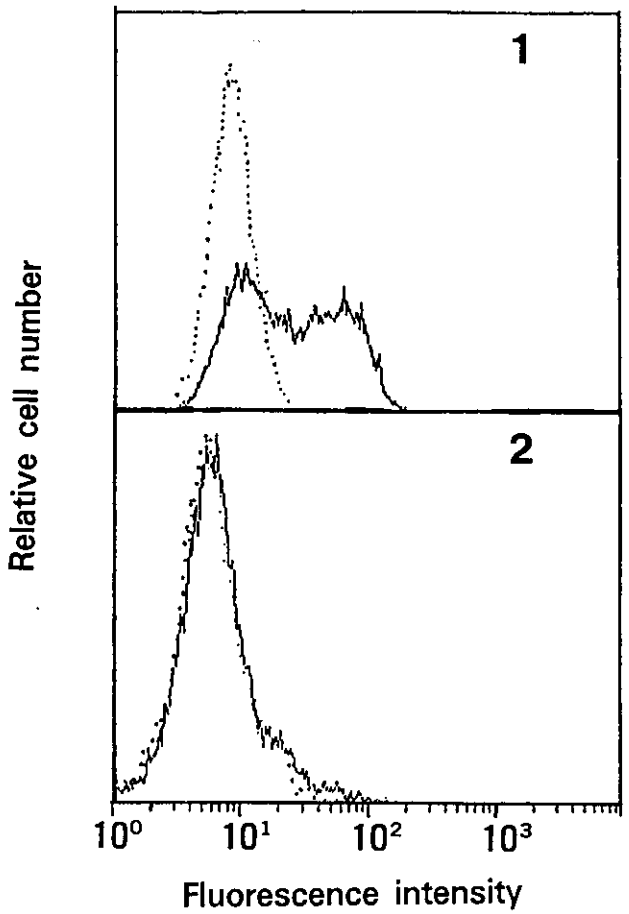

Fig. 1. Cell surface analysis of peripheral blood from CML. Peripheral blood from patients with lymphoid crisis of CML (1) and chronic phase of CML (2) were stained with 3H12 antibody (——) or isotype-matched control antibody (·····) followed by FITC-conjugated goat anti-human IgM. The fluorescence was analyzed by FACS with a logarithmic amplifier.

blastic leukemia cells of CML (3 out of 4 cases), but we could not detect any positive cells from myeloid crisis or chronic phase of CML patients (Table I). The antibody showed no reactivity to normal cells of hematopoietic origin to other types of leukemias including CLL, AML and ALL. Typical cell surface staining profiles examined by FACS on lymphoid and myeloid blast cells of CML are shown in Fig. 1. The results for lymphoblastic CML show biphasic patterns: positive cells were lymphoid blastic cells and negative cells were nonblastic cells. The staining profiles of the other two CML patients with lymphoid crisis showed similar fluorescence patterns. No significant positively stained cells were found in CML patients of myeloid blastic crisis.

This antibody had been initially established against SCLC and was found to be reactive to lymphoblastic

Table II. Reactivity of 3H12 to Various Leukemia and Lymphoma Cells

Cell line	Origin	% fluorescence-positive cells
T lymphocyte		
RPMI8402	ALL[a)]	0
DND-41	ALL	0
HPB-ALL	ALL	0
MOLT-13	ALL	0
JM	ALL	0
MOLT-3	ALL	0
MOLT-4	ALL	0
MOLT-16	ALL	0
MOLT-16	ALL	0
PEER	ALL	0
H9	ALL	0
MKB-1	AML	0
HPB-MLT	LY	0
A3/Kawakami	LY	0
MT-1	ATL	0
ED-S$^-$	ATL	0
HUT-78	SS	0
B lymphocyte		
NALM-1	CML	0
NALM-6	ALL	0
KOPN-8	ALL	0
BALL-KH	ALL	0
RAJI	BL	80
HRIK	BL	100
B35M	BL	10
DAUDI	BL	100
DG-75	BL	0
B46M	BL	0
Null lymphocyte		
REH	ALL	0
KM-3	ALL	0
Myelomonocyte		
KG-1	AML	0
ML-2	AML	0
MOLM-1	CML	0
GDM-1	CML	0
KU-812	CML	0
Immature hematopoietic cell		
K-562	CML	80
BV-173	CML	100

a) The abbreviations used are: ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; LY, lymphoma; ATL, adult T-cell leukemia; SS, Sézary syndrome; CML, chronic myelogenous leukemia; BL, Burkitt lymphoma.

cells. There are several reports that SCLC shares determinants of hematopoietic cell origin such as Leu-7.[14, 15] Moreover, Ruff and Pert suggested that SCLC originated

from hematopoietic stem cells, based on the reactivity of macrophage-specific monoclonal antibody to SCLC.[16] Thus, our findings tend to support the above observations.

