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ANTIGENIC ANALYSIS OF HEMATOPOIESIS

III. A Hematopoietic Progenitor Cell Surface Antigen Defined by a Monoclonal Antibody Raised against KG-1a Cells[1]

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The anti-My-10 mouse monoclonal antibody was raised against the immature human myeloid cell line KG-1a and was selected for nonreactivity with mature human granulocytes. Anti-My-10 immunoprecipitated a KG-1a cell surface protein with an apparent Mr of approximately 115 kD. We describe the binding of this antibody to human hematopoietic cell types and show that My-10 is expressed specifically on immature normal human marrow cells, including hematopoietic progenitor cells. My-10 is also expressed by leukemic marrow cells from a subpopulation of patients. Thus, this antibody allows the identification and purification of hematopoietic progenitor cells from normal human marrow and the subclassification of leukemia.

Monoclonal antibodies specific for immature as well as mature human lymphocyte cell-surface antigens have been described by several groups (1–4). We and others have developed monoclonal antibodies against mature human granulocytic and monocytic cells (1, 5–15). Similarly, selective probes to cell-surface antigens of early marrow cells are potentially useful for the identification and subsequent isolation of immature myeloid precursor cells or regulatory cells. Specific monoclonal antibodies might also identify receptors involved in interactions between hematopoietic progenitor cells and humoral or cellular regulatory elements. In addition, such antibodies should prove useful in the subclassification and additional study of leukemia.

Unfortunately, direct strategies to develop the requisite antibodies are formidable, because clonal progenitor cells constitute less than 1% of normal marrow cells and are specifically identifiable only by colony-forming assays (e.g., Reference 16). Mouse monoclonal antibodies raised against the K-562 cell line, however, have been shown to react with myeloid progenitor cells (17). In this study, we used the KG-1a human leukemic cell line as an immunogen in an attempt to produce antibodies against human blast cell-surface antigens. The KG-1 myeloblastic leukemic cell line was derived from a patient with nonlymphocytic leukemia (18), and the KG-1a cell line arose from it as a spontaneous tissue culture variant (19). KG-1a cells are phenotypically less differentiated than KG-1 cells and have the morphologic and cytochemical features of primitive hematopoietic blast cells (19). From a mouse immunized with KG-1a cells we developed a monoclonal antibody that reacted selectively with a small subpopulation of primitive human myeloid cells.

MATERIALS AND METHODS

Animals. Four- to 12-wk-old BALB/cJ female mice were obtained from The Jackson Laboratories (Bar Harbor, ME) and were utilized for the development and production of monoclonal antibodies.

Cell lines. Cell lines were obtained and cultured as described (20). In addition, the recently described HEL human erythroleukemia cell line (21) was generously provided by Dr. T. Papayannopoulou (Seattle, WA) and was cultivated similarly.

Fresh human cells. Heparinized (20 U/ml) peripheral blood was obtained from normal laboratory volunteers, and cell types were separated by several techniques. Platelets, red blood cells, and peripheral blood mononuclear cells (PBMC) were separated as described (5, 20) over Histopaque-1077 (Sigma Chemical Co., St. Louis, MO). Inasmuch as Todd *et al.* (14) had pointed out that monocytes may adsorb platelet fragments during conventional PBMC preparation, as above, defibrinated (rather than heparinized) blood samples were used when monocytes were to be evaluated. Lymphocytes or monocytes in a mixed population of PBMC could be separately analyzed for fluorescence by first selecting a "lymphocyte region" or "monocyte region" on the basis of forward and right angle light scatter (22) by using flow cytometry (Spectrum III cytofluorograph; Ortho Diagnostics, Raritan, N.J). In other studies, the monocytes/macrophages in PBMC preparations (1 million cells/ml complete growth medium) were labeled by incubation (37°C, 5% CO_2, 45 min) with latex microspheres (100 million/ml) (Dow Diagnostics, Indianapolis, IN). After washing, phagocytic mononuclear cells were identified microscopically (≥3 beads/cell).

