Murine Monoclonal Antibodies (MCS-1 and MCS-2) Reactive with Human Myeloid Leukemia Cells

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Received for publication June 21, 1995

Summary: In an attempt to identify the antigens expressed on human myeloid leukemia cells, two murine monoclonal antibodies (mAb) designated as MCS-1 (isotype; IgG3) and MCS-2 (IgG1) were raised. MCS-1 reacted with peripheral blood granulocytes, but not with monocytes, whereas MCS-2 reacted with both granulocytes and monocytes. The incidence of MCS-1 and MCS-2 reactivity with the cells from a total of 121 patients with various type of leukemias was as follows: 25/46 (54%) and 39/47 (83%) in acute myeloblastic leukemia and acute monoblastic leukemia, 8/16 and 16/16 in chronic myeloid leukemia in blastic crisis (CML-BC) of myeloid type, 0/7 and 2/7 in CML-BC of lymphoid type, 0/26 and 2/32 in acute lymphoblastic leukemia (ALL), 7/7 and 7/7 in chronic phase of CML, respectively. Two cases of MCS-2 positive ALL had a Philadelphia chromosome marker (Ph¹). In contrast, neither MCS-1 nor MCS-2 reacted with lymphocytes or any of leukemic and non-leukemic lymphoid cell lines tested. These results indicate that MCS-1 and MCS-2 mAb recognized two different differentiation antigens expressed on granulocytes and monocytes. These mAb would be useful reagents to determine differentiation antigens on the cells in granulocyte and monocyte lineage.

Key words: myeloid leukemia — myeloid antigen — monoclonal antibody — CD13 — CD15 — MCS-1 — MCS-2

Introduction

During the past decade the surface antigens on cells from human hematopoietic malignancies have been intensively studied with murine monoclonal anti-

bodies (mAb) (Kaplan et al. 1989; Kurec et al. 1991). However, the immunological analyses of the cells in acute myeloblastic and monoblastic leukemias (AML and AMoL) have not yet been well studied (Neame et al. 1986; Drexler, 1987; Merle-

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Beral et al. 1989). We have developed two mAb using two human myeloid leukemia cell lines (ML-3 and HL-60) as immunogens and a panel of myeloid and non-myeloid cell lines as a screening panel for antibody specificity. The present study describes the two anti-myeloid mAb, MCS-1 and MCS-2, that were subsequently determined as CD15 mAb and CD13 mAb, respectively, in the International Workshop on Human Leukocyte Differentiation Antigens.

Materials and Methods

Cell lines

A total of 55 cell lines (45 permanent human malignant hematopoietic cell lines and 10 EBV-transformed B-lymphoid cell lines) were used in the present study. The cells of these cell lines were properly used as immunogens or target cells for screening of hybridoma antibody reactivity. The immunological markers of these cell lines were previously described in detail (Minowada et al. 1982). They were maintained in RPMI1640 medium (GIBCO, Grand Island, NY) supplemented with 5% heat-inactivated fetal calf serum (FCS) (GIBCO), 100 U/ml penicillin (ICN, Cleveland, OH), and 50 μ g/ml streptomycin (ICN) in humidified 5% CO₂ in air at 37°C. Care was taken to use all cell lines for the study in an exponential growth phase in which the full expression of antigens was expected. The cells were negative for mycoplasma infection at the time of study.

Leukemia cells, thymocytes, and normal peripheral blood leukocytes

Leukemia cells were obtained from the heparinized venous blood or aspi-

rated bone marrow cells of the patients whose diagnoses were made by the conventional hematological and immunological methods (Minowada et al. 1982). Mononuclear cells were isolated by Ficoll-Hypaque discontinuous density gradient centrifugation. In all cases, the percentage of leukemic blasts in the isolated mononuclear cell population was more than 80%.

Thymocytes were prepared by mincing and filtrating through a stainless steel wire mesh from the thymuses which were obtained from the patients under corrective cardiac surgery who do not have hematological disorders. Informed consents were obtained for each case and the use of left-over cells after diagnostic procedure was approved by the Institutional Review Committee. Peripheral blood leukocytes in the heparinized venous blood obtained from the healthy volunteers were analyzed without separation of the cells by using immunofluorescence method and under FACScan® flow cytometry (Becton Dickinson Immunocytometry Systems, San Jose, CA) after lysing the erythrocytes.

