Differentiation Antigens of Human Hemopoietic Cells: Patterns of Reactivity of Two Monoclonal Antibodies

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ABSTRACT

Two mouse anti-human monoclonal antibodies (S3.13 and S5.7) raised against cells of acute myelogenous leukemia were found to react with antigens expressed on the surface of subsets of monocytes and lymphocytes.

S3.13 precipitates a peptide of M, 29,000, and S5.7 precipitates a peptide of M, 20,000 present on the surface of all the cell types tested. These two surface antigens were distributed on discrete subpopulations of normal hemopoietic cells. The antibodies reacted with all (S5.7) or a subpopulation (S3.13) of peripheral blood T-lymphocytes, and with a subset of monocytes. Both antibodies reacted with bone marrow blast cell progenitors of the myelomonocytes and erythroid lineage. S5.7 also reacted with non-T-lymphocytes and with cells of the eosinophilic lineage. Both antigens disappeared from the cell surface during normal myeloid and erythroid differentiation. Thus, these monoclonal antibodies define the molecular characteristics and the cellular distribution of two differentiation antigens present on cells of the hemopoietic lineage.

INTRODUCTION

Monoclonal antibodies (13) directed against antigens present on hemopoietic cells are being utilized to characterize the distribution of differentiation antigens on normal and leukemic cells (23). Lineage-specific differentiation antigens have been defined in several cases (12, 22), but it is often the case that the distribution of surface antigens present on normal and leukemic cells is not limited to a single lineage or to a specific cell differentiation stage. For example, DR surface antigen (a dimer of gp33 and gp28) is expressed by B-lymphocytes, monocytes, myeloid, erythroid, and B-lymphocyte progenitor cells and by a fraction of myeloid leukemias and ALLs (1, 8, 23). The reasons underlying such a complex distribution are not yet understood but probably reflect the peculiar and specific function of this antigen.

A knowledge of distribution of the various molecules on the surface of normal and leukemic hemopoietic cells will be of value in understanding lineage interrelationships and may help to shed some light on the function of these molecules. We have characterized the surface antigens recognized by 2 monoclonal antibodies generated by immunizing mice with AML cells. These antibodies are reactive with a large number of leukemic cell lines and have a complex distribution on hemopoietic cells.

MATERIALS AND METHODS

Hemopoietic Tissues. Normal peripheral blood and marrow for these investigations were obtained from healthy volunteer donors and approved by the Committee for the Protection of Human Subjects of The Wistar Institute.

Mononuclear cells were separated by Ficoll/Hypaque (D = 1.078) centrifugation (4), and the cells at the interface were suspended in PBS and incubated with the monoclonal antibodies as described below. Total peripheral blood leukocytes were prepared as described previously (7, 20). Thymus tissue was obtained at autopsy within 4 hr of death.

Cell Lines. HL-60, ML3, and KG1 (AML); THP1 (monocytic leukemia); U937 (histiocytoma); K562 (chronic myelogenous leukemia in blast crisis); Daudi and Raji (Burkitt’s lymphoma); Jurkat, HPB, and HUT 78 (T-ALL); and Nalm 1 (non-B- and non-T-ALL) are established human cell lines maintained in our laboratory as described previously (16, 20). The ML3 cell line (19), used as an immunogen for one of the fusions, was established from the peripheral blood of a 24-year-old male with acute myelogenous leukemia. ML3 cells have the morphological and histochemical markers of the myeloid lineage at the promyelocyte stage. P3-X63-Ag8.653 mouse myeloma line (10) and the nonhemopoietic cell lines described in the text were grown in Roswell Park Memorial Institute Medium 1640 containing 10% fetal bovine serum and incubated in a humidified 5% CO2-humidified atmosphere at 37°.

Selection of Monoclonal Antibodies. The hybrid clones reactive with the cells used for immunization were identified by radioimmunoadsorbent testing by testing the supernatant present in the colony-containing wells (16, 20). All clones that reacted with the leukemic cells were further screened for specificity by flow cytofluorimetric analysis (see below). Two monoclonal antibodies reacted strongly with leukemic cells. These monoclonal antibodies were identified as S3.13 (produced using AML cells from a patient) and S5.7 (produced using ML3 cells). Hybridoma cells (2 × 106) were injected i.p. into pristane-primed BALB/c mice to produce ascites ranging in titer from 1:104 to 1:105.

