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p26: A Cell Surface Antigen Expressed Selectively by Hematopoietic Cells

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ABSTRACT

A monoclonal antibody (MoAb) H5 (IgG2b) was obtained by fusion between SP2/0, mouse myeloma cell line and spleen cells from BC_3F1 mice immunized with HEL, a erythroleukemia cell line. MoAb H5 precipitated a 26 kilodalton (KD) polypeptide in reduced condition. It reacted with platelets, monocytes, eosinophiles, 13% of lymphocytes, thymocytes and colony-forming units-granulocyte/monocyte (CFU-GM), but not with neutrophiles, red blood cells (RBC) and burst-forming units-erythroid (BFU-E). It also stained T-lymphoid leukemia cell lines but not B cell lines. Thus this antigen was expressed selectively by both myeloid and lymphoid lineages.

Key words: *Monoclonal antibody, CFU-GM, Platelet*

Monoclonal antibodies (MoAb) have been established to identify antigens expressed by human myelomonocytic cells and their precursors[7,9,12]. Some of those have been shown to react with commited myeloid progenitors (CFU-GM)[7]. In attempts to generate MoAb reactive with hematopoietic progenitors, we used non-lymphoid leukemia cell lines as immunogens and have produced several kinds of monoclonal antibodies[10]. One of them (H5) have been studied more extensively. MoAb H5 is reactive with CFU-GM, monocytes, eosinophiles, platelets, 13% of peripheral blood lymphocytes, thymocytes and T-lymphoid leukemia cell line, but not with neutrophiles, BFU-E, RBC and B cell lines. This report describes our investigation with MoAb H5.

MATERIALS AND METHODS

Generation of MoAb

HEL, erythroleukemia line was used as an immunogen. Nine-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[15].

Cell preparation

Defibrinated blood from normal volunteers was used as a source of peripheral blood mononuclear cells (PBMC). PBMC were separated as described[11]. Monocytes were separated by Percoll continous gradient centrifugation[14]. Granulocytes were separated by Percoll discontinous gradient[10]. 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 the kind of granulocytes with antigen H5 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[4]. After standing for an hour, the cells were smeared on slide glasses and stained with Wright-Giemsa solution. The kind of rosette forming granulocytes was identified under the microscope.

Cell lines

In addition to HEL, ML-1, a myeloid leukemia cell line, U937, a monocytoid-histiocytic cell line, KG-1, a myeloblastic leukemia cell line, K562, an erythroid/myeloid line from a patient with chronic myelocytic leukemia (CML) in blastic crisis, and HL-60, a promyelocytic cell line, were used as non-lymphoid lines. For T leukemia cell lines, RPMI 8402, MT-1, Molt-4, Jurkat, and CEM were used. As B lymphoblastoid cell lines, Raji, Daudi, Josh-7, 8866P, $32a_1$ and SeD were used.

Bone marrow cells and bone marrow cultures

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 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 were 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[8] BFU-E were scored as hemoglobin-containing single or multiple colonies of greater than 64 cells on the 14th day. This culture also allowed CFU-GM colony formation. CFU-GM were scored as colonies of greater than 40 cells on the 14th day.

For the preparation of E^- sIg^- bone marrow cells, separated bone marrow cells were mixed with neuraminidase-treated sheep RBC[2] and anti-human IgM goat antibody-conjugated ox-RBC[4]. 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-cojugated 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 was added and a 20 min incubation at 4°C was performed. After three washings, cells were analyzed with a flow cytometry. 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[5]. 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[13].

RESULTS

Characterization of MoAb H5

MoAb H5 was established with HEL as an immunogen. By ELISA, MoAb H5 was typed to be IgG2b and it fixed complements. MoAb H5 precipitated a 26 KD molecule from ^{125}I-labeled platelets (Fig. 1, lane 2). A similar molecule was precipitated from HEL, the immunogen. This molecule was estimated from 15% SDS gel in reduced condition.