Immunological marker analysis

The leukemia cells were characterized by multiple immunological markers as previously described (Minowada et al. 1982). Briefly, E-, EA- and EAC-resetting cells were counted by using sheep erythrocytes (E), bovine erythrocyte-IgG antibody complex (EA), and bovine erythrocyte-IgM antibody-complement complex (EAC), respectively. Surface membrane immunoglobulins (SmIg) and cytoplasmic immunoglobulins (CyIg) were detected by direct immunofluorescence using fluorescein-isothiocyanate (FITC) conjugated goat anti-human immunoglob-

ulin light or heavy chain specific reagents (Cappel, Cochranville, PA). Human T-cell antigen (T-Ag) (Tsubota et al. 1977), Ia-like (gp 28, 33) antigen (Koyama et al. 1977), and antigen associated primarily with common form of ALL (cALL) (Koshiba et al. 1978) were assessed by indirect membrane immunofluorescence using respective polyclonal rabbit antibody reagents and FITC-conjugated goat anti-rabbit IgG reagent. A number of selected mAb were also used to substitute or/and supplement the results of polyclonal antibodies.

Preparation of murine mAb

Female BALB/cCR mice (RPMI West Seneca Laboratory, West Seneca, NY) were immunized intravenously with 10^7 viable cells of ML-3 or HL-60 five times at weekly intervals. Murine mAb were generated by a standard method as reported previously (Kohler and Milstein, 1975). The culture supernatants of hybridomas containing antibody against the cells of immunized cell lines (ML-3 or HL-60) were positively selected by indirect membrane immunofluorescence. Next, the culture supernatants of hybridomas showing antibody activity against the cells of three T cell lines (MOLT-4, HPB-ALL, and RPMI-8402), three non-T non-B cell lines (Reh, KM-3, and NALM-16), or three B cell lines (BALL-1, Ramos, and RPMI-8057) were identified and eliminated from the subsequent study.

Membrane immunofluorescence

A mAb designated as MCS-1 generated from the ML-3 immunized mice and another mAb designated as MCS-2 generated from the HL-60 immunized mice were thus selected, respectively. Immunoglobulin isotypes of MCS-1 and MCS-2 were

murine IgG3 and IgG1, respectively, determined by Ouchterlony double immunodiffusion method using relevant goat anti-mouse isotype specific antibodies. Surface markers were examined by indirect or direct immunofluorescence method as reported (Sagawa et al. 1995). The stained cells were analyzed by a UV microscope or FACScan® flow cytometry (Becton Dickinson Immunocytometry Systems, San Jose, CA). Peripheral blood leukocytes were first stained in the form of heparinized whole blood by immunofluorescence method, and then fluorescein profile of each leukocyte population was analyzed under FACScan® flow cytometry after lysing the erythrocytes.

Majority of cases in the fresh-uncultured cell population even in a monoclonal leukemia, purity of one type of cell population was never at 100% owing the presence of residual normal cells. Furthermore, myeloid leukemia cells present often in themselves heterogeneity that is characterized by morphological criteria. Therefore, if only a fraction of cell population was stained, 30% or greater than 30% was taken as positive.

Immunoprecipitation

Immunoprecipitation of cell surface antigens recognized by MCS-1 and MCS-2 was performed using ML-3 cells and HL-60 cells as cell source, respectively, by the standard method as reported previously (Sakai et al. 1987).

Results

Reactivity of MCS-1 and MCS-2 with various cell lines

Both MCS-1 and MCS-2 reacted to

both the three myeloid leukemia lines (ML-3, HL-60 and KG-1) and two monocytic leukemia lines (U937 and THP-1-0). In contrast, no reactivity of MCS-1 or MCS-2 was found with following cells; an erythroid precursor line (K562); any of 13 T-cell leukemia-lymphoma lines (CCRF-CEM, CCRF-HSB2, MOLT-3, MOLT-4, RPMI-8402, HPB-ALL, JM, Peer, DND41, HPB-MLT, TALL-1, HD-Mar2, and SKW-3); any of 10 non-T non-B ALL lines (NALM-1, NALM-6, NALM-17, KOPN-1, HPB-Null, LAZ-221, Reh, KM-3, NALL-1, and NALM-16); any of 10 Burkitt lymphoma lines (EB-3, Raji, HR-1K, B35M, Daudi, B46M, Ramos, DND39, Namalwa, and BJAB); and any of 10 EBV-transformed B cell lines (RPMI-8057, RPMI-8392, RPMI-8432, RPMI-1788, RPMI-8052, B87, B112, B130, B131, and BES-1).