To obtain enriched T and B lymphocyte populations, PBMC (5 million/ml in complete growth medium) were first depleted of monocytes and macrophages by incubation (37°C, 5% CO_2, 90 min) in plastic petri dishes (Falcon, Oxnard, CA.). The nonadherent PBMC were then washed and fractionated by using a sheep erythrocyte rosette formation (23). To isolate peripheral blood granulocytes, mononuclear cells were first removed by Histopaque-1077 density gradient centrifugation. The cells beneath the interface of the first gradient were washed once and granulocytes were then separated from red cells by dextran sedimentation. Small numbers of residual red cells did not interfere with later analysis of antibody binding to leukocytes; if large numbers (>25%) of red cells were present, they were lysed osmotically (24).

Marrow was aspirated from posterior iliac crests into α-medium (M. A. Bioproducts, Walkersville, MD) containing preservative-free heparin (100 U/ml Panheprin R; Abbott, Chicago, IL). Excess cells

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obtained from donor marrow harvested for allogeneic marrow transplantation or marrow cells from normal volunteers were utilized. Diluted marrow samples were centrifuged over Histopaque-1077. The interface cells were washed, suspended in complete growth medium, and incubated (37°C, 5% CO_2) in petri dishes for at least 90 min to remove plastic-adherent cells. The low density, plastic-non-adherent marrow cells were washed at least once again before use. Leukemic blast cells were obtained from patient diagnostic marrow samples, as described (5).

Monoclonal antibodies. Antibody secreting hybridomas were produced by fusion of mouse plasmacytoma cells with splenocytes by using the techniques of Kohler and Milstein (25) as modified (26). A BALB/CJ female mouse was hyperimmunized by i.p. injections (four injections over a 4-mo period) of approximately 10 million washed, viable KG-1a cells in saline: the fourth of these injections was 5 days before fusion. Three and 4 days before fusion, the mouse was boosted i.v. with KG-1a cells. Then, the mouse spleen cells were fused with non-Ig-producing SP-2/0-AG14 (SP-2) mouse plasmacytoma cells and were cultured in HAT[a] medium (26). Hybridomas were assayed, and the anti-My-10-producing clone was selected for binding to KG-1a cells but not to human peripheral blood granulocytes (see *Results*). All hybridoma antibodies utilized were derived from cells subcloned at least twice. Neat spent hybridoma culture supernatant was used as the source of antibody under conditions (determined in preliminary experiments) sufficient to saturate binding sites on KG-1a cells. The isotypes of all hybridoma- and plasmacytoma-derived antibodies used were determined as described (27).

The antibodies I2 (1: anti-HLA-DR), cALLa (3: anti-common acute lymphoblastic leukemia antigen), Mo2 (14: monocyte specific), T11 (28, 29: anti-sheep red blood cell receptor of T cells), and B1 (1: anti-pan B cell) were generously provided by Dr. L. Nadler (Sidney Farber Cancer Center, Boston, MA) and Dr. K. Kortwright (Coulter Diagnostics, Hialeah, FL). The anti-Leu-1 monoclonal antibody (30) was generously provided by Dr. R. Levy (Stanford, Palo Alto, CA). The MOPC 21 IgG1$_\kappa$ mouse myeloma protein, produced by the P3 × 63-AG8 cell line (American Type Tissue Collection, Rockville, MD) and having no known specificity, was utilized as a negative control antibody (culture supernatant). The 28/43/6 monoclonal antibody, which binds to lymphocytes from all donors tested (20), was used as a positive control.

Indirect immunofluorescence assay. Indirect immunofluorescence assays to measure the binding of monoclonal antibodies to cells were performed as described (5, 20). Binding was analyzed either by standard phase and fluorescence microscopy and/or by flow microfluorometry.

Radiolabeling of cells. KG-1a cells were radiolabeled vectorially with ^{125}I-iodide by using the method of Hubbard and Cohn (31). Briefly, 20 million cells in exponential growth were washed four times in 10 mM HEPES-0.15 M NaCl buffer, pH 7.4 (buffer A). The cell sediment was resuspended in 1 ml of buffer A containing 0.05 M glucose, 40 μl of (100 IU/ml) lactoperoxidase (Calbiochem-Behring, San Diego, CA), and 2.5 μl of freshly prepared (1 mg/ml) glucose oxidase (Millipore Corp., Freehold, NJ); 0.5 to 1 mCi of ^{125}I-iodide (New England Nuclear Corp., Boston, MA) was added and the cell suspension was incubated at 22°C for 20 min with gentle agitation. Then, 10 ml of buffer A containing 5 mM KI and 0.1% glucose were added to stop the reaction. After four washes with buffer A, the cell sediment was resuspended in 500 μl of disruption buffer (10 mM Tris-HCl, pH 7.5, containing 1% (v/v) Nonidet P-40, (NP-40), 200 μ/ml Trasylol, 10 mM EDTA, and 50 μg/ml Leupeptin (Sigma)) for 20 min on ice with periodic vortexing. The cell extract was then centrifuged (10 min, 15,600 × G, 4°C), and the supernatant was used for immunoprecipitation.

