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Monoclonal Antibody against Myeloid Leukemia Cell Line (KG-1)[1]

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ABSTRACT

A murine monoclonal antibody (WI-5) was produced against a myeloid leukemia cell line (KG-1). The antibody was immunoglobulin G3 κ. It reacted only against KG-1 cells and failed to react against 33 other cell lines representing fibroblasts, solid tumors, and cells of myeloid and lymphatic origin. It also showed no reaction against normal red blood cells, granulocytes, platelets, monocytes, and T- and B-lymphocytes. Similarly, there was no reaction against lymphocytes transformed by mitogens. Peripheral blood and bone marrow samples from acute granulocytic and acute lymphocytic leukemia, chronic granulocytic leukemia, chronic granulocytic leukemia in blastic crisis, and chronic lymphocytic leukemia failed to react with WI-5. It was suggested that WI-5 detected a unique antigen on KG-1 cells.

INTRODUCTION

Tumor-specific antigens have been shown in the murine system (6). The existence of antileukemic antibodies in patients with leukemia was indicated in earlier studies as reviewed by Harris (7). Antisera produced in animals against leukemia cells have demonstrated leukemia-associated antigens (16, 18, 19). Recently, it has become possible to detect cell differentiation and leukemia-specific antigens using the hybridoma technique (5, 17, 22). The myeloid leukemia cells have been used as antigens. The antibodies produced in this manner reacted with normal as well as leukemia cells (5, 17, 22). We report the characterization of a monoclonal antibody (WI-5) which was produced against a human myeloid leukemia cell line (KG-1) (10, 14).

MATERIALS AND METHODS

Cell Lines. Thirty-three cell lines of human origin (HL-60, K-562, KG-1, ML-3, RC2a, NALL-1, Reh, NALM-16, Molt4, RPMI-8402, NALM-1, NALM-6, RPMI-1788, RPMI-4098, RPMI-6410, AK-1, U-266, RPMI-8226, GM-1500, Daudi, Namalwa, U-937, U-Amnion, WISH, GM-45, HT-144, MeMo, G-361, Hep-2, HeLa, A-549, HT-29, and BT-20) were cultured in RPMI 1640[4] (Grand Island Biological Co., Grand Island, N. Y.) with 10 to 15% heat-inactivated fetal bovine serum (Grand Island Biological Co.) and used in these experiments. P-3-NS-1/1Ag4-1 (NS-1) cell line was cultured in RPMI 1640 with 10% heat-inactivated horse serum (Grand Island Biological Co.). The media were supplemented with gentamicin (50 μg/ml) (Grand Island Biological Co.). The cells were cultured at 37° in 5% CO_2 humidified atmosphere.

Preparation of Target Cells for Assays. Normal buffy coats, supplied by Wadley Central Blood Bank, were diluted 1:5 in RPMI 1640 and separated into mononuclear and nonmononuclear cells by the Ficoll:Hypaque (Sigma Chemical Co., St. Louis, Mo.) density gradient method (2). Cells obtained from the interface consisted of over 99% mononuclear cells when analyzed morphologically following Wright's stain. The mononuclear cells were further separated into monocytes, T-lymphocytes, and B-lymphocytes by a sequential method of adherence to Petri dishes and filtration through a nylon wool column as described elsewhere (1). The purity of monocytes and T-lymphocytes was tested by indirect immunofluorescence using murine monoclonal antibodies MO-2 and T-11 (Coulter Electronics, Hialeah, Fla.) and found to be 75 to 85% and 85 to 95%, respectively (8, 24).

Platelets. The platelet-rich plasma was obtained from diluted buffy coats by centrifugation at 200 × *g* for 10 min.

Granulocytes. The pellets of RBCs and granulocytes were collected from the bottom of the Ficoll:Hypaque gradient. RBCs were removed by dextran sedimentation followed by lysis of residual RBCs (1). The final preparation contained 95% granulocytes as determined morphologically.

Immature Cells. Peripheral blood from patients with leukemia was obtained in heparin, and bone marrow samples were collected in EDTA Vacutainer tubes (Vacutainer Systems, Rutherford, N. J.). The mononuclear and immature cells were obtained by Ficoll:Hypaque density gradient centrifugation (2). The mononuclear cells were placed into 100- x 14-mm Petri dishes in 25 ml RPMI 1640 with 15% heat-inactivated human AB serum (Grand Island Biological Co.) and cultured overnight at 37° in 5% CO_2 humidified atmosphere to remove monocytes. The nonadherent cells were identified morphologically following Wright's staining.

Other Cells. Lymph node, adenoid, thymus, and spleen cells were obtained from specimens removed surgically from patients for clinical reasons. Histological reports of these specimens were normal.

