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Expression of a 21KD Molecule on Hematopoietic Progenitor Cells

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ABSTRACT

A monoclonal antibody (MoAb) K15 (IgG3) was obtained by fusion between SP2/0, mouse myeloma cell line and spleen cells from BC_3F1 mice immunized with K562. a chronic myelocytic leukemia (CML) blastic crisis cell line. MoAb K15 precipitated a 21 kilodalton (KD) polypeptide in reduced condition. It stained lymphocytes, monocytes, eosinophiles and small bone marrow cells, but not neutrophiles, platelets, red blood cells (RBC) and large bone marrow cells. Depletion of $K15^+$ cells abolished both colony-forming units-granulocyte/monocyte (CFU-GM) and burst-forming units-erythroid (BFU-E). Thus MoAb K15 identifies antigens expressed on hematopoietic progenitor cells. The selective retention of this antigen by certain cell lineages may suggest some functional role of this antigen.

Key words: *Monoclonal antibody, CFU-GM, BFU-E*

Monoclonal antibodies (MoAb) against immature hematopoietic cells have been described[6]. We pay attention to the phenotype of human bone marrow progenitor cells. We are attempting to produce anti-progenitor cell reagents that can identify comitted progenitor cells of CFU-GM and BFU-E. This kind of reagents is useful for analysis of bone marrow progenitor cells. Utilizing non-lymphoid cell lines as immunogens, we have established a series of monoclonal antibodies and shown to have selective reactivity with non-lymphoid cells. One of them (K15) have been studied more extensively. MoAb K15 have the reactivity with progenitor cells of CFU-GM and BFU-E as well as with more mature hematopoietic cells. This report describes our investigation with MoAb K15.

MATERIALS AND METHODS

Generation of MoAb

K562, CML blastic crisis cell line was used as an immunogen. Ten-week-old BC_3F1 female mice were injected i.p. with 2×10^7 cells with 4mg alum as adjuvant. A second immunization was done 3 weeks later with 2×10^7 cells in phosphate buffered saline (PBS) i.p.. Three days later, spleen cells were fused with SP2/0 Ag 14 tumor cells with PEG 1000. Hybridomas were selected in HAT medium: their supernatant were screened by indirect immunofluorescence. The desired hybridomas were cloned on agarose. Details of these procedures have been described[11].

Cell preparation

Defibrinated blood from normal volunteers was used as a source of peripheral blood mononuclear cells (PBMC). PBMC were separated as described[8]. Monocytes were separated by Percoll continuous gradient centrifugation[10]. Granulocytes were separated by Percoll discontinuous gradient[7]. The purity of the granulocyte fraction was more than 97% as determined by Wright-Giemsa staining. Platelets were isolated from platelet-rich plasma and red blood cell (RBC) from the pellets of Ficoll-Hypaque gradients of peripheral blood cells.

Identification of antigen K15 bearing granulocytes determined by immune rosette method.

Separated granulocytes were incubated with hybridoma culture supernatant, washed three times with PBS and mixed with ox-RBC conjugated with anti-mouse Ig by $CrCl_3$ method[2]. After standing for an hour, the cells were smeared on slide glasses and stained with Wright-Giemsa solution. The type of rosette forming cells was determined under the microscope.

Bone marrow cells and bone marrow culture

Bone marrow cells were obtained from normal volunteers. They were separated by Ficoll-Hypaque density centrifugation to remove RBC and mature granulocytes. Separated bone marrow cells were subjected to an immune rosette method[2] to obtain cells reactive with MoAb K15, or they were subjected to a complement (C)-mediated cytolysis procedure to deplete the reactive cells. For the C-mediated killing procedure, 0.5 ml of 2×10^7/ml of bone marrow cells incubated with 0.5 ml of hybridoma culture supernatant for 30 min at 37°C. One milliliter of baby C (Pel Freez Biologicals, Rogers, AR, U.S.A.) was added and the mixture was incubated for 45 min at room temperature.

Dead cells were removed by centrifugation on a Ficoll-Hypaque gradient. Bone marrow cells were cultured in quadruplicates in Iscove's modified Dulbecco's medium containing 0.9% methylcellulose, 10% conditioned medium from human peripheral blood leukocytes stimulated with phytohemagglutinin, and 30% fetal bovine serum (FBS). This procedure was essentially as described by Messner et al[4] BFU-E were scored as hemoglobin-containing single or multiple colonies of more than 64 cells on the 14th day. This culture also allowed CFU-GM colony formation. CFU-GM were scored as colonies of more than 40 cells on the 14th day, since the growth of CFU-GM was slow in our cultures.