Reactivity of 3H12 to various leukemia and lymphoma cell lines Since 3H12 antibody seems to react preferentially to lymphoid but not to myeloid blastic cells, we examined the reactivity of 3H12 to various cultured hematopoietic cell lines in order to analyze further the 3H12 antigen expression. The results in Table II show that 3H12 did not react to most of the leukemic and lymphoma cell lines of various cell lineages and origins. However, it did react to immature cell lines with the capacity to differentiate into more than one lineage (K-562 and BV-173) and to some cell lines of Burkitt lymphomas (RAJI, HRIK and DAUDI). The immunofluorescence profiles of BV-173 showed the typical cell surface staining with a patchy pattern (Fig. 2). We further examined the various representative lineage markers (B lymphocyte markers, CD10, CD19 and CD20; T lymphocyte, CD2 and CD7; myeloid lineage, CD14 and CD33) and TdT activity on 3H12-positive cell lines and lymphoblastic cells from CML patients. The results in Table III show that the reactivity of 3H12 was not directly correlated with the lineage markers listed or the TdT activity, suggesting that this antibody might recognize a new marker expressed on immature hematopoietic cells.

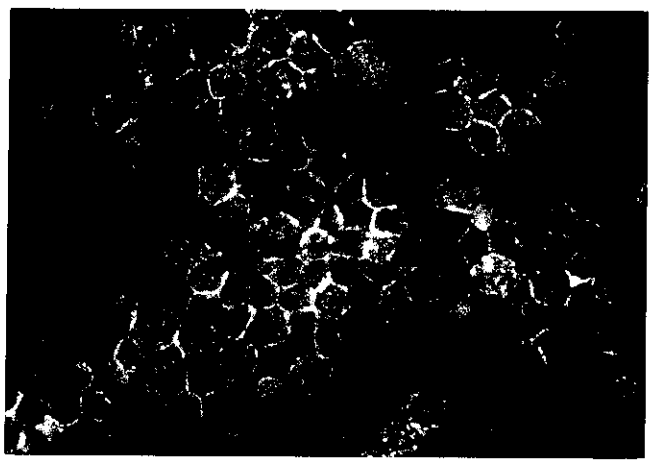

Fig. 2. Immunostaining of BV-173 with 3H12 antibody.

The reactivity of 3H12 to Burkitt lymphoma cells is not clearly understood at present. In fact, we could also detect the 3H12 antigen on bone marrow from a patient with Burkitt lymphoma (data not shown). Since the 3H12 antibody does not react to NALM-1 cells[17] which are considered to be in a similar differentiation stage of B cells to RAJI and DAUDI, the expression of 3H12 in Burkitt lymphomas seems not to be dependent on the B lymphocyte differentiation stages. Thus, we suggest that these cells express the 3H12 antigen, which, as reported, might be driven by trans-acting transcription activating factor(s) of EB (Epstein-Barr) virus origin.[18, 19]

Immunoprecipitation by 3H12 antibody In order to characterize the biochemical properties of the molecule recognized by 3H12 antibody, we performed immunoprecipitation experiments on BV-173 and DAUDI cell lines. As shown in Fig. 3, the 150 kDa molecule was precipitated from both cell lines under reducing condi-

Table III. Comparison of 3H12 Reactivity and Various Cell Surface Markers on Hematopoietic Cells

Cell	Type	Cell surface markers								
		3H12	TdT	lymphoid					myeloid	
				CD10	CD19	CD20	CD2	CD7	CD14	CD33
BV-173	immature	+	+	+	+	−	−	−	−	−
K-562	immature	+	−	−	+	−	+	+	+	−
MOLM-1	myeloid	−	−	−	−	−	−	−	−	+
GDM-1	myeloid	−	−	−	−	−	−	−	−	+
NALM-1	pre-B cell	−	+	+	+	+	−	−	−	−
MOLT-4	T cell	−	+	−	−	−	+	+	−	−
Blastic crisis of CML										
Lymphoid crisis pt[a)]		+	NT	+	+	NT	−	−	−	−
Lymphoid crisis pt		+	+	+	+	NT	NT	NT	+	+
Myeloid crisis pt		−	−	−	−	NT	−	−	+	+

a) The abbreviations used here: pt, patient; NT, not tested.
+/−: based on the data on the activity of TdT or the immunostaining profiles of the cells with various anti-CD monoclonal antibodies analyzed by FACS.