Immunoprecipitation with monoclonal antibodies. Immunoprecipitation was performed essentially as described by Lampson (32). For each monoclonal antibody to be tested, 300 μl of 10% fixed, whole, protein A-bearing Cowan strain Staphylococci (SA; Calbiochem-Behring) were washed three times by centrifugation (15,600 × G, 5 min; 4°C) in Lampson wash buffer (WB) (0.1 M phosphate-buffered saline, pH 8.6, containing 0.1% bovine serum albumin, 0.02% NaN3, 0.5% NP-40, 0.1% SDS). The SA sediment was then resuspended to the initial volume with goat anti-mouse IgG serum (Kierkegaard and Perry, Gaithersburg, MD) and was incubated 12 to 18 hr at 4°C. The SA-IgG complex was washed seven times in WB and resuspended with monoclonal antibody (hybridoma culture supernatant) to 10% (v/v). After 40 min incubation (22°C), the SA-IgG-monoclonal antibody complex was washed three times in WB and resuspended to the initial volume in WB. To this complex, 80 to 120 μl of cell extract was added, followed by incubation at 4°C for 12 to 18 hr. The SA-IgG-monoclonal antibody complex was then washed three times in WB and resuspended in 50 μl of WB plus 25 μl of Laemmli (33) sample buffer (0.0625 M Tris-HCl, pH 6.8, containing 12.5% glycerol, 1.25% 2-mercaptoethanol, 5% SDS, and 1 mM EDTA), boiled for 2 min, centrifuged (15, 600 × G, 5 min), and the supernatant was harvested for analysis by SDS-polyacrylamide gel electrophoresis.

SDS polyacrylamide gel electrophoresis. The samples were analyzed on 10% SDS-polyacrylamide gels under reducing conditions according to the method of Laemmli (33). After electrophoresis, the gel was stained with Coomassie Brilliant Blue, destained, dried onto filter paper, and exposed to x-ray AR film (Kodak, Rochester, NY) at −70°C.

Immune adherence and immune rosettes. The "panning" immune adherence technique of Engleman *et al.* (30) was utilized as described (20). Described procedures (34, 35) were used for direct and indirect immune rosetting.

Colony-forming assays. Day 14 multilineage colonies (36–38) were assayed in quadruplicate in medium containing 0.96% methylcellulose, 5% placenta-conditioned medium (16, 39), and 1 U/ml erythropoietin (Connaught, Toronto, Canada). The colony number was a linear function of total cells plated. It should be noted that in most experiments the cells were plated at several dilutions to obtain countable plates (20 to 200 colonies). This was particularly important with My-10-positive cell fractions, which were enriched in colony-forming cells.

Colonies were counted *in situ* by using a dissecting microscope (50 to 80×) or inverted phase microscope (200×), and gross colony and cellular morphology was recorded. Representative colonies were plucked by using a Pasteur pipette. Stained cytocentrifuge preparations were analyzed for confirmation of cell type(s) within the colonies.

Cell staining. Smeared or cytocentrifuged preparations of whole or separated marrow cells or colonies were stained either with Wright-Giemsa stain or with a double-esterase (α-naphthyl acetate and naphthol AS-D chloroacetate esterases) cytochemical stain and Mayer's Hematoxylin counterstain for differential counting, or with other cytochemical stains (40).

RESULTS

Development of the anti-My-10 monoclonal antibody. Monoclonal antibody anti-My-10 was produced by hybridizing SP-2 plasmacytoma cells with splenocytes from a BALB/cJ mouse that had been repeatedly immunized with viable KG-1a cells. By 2 wk, macroscopic colonies were observed in all 48 cultures: the culture supernatants were tested in indirect immunofluorescence assays on KG-1a cells as well as on granulocytes from several normal donors. Four of these initial culture supernatants were strongly reactive (at least five times background) with KG-1a cells but did not react with granulocytes from any donor tested. The hybridoma culture producing the anti-My-10 monoclonal antibody was cloned twice in soft agarose (27). Anti-My-10 was shown to be an IgG1$_\kappa$ antibody by enzyme-linked immunosorbent assay (EIA) (27) by using isotype-specific antibodies (Zymed Laboratories, Burlingame, CA).