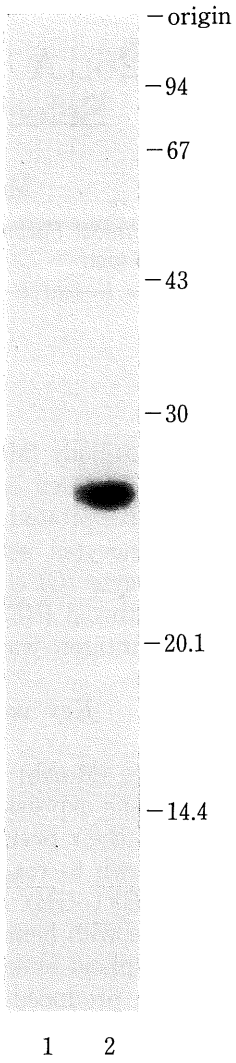

Fig. 1. The 26KD polypeptide was precipitated by MoAb H5 from ^{125}I-labeled platelets (lane 2). No band was seen with MoAb NS 8. 1. (IgG2b, anti-sheep RBC) as a control antibody (lane 1).

Peripheral blood cell distribution of antigen H5.

The cellular distribution of the reactive antigen by H5 was analyzed by immunofluorescence with a flow cytometry. The results are shown in Table 1. MoAb H5 stained almost all platelets, almost all monocytes, 13% of lymphocytes, 7.5% of granulocytes and thymocytes, but not RBC. Stained granulocytes were identified as eosinophiles by immune rosette method.

Hematopoietic cell lines

Antigen expression on various human hematopoietic cell lines are shown in Table 2. MoAb H5 reacted only one non-lymphoid leukemia cell line, HEL out of six cell lines. It also stained three T-lymphoid leukemia cell lines, RPMI 8402, MT-1 and Molt-4 out of 5 cell lines but not B-lymphoid cell lines.

Table 1. Reactivity of MoAb H5 with normal hematopoietic cells

Cells		MoAb H5 (%)	MoAb T.E. (CD2) (%)	MoAb Josh524 (HLA-DR) (%)
Lymphocytes	(6)[a]	13.0 ± 3.0[b]	87.4 ± 2.9	11.6 ± 3.3
Monocytes	(6)	92.6 ± 2.7	3.0 ± 1.8	92.6 ± 2.8
Granulocytes	(6)	7.5 ± 3.6[c]	<1	<1
Platelets	(6)	94.0 ± 3.8	<1	<1
RBC	(6)	<1	<1	<1
Thymocytes	(3)	29.0 ± 9.6	88.4 ± 8.4	<1

[a]The number of cell samples were analyzed.
[b]This represents the mean and the standard deviation of determinations of samples.
[c]The stained granulocytes were predominantly eosinophils.

Table 2. H5 antigen expression on various human hematopoietic cell lines by indirect immunofluorescence

Cell line	Cell type	%positive cell
ML-1	AML	<1[a]
U937	Monocytic	<1
KG-1	Myelocytic	<1
HEL	Erythroleukemia	41.7 ± 4.7
K562	Myeloid/Erythroid	<1
HL-60	Promyelocytic	<1
RPMI 8402	T-lymphoid	72.1 ± 5.3
MT-1	T-lymphoid	97.1 ± 1.1
Molt-4	T-lymphoid	83.0 ± 3.9
Jurkat	T-lymphoid	<1
CEM	T-lymphoid	<1
Raji	B-lymphoid	<1
Daudi	B-lymphoid	<1
Josh-7	B-lymphoid	<1
8866P	B-lymphoid	<1
32a1	B-lymphoid	<1
SeD	B-lymphoid	<1

[a]Data were obtained from 3 separated experiments for each target cells.

Antigen expression of bone marrow cells

The staining of bone marrow cells was analyzed by a flow fluorocytometry. The bone marrow cells were resolved into two populations by light-scattering analysis with forward and 90-degree light scattering. One population with more light scattering is arbitrarily termed the "large cell" population, while that with less light scattering is referred to as the "small cells". These two populations were anlyzed with a panel of MoAb (Table 3).

Most of large cells belong to the category of a myeloid cell lineage as described previously[10]. MoAb H5 reacts with 17.8% of small cells but not with large cells. It is apparent that the small cell population of unfractionated bone marrow cells contain mature lymphocytes, T and B cell lineages. Therefore, E^- sIg^- bone marrow cells were analyzed to investigate the reactivity with immature small cells. It was shown that E^- sIg^- bone marrow cells contained only 3.2% of T cells, less than 1% of B cells and 60.5% of HLA-DR^+ cells. These finding indicated that the mature lymphocytes were removed and immature cells were enriched in E^- sIg^- bone marrow cells. MoAb H5 reacted with 33.2% of E^- sIg^- bone marrow cells. These findings suggest that MoAb H5 reacts with immature small bone marrow cells but not with large bone marrow cells (promyelocyte-metamyelocyte).