For the preparation of E^- sIg^- bone marrow cells, separated bone marrow cells were mixed with neuraminidase-treated sheep RBC[1] and anti-human IgM goat antibody-conjugated ox-RBC[2]. After one hour incubation at 4°C, cells were subjected to Ficoll-Hypaque density centrifugation to deplete the cells which formed rosettes with neuraminidase-treated sheep RBC and anti-human IgM-conjugated ox-RBC.

Immunofluorescence studies

Cells (0.05 to 1 × 10^6) were first incubated with hybridoma culture supernatants for 20 min at 4°C. After three washings with PBS containing 1% bovine plasma albumin, fluorescein-labeled F $(ab')_2$ anti-mouse Ig goat antibody (Tago, Burlingame, CA, U.S.A.) was added and a 20 min incubation at 4°C was performed. After three washings, cells were analyzed with a Epics V flow cytometry (Coulter Electronics). Integrated fluorescence of the population gated by forward angle light scatter and right angle light scatter was measured and 10,000 cells were analyzed.

Iodination and immunoprecipitation

Cells were iodinated in suspension by the method of Hubbard and Cohn[3]. In brief, 2 × 10^7 K562 cells were incubated with 1mCi/ml $Na^{125}I$, 50mU/ml type V glucose oxidase (Sigma Chemical Co., St. Louis, Mo, U.S.A.) and 10μg/ml lactoperoxidase (Calbiochem-Behring Co., San Diego, CA, U.S.A.) for 5 min on ice. The reaction was terminated by aspirating the supernatant and by repeated washings with RPMI 1640. After iodination, Immunoprecipitation, gel electrophoresis, and autoradiography were performed as described[9].

RESULTS

Characterization of MoAb K15

MoAb K15 was established with K562 cells as an immunogen. By ELISA, MoAb K15 was typed to be IgG3. MoAb K15 precipitated a molecule with 21 KD from ^{125}I-labeled K562 cells under reduced condition (Fig. 1, lane 1).

Distribution of antigen K15 on peripheral blood cells

The cellular distribution of the reactive antigens by MoAb K 15 was analyzed by immunofluorescence with a flow cytometry. The results are shown in Fig. 2 and Table 1. MoAb K 15 stained most of lymphocytes and monocytes weakly. It did not stain most of granulocytes but it stained small population (4.7 ± 2.5%) of granulocytes. Stained granulocytes were identified as eosinophiles by immune rosette method. MoAb K15 did not react with platelets and RBC.

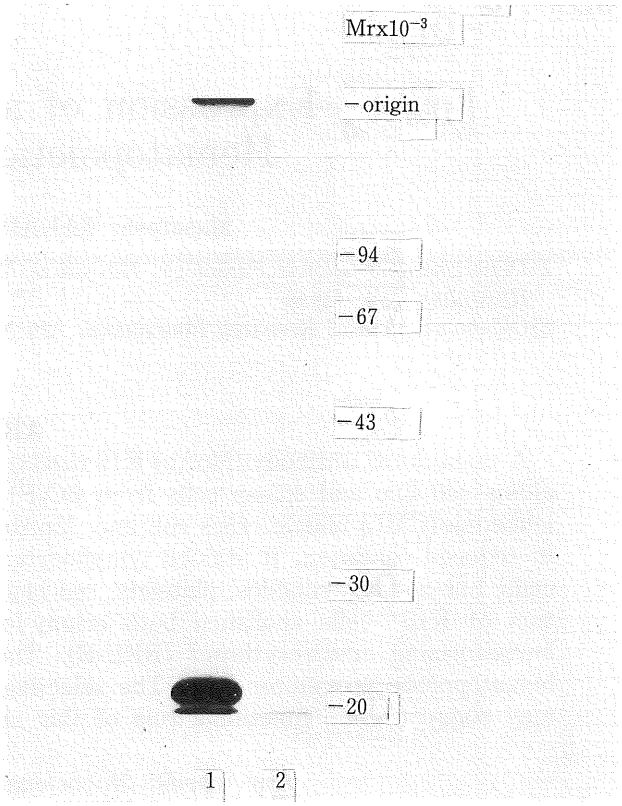

Fig. 1. The 21KD polypeptide was precipitated by MoAb K15 from ^{125}I-labeled K562 cell line (lane 1). No band was seen with MoAb NS 7 (IgG3, anti-sheep RBC) as a control antibody (lane 2).

Antigen expression on bone marrow cells

$K15^+$ cells and $K15^-$ cells were separated by immune rosette method. These cells were stained with Wright-Giemsa solution and classified morphologically. Myeloblast, monocytes and lymphocytic cells were enriched in $K15^+$ cells (Table 2). The reactivity of MoAb K15 with megakaryocytes could not be demonstrated in Table 2, as no megakaryocytes could be found in that experiment. But in other experiments, megakaryocytes were found in $K15^+$ population.