Immunoprecipitation of a radiolabeled KG-1a antigen by anti-My-10. Vectorial labeling of the plasma membrane of intact cells with ^{125}I-iodide, followed by immunoprecipitation with SA-bound monoclonal antibody, SDS-PAGE analysis, and visualization of antigen by autoradiography, was utilized to identify the KG-1a membrane protein detected by anti-My-10 (Fig. 1). Under reducing as well as nonreducing conditions, My-10 antigen had a molecular radius (Mr) of approximately 115 kilodaltons (kD), indicating the absence of disulfide-linked oligomers.

Expression of My-10 antigen on myeloid cell lines and normal human blood and marrow cells. Figure 2 shows fluorescence-activated cell sorter (FACS II) histo-

[a] Abbreviations used in this paper: Mr, molecular radius; SA, Staphylococcal protein A; FACS, fluorescence-activated cell sorter; EIA, enzyme-linked immunoassay; HAT, hypoxanthine-aminopterin-thymidine; CFC-GM, colony-forming cell-granulocyte/monocyte; kD, kilodalton; BFU-E, burst-forming unit-erythroid; PBMC, peripheral blood mononuclear cells.

Figure 1. Autoradiograph of radiolabeled cells and immunoprecipitated My-10 (Mr 115,000). The *horizontal lines* on *left* indicate m.w. standards that from top to bottom are: myosin, 200 kD; phosphorylase b, 92.5 kD; pyruvate kinase, 60 kD; and actin, 43 kD.

grams of a panel of myeloid cell lines after reaction with the anti-My-10 and control monoclonal antibodies (indirect immunofluorescence assay). Large quantities of cell surface My-10 antigen were detected on KG-1a cells. The anti-My-10 labeled KG-1a cell population was approximately as intensely fluorescent as the (positive control) 28/43/6-labeled sample. In contrast, when the other cell lines were labeled with anti-My-10, the fluorescence histograms were not greatly different from the negative control (MOPC 21) profile. (Daudi and K-562 cells were not detectably labeled with the positive control 28/43/6 antibody. This is consistent with the thesis that this antibody detects a framework epitope of the HLA-A,B molecule, because HLA-A,B is not expressed on Daudi or K-562 cells (20)). In this experiment, Daudi cells appeared slightly positive for My-10. In other experiments, however, all of these cell lines (except KG-1a) were clearly negative for anti-My-10 binding. The same conclusions were reached when whole viable cells were tested by EIA, and when purified anti-My-10 was used rather than tissue culture supernatant.

Figure 3 shows FACS II fluorescence data of isolated peripheral blood granulocytes, plastic-adherent monocytes (86% monocytes by Wright-Giemsa stain), and nonadherent "lymphocytes" (66% lymphocytes by Wright-