Mitogen-induced Transformation. Transformed human lymphocytes were obtained after stimulation with phytohemagglutinin (Burroughs Wellcome Ltd., Beckenham, England), pokeweed mitogen (Sigma, St. Louis, Mo.), and concanavalin A (Pharmacia, Uppsala, Sweden), utilizing the conditions described previously (11).

Panel of Lymphocytes for HLA Typing. The antibody (WI-5) was tested by the complement-dependent microcytotoxicity test (20) against a panel of lymphocytes from 53 individuals, which were routinely used for HLA typing.

Immunization. A 6-week-old female BALB/c mouse was immunized against KG-1 cells by 2 i.v. injections of 1×10^7 cells in 0.1 ml phosphate-buffered saline given 10 days apart. The spleen was removed 4 days after the second injection. Hybridization was carried out according to the methods of Kohler and Milstein (15) and Kennett *et al.* (9) with minor modifications as described previously (1).

Hybridoma Screening. The hybridoma supernatants were tested for antibody against KG-1 cells by the complement-dependent microcytotoxicity test, using the Terasaki technique described in Ref. 20. Unrelated hybridoma supernatant containing IgG and IgM were used as negative controls.

Cloning and Propagation of Hybridomas. One of the positive hybridomas (WI-5) was cloned and subcloned using the limiting dilution technique. It was maintained in RPMI 1640 containing 10% head-inactivated horse serum and gentamicin (50 μg/ml). The immunoglobulin class of WI-5 was determined by the Ouchterlony method using goat anti-mouse heavy or light chain-specific sera (Meloy Laboratories, Springfield, Va., and Miles Laboratories, Inc., Elkhart, Ind.).

The hybridoma cells (1×10^7) were also injected i.p. into BALB/c

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[4] The abbreviation used is: RPMI 1640, Roswell Park Memorial Institute Tissue Culture Medium 1640.

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mice 2 to 3 weeks after priming with an i.p. injection of 0.5 ml of 2,6,10,14-tetramethylpentadecane (Pristane) (Sigma). The ascitic fluid was removed 10 to 14 days after the injection of cells.

Indirect and Direct Immunofluorescence Tests. The indirect immunofluorescence test was performed according to the method described by Brooks *et al.* (3) with minor modifications. The incubation periods with the first and the second antibody were increased to 30 min each at 4°. The fluoresceinated IgG fraction of goat anti-mouse IgG (Meloy) was used as the second antibody. Immunofluorescence studies against all target cells were done with mouse ascites fluid (1:10 dilution). The indirect immunofluorescence titer of mouse ascites fluid of WI-5 was 1:500 against KG-1 cells. Unrelated hybridoma mouse ascites fluid was used as negative control. The cells were read by fluorescent microscopy using an American Optical microscope (American Optical Scientific Instruments, Buffalo, N. Y.). At least 200 cells were counted for each sample.

Immunoprecipitation of Antigens. KG-1 cells were labeled by biosynthetic incorporation of [^{3}H]leucine (New England Nuclear, Boston, Mass.) (25). Cells were lysed, and the antigen was immunoprecipitated with WI-5 monoclonal antibody against HLA loci designated as A, B, and C (Cappel Laboratories, Cochranville, PA.) and an unrelated control monoclonal antibody (IgG).

The precipitate was washed 3 times and analyzed by sodium dodecyl sulfate:polyacrylamide gel electrophoresis (12). The gel was cut lengthwise, obtaining 2.5-mm slices. Radioactivity in each slice was counted in a scintillation counter using a liquid scintillation fluid (Handifluor; Mallinckrodt Chemical Works, Paris, Ky.). A gel containing low-molecular-weight protein standard (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) with added β_2-microglobulin (labeled with ^{125}I) was obtained from Pharmacia. The molecular weight of the antigen, as defined by the peak of radioactivity in the gel slices, was calculated by comparison with the standards.

Absorption Test. WI-5 ascites fluid, 0.5 ml of 1:10 dilution, was absorbed with 5×10^6 to 2×10^8 KG-1 cells/ml at room temperature for 1 hr. After incubation, cells were centrifuged at $400 \times g$ for 5 min. The supernatant of each tube was tested for reactivity against KG-1 cells by indirect immunofluorescence.

RESULTS

Characterization of Monoclonal Antibody. The antibody, WI-5, gave 4+ reaction against KG-1 cells in microcytotoxicity and indirect immunofluorescence tests. WI-5 was found to be IgG κ as determined by the Ouchterlony method. In the absorption test, when 7.5×10^7 KG-1 cells were used, WI-5 lost activity against KG-1 cells. The hybridoma has been stable for the past 15 months and is still secreting antibody.

Reactivity against Cell Lines. WI-5 reacted against KG-1 cells but failed to react against other leukemia, myeloma, lymphoid, fibroblast, and solid tumor cell lines (Tables 1 and 2).