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

MoAb		Unfractionated small (%)	Unfractionated large (%)	E^- sIg^-[a] small (%)	E^- sIg^-[a] large (%)
T.E.	(CD2)	53.5	<1	3.2	<1
Josh524	(HLA-DR)	30.3	2.8	60.5	2.0
Leu-10	(HLA-DQ)	23.2	<1	37.2	<1
anti-μ		12.6	<1	<1	<1
B1		12.5	<1	<1	<1
H5		17.8	3.8	33.2	<1

[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. Expression of antigen H5 on BFU-E and CFU-GM as determined by C-mediated lysis

Expt.	No. colonies/10^5 cells plated BFU-E	CFU-GM
1. Untreated	242	76
C alone	224	83
MoAb H5+C	229	16
2. Untreated	245	163
C alone	277	172
MoAb H5+C	260	12

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

Presence of antigen H5 on CFU-GM progenitors but not on BFU-E progenitors

MoAb H5 was found to fix C. By using C-mediated cytolysis, antigen H5 was found to be present on CFU-GM but not on BFU-E (Table 4). In two experiments, treatment of bone marrow cells by MoAb H5 in the presence of C eliminated most of CFU-GM progenitors. This treatment did not change the number of BFU-E substantially.

DISCUSSION

Differentiation from stem cells through intermediate stages to functional end cells is seen as an orderly progression of cells along a pathway containing a series of branch points. At such a branch point a cell takes one of 2 or more alternative paths and is committed to, for instance, lymphoid or myeloid, T cell or B cell lineage. Analysis of normal cells, leukemia cells and cell lines has been used in attempts to trace these differentiation pathways. Surface markers are among the more informative features of cells which help map the differentiation pathways. However, it is clear that individual markers can disappear and reappear at different stages of maturation and differentiation.

MoAb H5 identifies a 26 KD polypeptide. It reacts with 33.2% of small E^- sIg^- bone marrow cells which are clearly demonstrate not to contain mature lymphocytes because of the absence of T cell marker (CD2) and B cell marker (sIgM and B1). The reactivity of small E^- sIg^- bone marrow cells with anti-HLA-DR support this finding. Thus, MoAb H5 may react with the progenitor cells. One of progenitor cells, CFU-GM was shown to be reactive with MoAb H5 (Table 4), but not BFU-E. MoAb H5 also reacted with monocytes, eosinophiles and platelets but not with large bone marrow cells (promyelocyte-metamyelocyte) and neutrophiles. These finding suggest that antigen H5 exists on CFU-GM progenitor cells but it disappears through promyelocyte-metamyelocyte during the maturation to neutrophiles, while it may be retained during the maturation to monocytes, eosinophiles and platelets. Thus in the myeloid lineage, MoAb H5 is useful as a marker of maturity. It also reacted with T lymphoid leukemia cell lines, thymocytes and 13.0% of peripheral blood lymphocytes but not with B lymphoid cell lines. These finding suggest that antigen H5 is expressed on immature T lineage cells and retained on small population of mature lymphocytes.

The wide but selective distribution of the antigen renders MoAb H5 potentially useful, together with other monoclonal antibodies, in the delineation of differentiation pathways.

MoAb H5 does not appear to be directed against the antigens detected by MoAb FMC-8, CALL-1 and BA-2, although the three antigens have similar molecular sizes. MoAb FMC-8 reaced with neutrophiles and K562 cell line but not with T-lymphoid leukemia cell lines unlike MoAb H5, while it was reactive with monocytes, platelets and small population of lymphocytes like MoAb H5[1]. CALL-1 did not reat with monocytes, lymphocytes and CFU-GM unlike MoAb H5[3]. BA-2 did not react with peripheral blood lymphocytes, monocytes and platelets unlike MoAb H5[6].

MoAb H5 is useful for the separation of eosinophiles from granulocytes. It also can potentially be used for the markers of lymphocyte subsets, since it reacts with small population of peripheral blood lymphocytes. But for this purpose, further study will be needed.

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