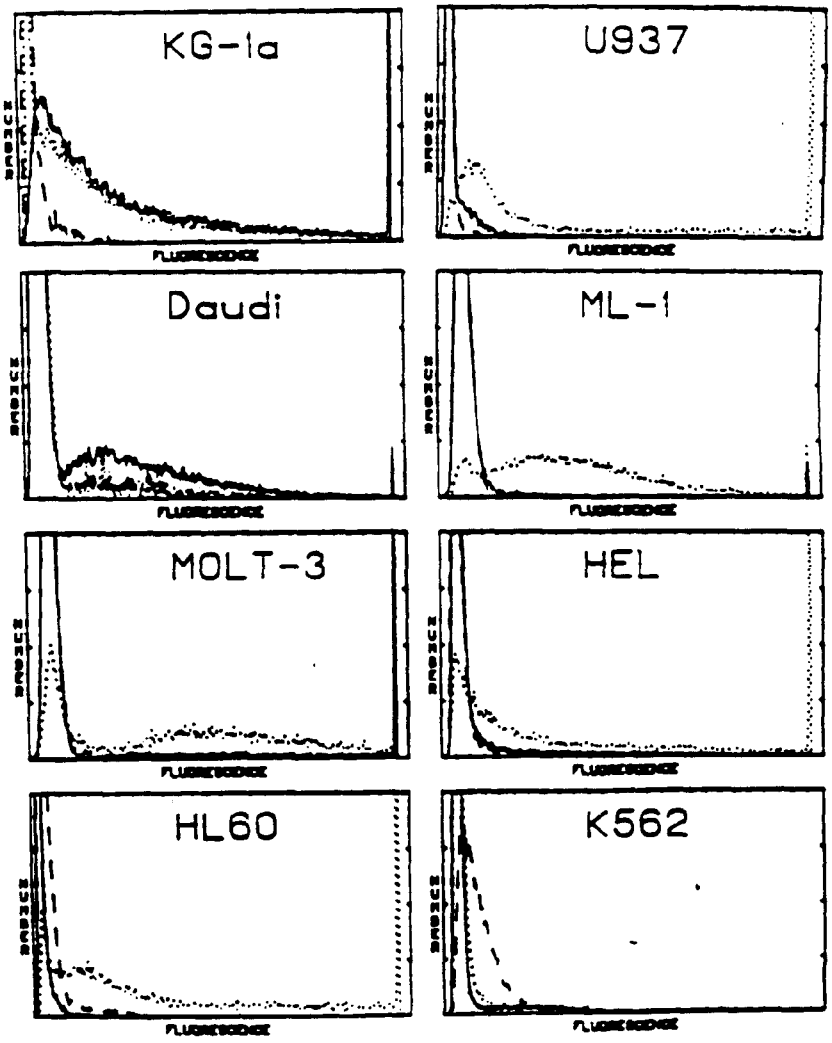

Figure 2. My-10 antigen expression on human leukemic cell lines: fluorescence histograms. Cells from continuous human leukemic cell lines were tested for antigen expression by indirect immunofluorescence with FACS II analysis. Dead cells were excluded from analysis of fluorescence on the basis of light scatter properties (42). Fluorescence histograms are shown with *ordinate* in number of cells (1 *hatchmark* = 100 cells), *abscissa* in FACS II fluorescence channels (1 *hatchmark* = 10 channels. Laser power = 400 mW; PMT voltage = 700; preamplification = 4.0 (coarse), 1.0 (fine)). Monoclonal antibodies were: anti-My-10 (*solid lines*), MOPC 21 (negative control: *dashed lines*), and 28/43/6 (positive control: *dotted lines*).

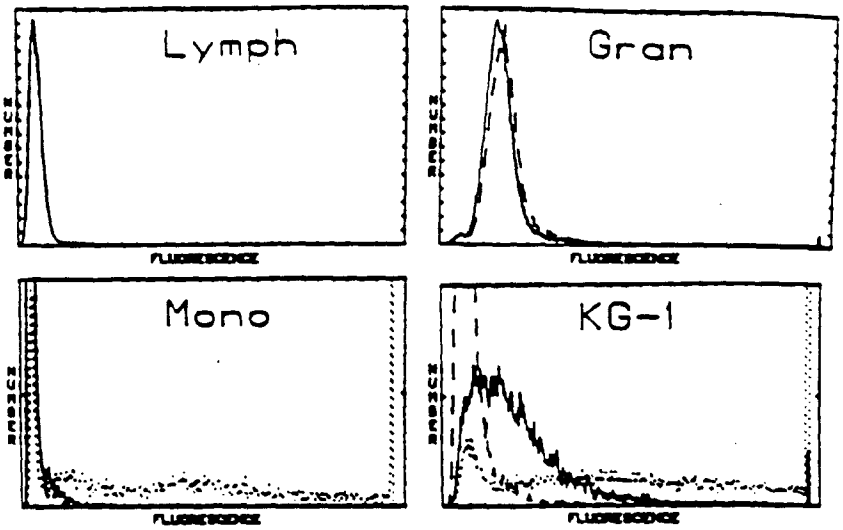

Figure 3. My-10 antigen expression on normal human blood cells and KG-1 cells: fluorescence histograms. KG-1 cells and purified populations of normal donor blood cells were tested for antigen expression by indirect immunofluorescence. On the basis of light scatter properties, dead and red cells were excluded from fluorescence analysis. Light scatter windows were also chosen to maximize purity of the analyzed populations (e.g., contaminating lymphocytes in the monocyte preparation were excluded from fluorescence analysis) (22, 42). FACS II fluorescence histograms are shown with FACS II settings and symbols as in *Figure 1*, except that lymphocytes and granulocytes were analyzed at a PMT voltage = 900, and the 28/43/6 antibody was not tested on lymphocytes or granulocytes in these experiments.