Isotype Characterization. Immunoglobulin isotype was determined by immunodiffusion in Ouchterlony plates using goat anti-mouse immunoglobulin class- and subclass-specific antibodies (Meloy Laboratories, Springfield, Va.).

Flow Cytocfluorimetric Analysis. Target cells were treated with 20 μl of the antibody at a chosen dilution, incubated at room temperature for 30 min, and washed 3 times. The cells were then incubated in 20 μl of FITC goat F(ab')2 anti-mouse F(ab')2 antiserum (Cappel Laboratories).
Control cultures were treated with the supernatant of the parental mouse myeloma and FITC-conjugated antibodies or with an irrelevant monoclonal antibody of the same isotype. An Ortho Cytotfluorographic System 50HH connected to a Data General MT-200 microprocessor was used for analysis of the fluorescent cells. A 2-watt argon laser (488 nm) was the source for generation of scatter signals and fluorescence activation. Fluorescence profiles were generated as histograms of number of cells in each channel (y-axis) and relative intensity of the fluorescence over 200 or 1000 channels (x-axis), or as type of cells resolved by scattering in the x- and y-axes and number of cells (fluorescent or not fluorescent) in the z-axis.

For flow cytometric analysis, 3 x 10^5 cells/sample were used. Marrow and peripheral blood subpopulations were identified on the basis of right- and forward-angle scatter (7, 16). The leukemic cell line populations were identified by their scattering as a homogeneous population. For sorting experiments, 2 x 10^6 cells/sample were used and, after indirect immunofluorescence labeling, they were resuspended in 1 ml of PBS.

For both cytometric and sorting analysis, the threshold for fluorescence intensity was established at the level at which 99% of the total cell population treated with control supernatant from the parental myeloma cell line and FITC-conjugated anti-mouse antibody were negative. Sorting was performed at a rate of 3 x 10^5 cells/sec. Positive and negative cells were separately collected into tubes containing 1 ml of PBS supplemented with 10% fetal bovine serum. Cytocentrifuge smears were stained with either Wright-Giemsa or May-Grünwald-Giemsa. At least 300 cells/slide were identified morphologically, and at least 3 separate sorting experiments on different bone marrows were performed with each of the 2 monoclonal antibodies tested.

Preparation of Erythrocyte Rosettes. Mononuclear leukocytes obtained after Ficol-Hypaque gradient centrifugation of normal peripheral blood were allowed to adhere to plastic dishes for 1 hr at 37°C. Nonadherent cells were then incubated with neuraminidase-treated sheep erythrocytes for 30 min on ice and were centrifuged on a Ficol-Hypaque gradient. Erythrocyte rosette-positive (pellet) and erythrocyte rosette-negative (interface) cells were processed for immunofluorescence as described above.

Radiolabeling and Immunoprecipitation. Exponentially growing Jurkat and HPB cell lines or primary leukemic cells freshly obtained from an AML patient or normal peripheral blood lymphocytes were used for labeling experiments. Cell surface iodination with Na^125I was catalyzed by lactoperoxidase and hydrogen peroxide (18). For metabolic labeling, the cells were washed once in Roswell Park Memorial Institute 1640 Tissue Culture Medium without L-methionine (Grand Island Biological Co., Grand Island, N. Y.) and cultured for 14 hr at 5 x 10^6 cells/ml in the appropriate medium supplemented with 10% dialyzed fetal calf serum in the presence of L-[35S]methionine (400 Ci/mmol; Amersham/Searle Corp., Arlington Heights, III.).

After labeling, cells were lysed with 1% Nonidet P-40 (Particle Data Laboratories, Elmhurst, Ill.) in 0.01 M Tris-HCl (pH 7.8):0.15 M NaCl:1 mM phenylmethylsulfonyl fluoride:ovomucoid trypsin inhibitor (0.02 mg/ml) (Sigma Chemical Co., St. Louis, Mo.) (17). The lysates were centrifuged at 13,000 x g for 15 min to remove nuclei. The supernatant fluid was subjected to autoradiography and to electrophoresis as described (3).
promyelocytic leukemia cell lines. S5.7 reacted with T-, B-, null, and myeloid cell lines but reacted very weakly with U937 histiocytic leukemic cells. S3.13 and S5.7 did not react with human fibroblasts, melanoma, colon carcinoma, teratocarcinoma, hepatoma, gastric carcinoma, or astrocytoma cell lines, but both reacted with 4 neuroblastoma cell lines (data not shown).