Lack of Reactivity against Hemopoietic Cells and Transformed Lymphocytes. Table 3 shows that WI-5 failed to react against normal human RBCs, mature granulocytes, T-cells, B-cells, monocytes, platelets, splenocytes, thymocytes, and lymph node and adenoid cells. The antibody was tested by the microcytotoxicity assay against a panel of lymphocytes from 53 individuals, which is used for HLA typing. No cytotoxicity was seen with the cells in the panel. Lectin-induced normal human lymphoblasts did not react with WI-5 as shown in Table 3.

Reactivity of WI-5 against Peripheral Blood Cells from Patients with Leukemia. The nucleated cells collected by the Ficoll:Hypaque method were found to consist of blasts, promyelocytes, myelocytes, some metamyelocytes, monocytes, and lymphocytes. Table 4 shows the ranges and the mean percentages of myeloblasts and promyelocytes in the Ficoll:Hypaque-separated mononuclear cells and reactivity of WI-5. There were 7 cases of acute granulocytic leukemia in the active phase of the disease. None of the samples reacted with WI-5.

Table 1
Reactivity of WI-5 against hematopoietic cell lines

Cell line	Cell type	Cytotoxicity (%)	Immunofluorescence[a]
KG-1	Acute granulocytic leukemia	100	4+ (100%)
ML-3	Actue granulocytic leukemia	0	0
RC2a	Acute myelomonocytic leukemia	0	0
HL-60	Acute promyelocytic leukemia	0	0
K-562	Erythroid, myeloid	0	0
Reh	Null	0	0
NALL-1	Null	0	0
NALM-16	Null	0	0
Molt-4	T-cell	0	0
RPMI-8401	T-cell	0	0
NALM-1	Pre-B	0	0
NALM-6	Pre-B	0	0
RPMI-1788	B-cell	0	0
RPMI-4098	B-cell	0	0
RPMI-6410	B-cell	0	0
AK-1	B-cell	0	0
U-266	Myeloma	0	0
RPMI-8226	Myeloma	0	0
GM-1500	Myeloma	0	0
Daudi	Burkitt's lymphoma	0	0
Namalwa	Burkitt's lymphoma	0	0
U-937	Histiocytic lymphoma	0	0

[a] Intensity score fluorescence: 0, no fluorescence above background; 1+, faint fluorescence; 2+, weakly bright fluorescence; 3+, moderately bright fluorescence; 4+, strongly bright fluorescence.

Table 2
Reaction of WI-5 against cell lines growing in monolayers

Cell line	Tissue	Immunofluorescence
U-Amnion	Normal amnion	0
WISH	Normal amnion	0
GM-45	Normal fibroblast	0
HT-144	Malignant melanoma	0
MeMo	Malignant melanoma	0
G-361	Malignant melanoma	0
Hep-2	Epidermoid cancer	0
Hela	Cervical cancer	0
A-549	Lung cancer (adenocarcinoma type)	0
HT-29	Colon cancer (adenocarcinoma type)	0
BT-20	Breast cancer (adenocarcinoma type)	0

Table 3
Reactivity of WI-5 against various human cells

Cells	No. of samples tested	Immunofluorescence (%)
RBCs	30	0
Granulocytes	30	0
Lymphocytes	30	0
T-cells	5	0
B-cells	5	0
Spleen cells	3	0
Lymph node cells	3	0
Lymph node from Burkitt's lymphoma	1	0
Adenoid cells	3	0
Thymocytes	1	0
Monocytes	10	0
Platelets	10	0
Mitogen[a]-induced lymphoblasts	18	0
Lymphocyte panel cells for HLA typing[b]	53	0

[a] Phytohemagglutinin, pokeweed, and concanavalin A were used as mitogens.
[b] Tested by microcytotoxicity technique.

Table 4
Reactivity of WI-5 against peripheral blood cells

Diagnosis	No. of samples tested	Immunofluorescence test	% of immature cells[a]
AGL[b] (remission)	6	Negative	0
AGL (relapse)	7	Negative	19–71 (45)[c]
ALL (remission)	10	Negative	0
ALL (relapse)	3	Negative	29–72 (46)
CGL	4	Negative	9–40 (21)
CGL (blast crisis)	5	Negative	76–94 (80)
CLL	5	Negative	0

[a] Immature cells include breast, promyelocytes, and myelocytes.
[b] AGL, acute granulocytic leukemia; ALL, acute lymphocytic leukemia; CGL, chronic granulocytic leukemia; CLL, chronic lymphocytic leukemia.
[c] Numbers in parentheses, mean range.