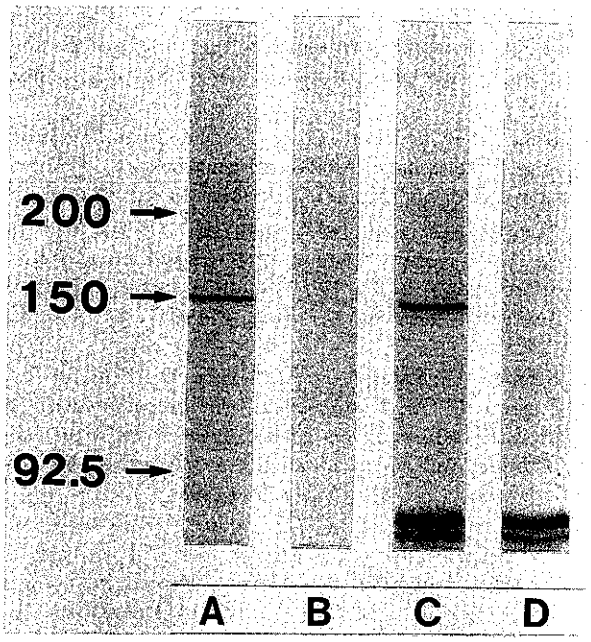

Fig. 3. Immunoprecipitation analysis on BV-173 and DAUDI cells with 3H12 antibody. [^{35}S]Cysteine-labeled BV-173 (lanes A and B) and DAUDI cells (lanes C and D) were immunoprecipitated with 3H12 antibody (lanes A and C) or isotype-matched control antibody (lanes B and D). The immunoprecipitated materials were subjected to 5% acrylamide SDS-PAGE under reducing conditions. The molecular weights were determined by using standard proteins as markers.

tions. The molecule migrated to the same position even under nonreducing conditions, suggesting that the 3H12 antigen is composed of a single molecule. Since the molecule recognized by the 3H12 antibody on SCLC is also 150 kDa, the same molecule is considered to be present on the above cells.

We have surveyed the known human leucocyte differentiation antigens and conclude that the 3H12 antigen can not be classified into any of the reported Clusters of Differentiation.[12] CD21, which has recently been recognized as the C3d receptor (also EB virus receptor) with a molecular mass of 145 kDa,[20] is relatively close to the molecular size precipitated by the 3H12 antibody. However, the 3H12 antigen is different from CD21 in that the latter is expressed on most B lymphomas, B cell CLL and 50% of bone marrow cells whereas the 3H12 antigen is not. Our recent preliminary studies on sequential immunoprecipitations using two antibodies indicate that CD21 is different from 3H12, despite the similarity of the molecular weights of the two molecules. The panel study with other CD antigens in Table III indicates that CD19 reactivity is relatively similar to the specificity of 3H12.[21] However, upon further analysis of the cell reactivities and molecular masses precipitated by the antibodies (CD19 is a 95 kDa glycoprotein expressed on normal and malignant B cells), it was clear that they are not identical. CD10, which is described as a common acute lymphoblastic leukemia antigen (CALLA), is expressed on lymphoblasts from patients with ALL and CML in lymphoid crisis and on cell lines of Burkitt lymphomas.[22,23] However, CD10 is also expressed in neuroblastoma cell lines, GOTO and NB-1, but 3H12 does not react to these cells,[7] or to ALL. Moreover, the molecular weight of CALLA is 100 kDa which is different from that of the antigen defined by 3H12. Thus, the 3H12 antigen seems to be a unique cell surface marker and might be a representative of a new cluster of differentiation antigens. Since this human antibody detects an antigen specifically expressed on lymphoblastic cells, it should be a useful tool for the diagnosis of lymphoblastic crisis of CML and may potentially be applicable therapeutically.

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