**Monoclonal Antibodies S5.7 and S3.13 Recognize Different Target Structures.** As shown in Fig. 1, monoclonal antibody S5.7 immunoprecipitates a target protein of M, 20,000 which can be labeled by either cell surface radioiodination (Fig. 1, Lane C) or metabolically by L-[35S]methionine (Fig. 1, Lane G). The T-cell-specific glycoprotein T3, detected by monoclonal antibody Leu-4 (Fig. 1, Lanes B and F), has a similar molecular weight. In contrast, S3.13 precipitates a protein of M, 29,000 when the cells are metabolically labeled with L-[35S]methionine (Fig. 1, Lane H). In several attempts, we were unable to detect the target antigen for S3.13 after cell surface radioiodination (Fig. 1, Lane D). The proteins immunoprecipitated, respectively, could also be immunoprecipitated by S5.7, and by S3.13, they could also be immunoprecipitated from T-cell lines, AML cells, and normal T-cells.

**DISCUSSION**

We have characterized the reactivity of 2 monoclonal antibodies (S3.13 and S5.7) with a variety of human leukemic cells as well as with normal hematopoietic cells. The same molecules (M, 29,000 and 20,000), which are precipitated from the HPB T-cell line and from the other T-lines, have been also precipitated from normal T-cells and AML cell lines (data not shown). Therefore, the antigens represent polypeptides that are expressed early during the process of hemopoietic differentiation and remain expressed in a few cell subsets during maturation. Monoclonal antibodies reactive with M, 20,000 to 30,000 polypeptides have been described by Hercend et al. (6) and by Kersey et al. (11). Antibody J2 recognizes a M, 26,000 glycoprotein present in activated T-cells but absent in resting T-cells (6). J2 also reacts with platelets, but not with monocytes, and is therefore clearly distinct from antibodies S3.13 or S5.7.

Antibody BA2 immunoprecipitates a polypeptide of M, 24,000 (11). This peptide is detectable on the surface of normal bone marrow lymphopoietic precursors in 77% of non-T- and non-B-ALL and 18% of T-ALL, but it is not present on peripheral blood leukocytes.

Zipl et al. (25) have described 2 monoclonal antibodies (1E7 and 17F12) that react with the normal T-lymphocytes. The reactivity patterns of S3.13 and S5.7 partially overlap with those of 1E7 and 17F12, since all 4 antibodies react with thymocytes and peripheral blood T-cells. However, S3.13 and S5.7 also react with a subset of monocytes.

Antibody OKT10, like S5.7 and S3.13, reacts with a discrete number of immature bone marrow cells (myeloid and lymphoid). However, OKT10 does not react with normal mature T-cells or monocytes (9).

Antibody TA-1 is reactive with all T-lymphocytes and monocytes as well as with 13% of nucleated marrow cells (15). However, TA-1 is nonreactive with cells of the eosinophilic or B-cell lineage. The reactivity of S3.13 appears to overlap, in many respects, the reactivity of monoclonal antibody RFB-1 described by Bodger et al. (2). Both antibodies react with a large fraction of peripheral T-cells and myeloblasts but react poorly with promyelocytes and a fraction of thymocytes. Both antibodies also bind to colony-forming cells (5) but do not react with pre-B-blasts or B-lymphocytes. Unlike RFB-1, however, S3.13 reacts weakly with a fraction of monocytes.

The 2 antibodies reported here define antigens that are abundantly expressed in human leukemic cell lines, as well as on acute leukemia cells freshly obtained from patients (21). These antigens are also present in hemopoietic progenitor cells of various lineages (5), showing a complex pattern of distribution during the process of terminal differentiation. Work is in progress to define the functions of such molecules, since this information would be useful in understanding the significance of appearance and disappearance of the antigens at a defined differentiation stage in each cell lineage.

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