Giemsa stain) after reaction with anti-My-10. No specific fluorescence was detected on peripheral blood cells in a system in which (as evidenced by the experiments (Fig. 4) with anti-My-10 and marrow cells, as well as in other unpublished experiments) an My-10-positive subpopulation consisting of as few as approximately 1 to 2% of the cells would have been detected. Positive My-10 expression of KG-1 cells is shown for comparison. In additional (immunofluorescence and EIA) assays, anti-My-10 did not label peripheral blood granulocytes, mononuclear cells (including E rosette-positive and E rosette-negative cells and latex bead-labeled phagocytic cells, analyzed individually), red cells, or platelets from any of nine normal human blood donors. Thus, My-10 was not expressed on a detectable subpopulation of any peripheral blood cell type. In addition, phytohemagglutinin-stimulated PBMC were not detectably labeled with anti-My-10 (data not shown).

Low-density, plastic-nonadherent, marrow cells from normal human donors were analyzed for cell surface expression of My-10 antigen by indirect immunofluorescence by using visual microscopic detection. A small but definite (1.3% mean) subpopulation of marrow cells was fluorescent over MOPC 21 background in eight experiments. Figure 4 illustrates that a small subpopulation of My-10-positive marrow leukocytes was also identified by flow cytometry. KG-1a cells, tested in the same experiment, are shown for comparison. In both the KG-1a cells and the My-10-positive marrow cells, there is cellular heterogeneity with regard to My-10 antigen cell-surface density, from near background to off-scale at these instrument settings. Mean fluorescence intensity of the anti-My-10-treated marrow cells was 1.2, compared with 0.8 with MOPC 21 and 15.6 with 28/43/6; 2.1% of anti-My-10-treated marrow cells were more fluorescent than the 99.9 percentile cell treated with MOPC 21.

FACS II sorting of My-10-treated marrow cells. Under aseptic conditions, normal low density, nonadherent marrow cells were incubated with centrifuged anti-My-10, were washed, and were then reacted with centrifuged,

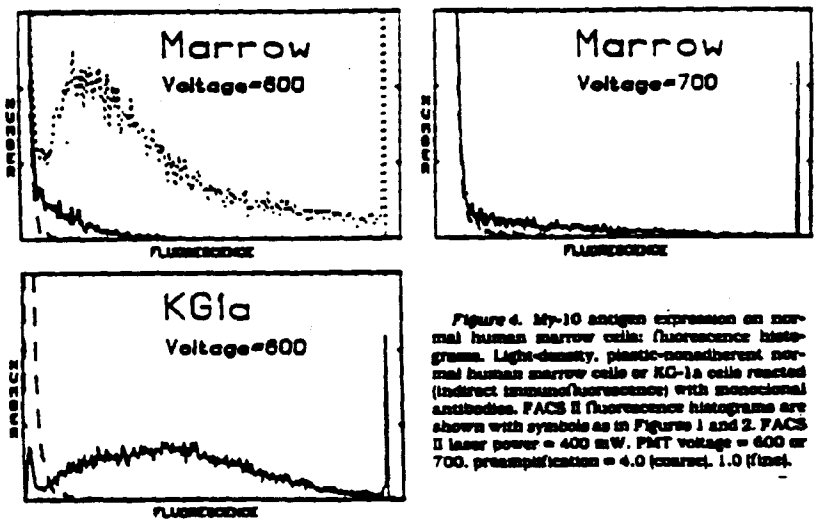

Figure 4. My-10 antigen expression on normal human marrow cells: fluorescence histograms. Light-density, plastic-nonadherent normal human marrow cells or KG-1a cells reacted (indirect immunofluorescence) with monoclonal antibodies. FACS II fluorescence histograms are shown with symbols as in *Figures 1* and *2*. FACS II laser power = 400 mW. PMT voltage = 600 or 700, preamplification = 4.0 (coarse), 1.0 (fine).