The bone marrow cells were resolved into two populations by light scattering analysis with forward and 90-degree light scattering (Fig. 3). One population with more light scattering is arbitrarily termed the "large cell" population, while that with less light scattering is reffered to as the "small cell". MoAb K15 reacted primarily with "small cell" population. It is of considerable interest to note that MoAb K15 stained a subpopulation of small cells very brightly. Analysis by log integrated green flourescence indicated that this population

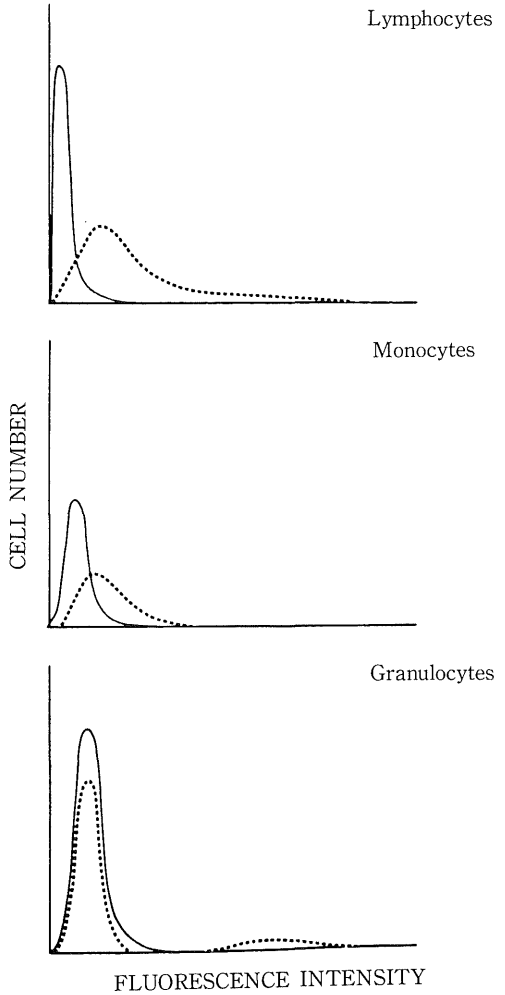

Fig. 2. Fluorocytometric analysis of staining patterns by MoAb K15

Table 1. Reactivity of MoAb K15 with normal hematopoietic cells

Cells	MoAb		
	K15	T.E. (CD2)	Josh524 (HLA-DR)
	(%)	(%)	(%)
Lymphocytes	95.4 ± 3.3[a]	87.4 ± 2.9	11.6 ± 3.3
Monocytes	>80	3.0 ± 1.8	92.6 ± 2.8
Granulocytes	4.7 ± 2.5[b]	<1	<1
Platelets	<1	<1	<1
RBC	<1	<1	<1

[a] This represents the mean and the standard deviation of determinations of samples from six individuals.
[b] The stained granulocytes were predominantly eosinophils.

was distinct from the peripheral blood lymphocytes. These two population were analyzed with a panel of MoAb (Table 3). MoAb K15 reacted with 70.4% of small bone marrow cells, while it reacted only 3.6% of large marrow cells. It is apparent that the small cell population of unfractionated bone marrow cells contain mature lymphocytes, T and B lineages. Therefore, E^- sIg^- bone marrow cells were analyzed to investigate the reactivity with immature small cells. It was shown that small cell population of E^- sIg^- bone marrow cells contained 4.3% of T cells, 2.4% of mature B cells and 48.3% of $HLA\text{-}DR^-$ cells. These findings indicated that most of mature lymphocytes were removed and immature cells were enriched in E^- sIg^- bone marrow cells. MoAb K15 reacted with 59.4% of small cell population of E^- sIg^- bone marrow cells. These finding suggest that antigen K15 is expressed on immature small bone marrow cells.

Table 2. Differential blood counts[a] of K15 antigen-positive vs-negative marrow cells

Marrow cells	$K15^{+b}$	$K15^-$
	(%)	(%)
Myeloblasts	5.7	0
Promyelocytes	2.0	6.8
Myelocytes	1.8	19.6
Metamyelocytes	1.7	32.7
Neutrophiles	0	22.7
Basophils	0	0
Eosinophils	0.7	0
Monocytes	12.8	0.8
Lymphocytes	74.6	0.4
Erythroblasts	0.7	17.0
Megakaryocytes	0	0

[a] Percent of > 500 Wright-Giemsa stained cells counted.
[b] $K15^+$ cells were obtained from rosette forming cells by immune rosette method.