Table 5
Reactivity of WI-5 against bone marrow cells

Diagnosis	No. of samples tested	Immunofluorescence test	% of immature cells[a]
Normal	2	Negative	43–44 (43.5)[b]
AGL[c]	8	Negative	39–75 (49.2)[b]
ALL	8	Negative	40–68 (51.2)[b]
CGL	7	Negative	71–84 (79.4)[b]
CLL	2	Negative	25–26 (25.5)[b]

[a] Immature cells include blasts, promyelocytes, and myelocytes.
[b] Numbers in parentheses, mean range.
[c] AGL, acute granulocytic leukemia; ALL, acute lymphocytic leukemia; CGL, chronic granulocytic leukemia; CLL, chronic lymphocytic leukemia.

Reactivity of WI-5 against Bone Marrow Cells. The nucleated cells collected by the Ficoll:Hypaque method were found to consist of blasts, promyelocytes, myelocytes, some metamyelocytes, monocytes, lymphocytes, plasma cells, and immature cells of the erythroid series. WI-5 failed to react with these cells as shown in Table 5.

Characterization of the Antigen. The biosynthetically labeled antigen was immunoprecipitated with WI-5. Chart 1 shows a single band of immunoprecipitated antigen with a molecular weight of 44,000. A monoclonal antibody (mouse IgG), not reactive against KG-1 cells, was used as a negative control which did not show immunoprecipitation. Anti-HLA loci designated as A, B, and C gave 2 peaks, one at M_r 44,000 and the other at about M_r 12,000. The low-molecular-weight peak corresponded to the peak of radioactivity in the protein standard which represented β_2-microglobulin.

DISCUSSION

In this study, we examined the reaction pattern of monoclonal antibody (WI-5) against KG-1 cells. WI-5 failed to react with normal and abnormal hemopoietic cells, transformed cells, and solid tumor cell lines. The reaction between WI-5 and KG-1 cells was confirmed by microcytotoxicity, immunofluorescence, and absorption tests. The KG-1 cells did not possess Fc receptors; therefore, WI-5 could not have bound to KG-1 cells through Fc receptors (13). The molecular weight of the antigen, reacting with WI-5, was 44,000. This molecular weight is similar to that of the HLA heavy chain (23). However, WI-5 did not react with a panel of lymphocytes which included the HLA antigens of KG-1 cells (14). The analysis of antigen with WI-5 showed a single peak, but the anti-HLA antibody showed

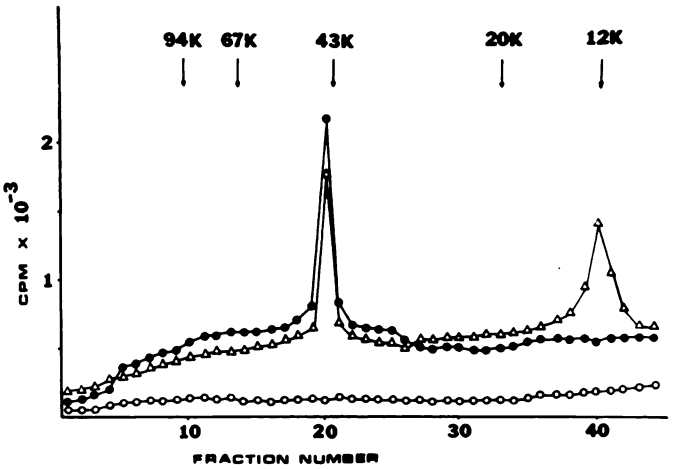

Chart 1. Sodium dodecyl sulfate:polyacrylamide gel electrophoresis of biosynthetically labeled ([^{3}H]leucine) and immunoprecipitated antigen of KG-1 cells by WI-5. ●, precipitated with WI-5; △, precipitated with anti-HLA loci designated as A, B, and C; ○, precipitated with control antibody which did not react with KG-1 cells. The molecular weight of the corresponding protein standard is given (*top*).

a second peak which corresponded to β_2-microglobulin (Chart 1). These observations suggested that WI-5 reacted with an antigen other than HLA.

In the mouse tumor systems, the existence of individual tumor-specific antigens has been reported (21). In humans, a unique antigen was defined on malignant melanoma (4). Recently, it was shown that murine monoclonal antibodies, against myeloid leukemia, recognized differentiation antigens of the myeloid series. These antibodies did not detect leukemia-specific antigen (5, 17). The KG-1 cell line, which was kindly supplied by Dr. D. W. Golde, did not contain virus (13), and we also failed to detect virus particles on electron microscopy. Seven samples of peripheral blood and 8 samples of bone marrow from acute granulocytic leukemia patients had no reaction with WI-5. It is therefore possible that WI-5 detected an individually specific antigen. It is also possible that specimens from other patients with acute granulocytic leukemia may react with WI-5 when a larger number of samples is tested.

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