Reactivity of MCS-1 and MCS-2 with lymphocytes, granulocytes, monocytes, and thymocytes

Peripheral blood leukocytes in whole venous blood obtained from healthy volunteers were stained with MCS-1 and MCS-2 by indirect or direct immunofluo-

rescence method without separation of the cells to each leukocyte population. After lysing the erythrocytes with 0.83% NH_4Cl reagent, each leukocyte population was analyzed under the FACScan® flow cytometry. MCS-1 reacted to only granulocytes, whereas MCS-2 reacted to both granulocytes and monocytes (Table 1 and Fig. 1). Neither of the two mAb reacted with either lymphocytes or thymocytes.

Reactivity of MCS-1 and MCS-2 with leukemia cells

MCS-1 reacted to the cells from 19 out of 38 AML cases and 6 out of 8 AMoL cases, whereas MCS-2 reacted with the cells from 32 out of 39 AML cases and 7 out of 8 AMoL cases (Table 2). The positive incidence of MCS-1 and MCS-2 reactivity among total AML and AMoL cases were thus 54% and 83%, respectively. MCS-1 stained the cells from 8 out of 16 cases of CML-BC of myeloid type and none of the 7 cases of CML-BC of lymphoid type, whereas MCS-2 stained the cells from 16 out of 16 cases of CML-BC of myeloid type and 2 out of 7 cases of

TABLE 1.
Reactivity of MCS-1 and MCS-2 with lymphocytes, granulocytes, monocytes, and thymocytes

Cells tested	Monoclonal antibody		
	Negative control	MCS-1	MCS-2
Lymphocytes*	2.6±0.8	3.5±0.8	4.0±0.9
Granulocytes*	2.5±1.5	99.3±0.7	98.6±1.8
Monocytes*	1.8±0.3	5.7±3.3	97.5±1.5
Thymocytes**	0	0	0

(% positive cells)

*: n=5, **: n=6

Fig. 1. Reactivity of MCS-1 and MCS-2 to peripheral blood leukocytes. The heparinized whole venous peripheral blood was stained with FITC conjugated H31 mAb (negative control), H94 mAb (positive control), MCS-1 mAb, or MCS-2 mAb by direct immunofluorescence method. The fluorescence positive cells in each leukocyte population were assessed under the FACScan® flow cytometry after lysing the erythrocytes.

TABLE 2.
Incidence of positive reactivity* of MCS-1 and MCS-2
with leukemia cells

Type of leukemia †	MCS-1	MCS-2
AML	19/38	32/39
AMoL	6/8	7/8
CML-BC-myeloid	8/16	16/16
CML-BC-lymphoid	0/7	2/7
CML-CP	7/7	7/7
ALL	0/26	2/32**
CLL	0/12	0/12

(positive cases/tested cases)

*: When more than 30% of the blasts were stained, the case was interpreted to be positive.

†: AML; acute myeloblastic leukemia, AMoL; acute monoblastic leukemia, CML-BC-myeloid; chronic myeloid leukemia-in blastic crisis-myeloid type, CML-BC-lymphoid; chronic myeloid leukemia-in blastic crisis-lymphoid type (Common-ALL antigen positive cells are more than 70%), CML-CP; chronic myeloid leukemia in chronic phase, ALL; acute lymphoblastic leukemia, CLL; chronic lymphocytic leukemia

** : Ph¹ chromosome anomaly was found in these 2 cases of non-T non-B ALL.

Fig. 2. Immunoprecipitation of MCS-2 antigen. Immunoprecipitation of (35 S) methionine labeled HL-60 cells with negative control ascites (lane A), MCS-2 ascites (lane B), and MY7 ascites (lane C). There is specific immunoprecipitation of a polypeptide of 130,000 dalton apparent molecular weight by MCS-2 and MY7. A band at 100,000 dalton apparent molecular weight is also immunoprecipitated by MCS-2. The numbers at the right-hand side indicate the markers of molecular weights ($\times 1,000$ dalton).

CML-BC of lymphoid type. The cells from all the 7 cases of CML in chronic phase were stained both with MCS-1 and MCS-2.

In contrast, neither MCS-1 nor MCS-2 reacted to the 30 ALL cases. The blast cells from two cases of ALL, where Ph¹ chromosome was present, showed reactivity with MCS-2, but not MCS-1. Neither

MCS-1 nor MCS-2 stained the cells from 12 patients with B-cell type CLL.

Molecular weight of antigen recognized by MCS-2

The result of immunoprecipitation of HL-60 cell lysate by the MCS-2 mAb is shown in Fig. 2. Specific bands of apparent molecular weight 100,000 dalton and 130,000 dalton were present when MCS-2 was used for the immunoprecipitation (Fig. 2, lane B). Parallel preparations using MY7 (CD13) (Griffin et al. 1981) revealed an identical band at apparent molecular weight of 130,000 dalton, but the band at 100,000 dalton was not observed (Fig. 2, lane C). Attempts were also made to immunoprecipitate the molecules recognized by MCS-1 antibody using ML-3 cells as cell source. However, no immunoprecipitations were obtained by the above mentioned method (data not shown).