fluorescein-conjugated, anti-mouse IgG (as above for analytical indirect immunofluorescence). After washing, the cells were analyzed and sorted on the basis of fluorescence intensity (FACS II). Dot plots of the marrow cells after reaction with the MOPC 21 control IgG (Fig. 5A) are compared with My-10-treated marrow cells (Fig. 5B). Figure 5D shows comparative fluorescence histograms. "My-10-bright" cells were defined as >50 channels fluorescence intensity (1.93% of total My-10-treated cells; in contrast, 0.05% of the MOPC 21-treated cells were brighter than 50 channel units). The FACS II was adjusted to deflect anti-My-10-treated cells with fluorescence intensity <30 channels into the "My-10-dull" fraction (97.14% of total cells). A "window" of cells between 30 to 50 channels fluorescence intensity (0.93% of total My-10-treated cells) was discarded to minimize overlap, and is illustrated by the dark horizontal band in Figure 5C. The fluorescence profiles of the (reanalyzed) sorted cells are shown in Figure 5D. The My-10-bright fraction consisted almost entirely (87%) of morphologically-defined blast cells (Fig. 6, Table I). Cytochemical assays (Table II) suggested that the FACS-separated My-10-positive blast cell fraction contained both monoblasts (nonspecific esterase positive) and myeloblasts (NASD chloroacetate esterase positive), and probably cells of other lineages (the majority of cells were "primitive" in that they stained for none of the tested substances).

The FACS-separated cells were also plated in methylcellulose cultures, and colony-forming cells-granulocyte/monocyte (CFC-GM) and burst-forming units-erythroid (BFU-E) were enumerated at day 14. The My-10-positive fraction contained essentially all of the hematopoietic CFC, and was approximately 50-fold enriched for these progenitor cell types (Table III); 18% of the My-10-positive cells formed colonies detectable in this culture system. Cell recovery was 41%; CFC recovery was slightly higher in this experiment, but this was not consistently observed in other experiments in which the percent CFC recovery approximated the percent recovery of total cells. These FACS results (replicated in two additional experiments) are also in agreement with the results with the use of the panning methodology (20) and immune rosetting (34, 35), except that compared with panning the FACS apparently yielded a population of My-10-positive cells that was more enriched in primitive and clonogenic cells (data not shown). By FACS, the yield of initial total cells and CFC was consistently approximately 50%. Although the purity was lower (30 to 80% blasts, 10- to 30-fold progenitor cell enrichment) by panning, the yield was almost 80%. There was no consistent suggestion, by either methodology, of partition of a hematopoietic helper or suppressor cell population, because in the majority of experiments, the expected total number of progenitor cells was recovered (all in the My-10-positive cell fraction).

Reactivity of anti-My-10 with diagnostic specimens from patients with acute leukemia. Initial diagnostic marrow specimens from John Hopkins Oncology Center