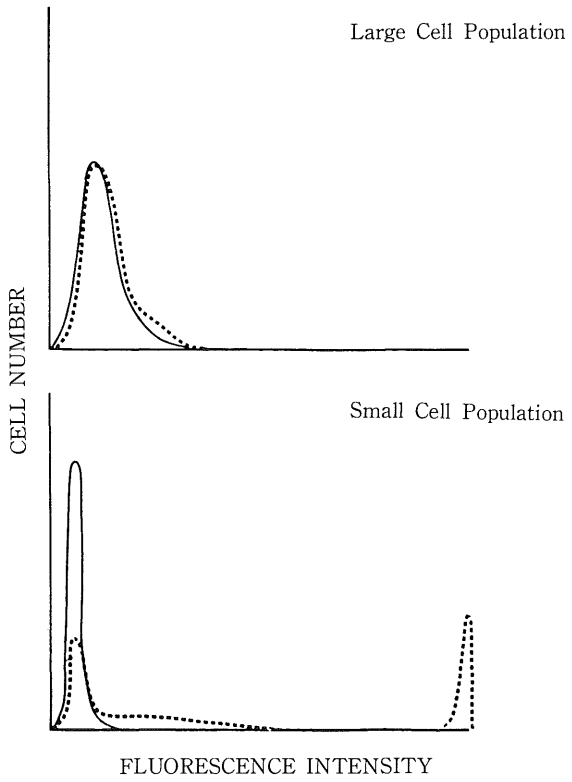

Fig. 3. Staining patterns of bone marrow

Presence of antigen K 15 on BFU-E and CFU-GM progenitors

MoAb K 15 was found to fix C. By using C-mediated cytolysis, antigen K 15 found to be

Table 3. Antigenic expression by two populations of bone marrow cells resolved by light scattering analysis

MoAb	Unfractionated		E^- sIg^{-a}	
	small	large	small	large
	(%)	(%)	(%)	(%)
T.E. (CD2)	53.0	<1	4.3	<1
Josh524 (HLA-DR)	25.5	6.3	48.3	6.1
Leu-10 (HLA-DQ)	18.6	<1	26.0	<1
anti-μ	11.1	<1	2.4	<1
B1	11.8	<1	10.4	<1
K15	70.4	3.6	59.4	4.0

[a] E^- sIg^- bone marrow cells were prepared by depletion of rosette forming cells with sheep RBC and anti-μ antibody conjugated ox-RBC.

Table 4. Bone marrow BFU-E and CFU-GM progenitor cells expressed K15 antigen

Expt.	%dead cells	No. Colonies/10^5 cells	
		BFU-E	CFU-GM
1. Untreated	2.9	88[a]	46
C alone	2.8	106	52
MoAb K15+C	17.8	9	5
2. Untreated	1.2	245	163
C alone	2.4	276	173
MoAb K15+C	16.5	30	2

[a] Data are presented as the mean of quadruplicate plates.

present on BFU-E and CFU-GM (Table 4). In two experiments of bone marrow cells, MoAb K 15 in the presence of C eliminated over 85% of BFU-E and CFU-GM progenitors.

DISCUSSION

MoAb K15 identifies a 21 KD polypeptide. It reacts with the progenitors of BFU-E and CFU-GM. Antigen K15 is expressed on myeloblasts, lymphocytes, monocytes and eosinophils. This antigen appears to be similar to that described by Pessano el al[5]. They reported that S 5.7 precipitated a 20 KD polypeptide and it reacted almost all lymphocytes, monocytes and eosinophiles, but not neutrophiles, RBC and platelets. However they did not show whether this antigen is present on BFU-E and CFU-GM progenitor cells or not.

The 21 KD antigen is expressed on 59.4% of small cell population of E^- sIg^- bone marrow cells and a subpopulation of these cells is stained brightly (Fig. 3, Table 3). This finding raised the possibility that the brightly stained cell population is markedly enriched for progenitor cells and that MoAb K15 can be used for enriching the progenitor cells. However, there is another possibility that the progenitor cells belong to large cell population of bone marrow cells, since MoAb K15 reacts with 3.6% of large cell population of unfractionated bone marrow cells.

We could not demonstrate the reactivity of MoAb K15 with multipotent stem cells, since colony-forming unit-granulocyte/erythrocyte/monocyte/megakaryocyte (CFU-GEMM) failed to grow in our bone marrow cultures. But there is the possibility that 21 KD polypeptide is present on the multipotent stem cells. If this is the case, during the differentiation of the stem cells to cells of various lineages, 21KD polypeptide is preferentially retained by certain cells. This is of considerable interest in view of the finding that eosinophils express this antigen while neutrophils do not. The differential retention may have certain functional significance.

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