Discussion

The peripheral blood granulocytes were stained with both MCS-1 and MCS-2, but monocytes were stained with only MCS-2. Neither lymphocytes nor thymocytes were stained with MCS-2. These results indicate a rather narrow reactivity repertoire of MCS-1, which seems to be confined to granulocyte lineage, in contrast to that of MCS-2, which seems to react broadly with the cells of both granulocyte and monocyte lineages.

Among 47 cases of AML and AMoL tested, MCS-1 reacted to 54% (25/46) and MCS-2 reacted to 83% (39/47), respectively. At any rate, the MCS-2 antigen has a broader distribution than the MCS-1

antigen among the leukemia cells tested.

In CML-BC cases of myeloid type, MCS-1 reacted to blasts from 8 out of 16 cases, and MCS-2 all of them. The blasts from 5 out of 7 cases of CML-BC of lymphoid type showed no reactivity with either MCS-1 or MCS-2. It should be noted, however, that the remaining 2 cases of CML-BC of lymphoid type reacted with MCS-2 but not MCS-1. In these 2 cases of CML-BC of lymphoid type and 2 cases of Ph¹ positive ALL, the same leukemia cells expressed both common ALL antigen and MCS-2 antigen. The leukemia cells in some CML-BC of lymphoid type or Ph¹ positive ALL cases may arise from a point in differentiation more closely related to myeloid lineage than those in common non-T non-B cell ALL. Such cases demonstrate the potential value of MCS-2 antibody as a predictive marker for Ph¹ anomaly in cases of ALL.

The non-reactivity of MCS-1 or MCS-2 with the cells of the 50 non-myeloid lines appears to fulfill the prerequisite for their specificity within the non-lymphoid lineage. The non-reactivity of MCS-1 or MCS-2 with the cells from an erythroid precursor cell line (K562) (Lozzio and Lozzio, 1975; Andersson et al. 1979) may further support indirectly its properties of erythroid lineage distinct from myeloid lineage.

Several reports have already dealt with mAb detecting myelomonocyte antigens using various antigen sources including myeloid leukemia cell line cells, fresh leukemia cells, or normal peripheral blood cells (Bread et al. 1980; Raff et al. 1980; Ugolini et al. 1980; Civin et al. 1981; Griffin et al. 1981; Majdic et al. 1981; Zola et al. 1981). Irrespective of antigen sources, all

recognized normal differentiation antigens, rather than leukemia-specific antigen. One can divide all reported mAb to myelomonocyte cells into three groups. First, one group of antibodies is reactive with both the cells in granulocyte and monocyte lineage. The reactivity of mAb in the second group is limited within the cells of granulocyte-lineage, and the reactivity of the third group mAb is limited within the cells of monocyte-lineage. The MCS-2 belongs to the first group, and MCS-1 belongs to the second group.

Our observation with a few myeloid leukemia cell lines, fresh AML cells and peripheral blood leukocytes suggested that MCS-1 and MCS-2 mAb recognize differentiation antigens rather than leukemia specific antigens. The MCS-2 and MY7 (Griffin et al. 1981) mAb bind a polypeptide of 130,000 dalton apparent molecular weight. It may be assumed that they bind to different epitopes because MCS-2 also binds to a polypeptide of 100,000 dalton molecular weight.

The present study describes two anti-myeloid mAb, MCS-1 and MCS-2, that were subsequently determined as CD15 mAb and CD13 mAb, respectively, in the International Workshop on Human Leukocyte Differentiation Antigens. The availability of anti-myeloid-monocyte cell antibodies such as MCS-1 and MCS-2 described in this study will provide further insight into the understanding of myeloid cell biology and classification of myeloid malignancies.

Acknowledgments: This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, by Grant No.

CA-14413 from the National Institutes of Health USA and by a grant from the Association for Research of Childhood Cancer, USA. We thank Dr. H. Fuji, Dr. T. Sugimoto, Dr. K. Minato and Dr. R. Bankert for valuable advice, Ms A. Gifaldi, L. K. Kover, L. Zgoda, D. Gould, L. Prestine and K. Katagiri for excellent technical assistance and Ms C. Wylie for clerical assistance, and Dr. J. D. Armitage and Dr. K. Itoh for enlightening discussion.

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