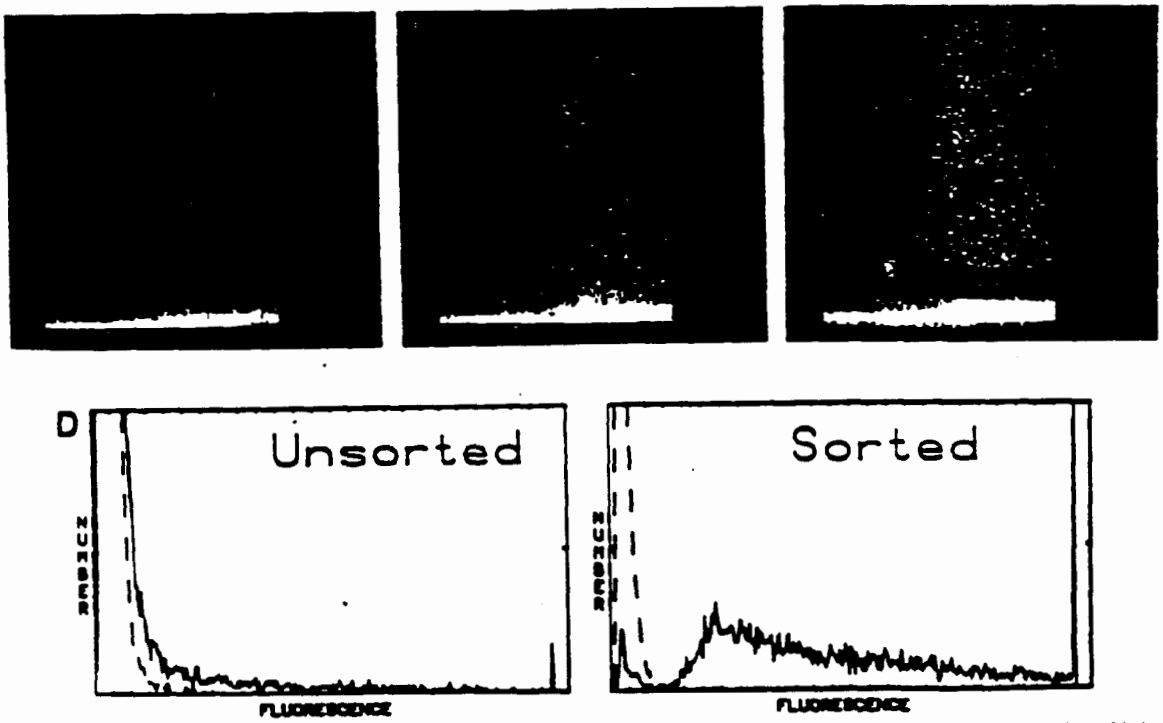

Figure 5. My-10 antigen expression on normal human marrow cells. *A–C.* FACS II oscilloscope fluorescence vs light scatter dot plots of light-density normal human marrow cells reacted (indirect immunofluorescence) with monoclonal antibodies. FACS II laser power = 400 mW; PMT voltage = 700; preamplification = 6 (coarse), 1 (fine). *A.* MOPC 21. *B.* Anti-My-10. *C.* Anti-My-10; dark *horizontal band* divides the sorted cell populations into "dull" and "bright." *D.* FACS II fluorescence histograms of the same labeled marrow cells. *Left.* Anti-My-10-treated (*solid line*) or MOPC-21-treated (*dotted line*) cells before separation. *Right.* "Dull" (*dashed line*) and "bright" (*solid line*) sorted anti-My-10-treated populations upon reanalysis after cell sorting.

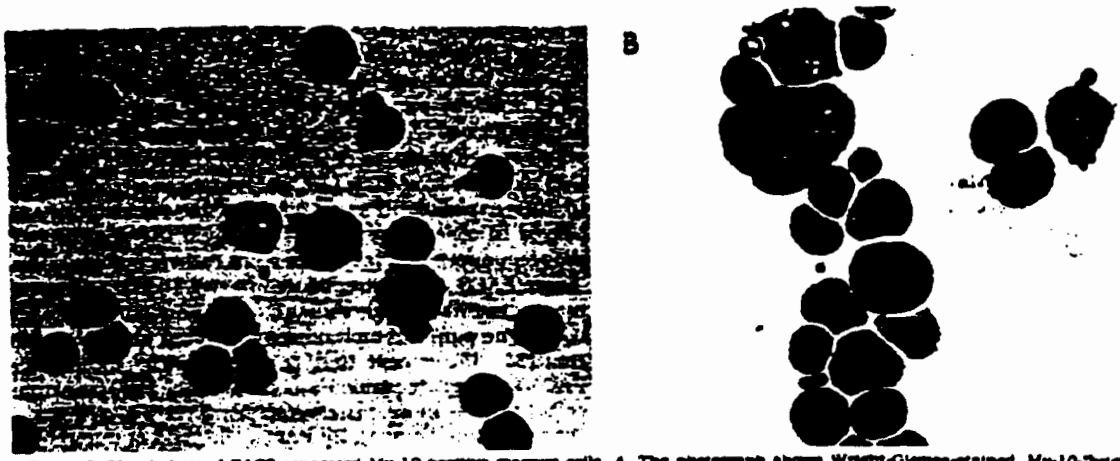

Figure 6. Morphology of FACS-separated My-10-positive marrow cells. *A.* The photograph shows Wright-Giemsa-stained, My-10-"bright" cells obtained by cell sorting: 17 blasts, two promyelocytes, and one metamyelocyte are shown in this photograph. *B.* This photograph is of Wright-Giemsa-stained My-10-positive cells obtained by panning. Note the heterogeneity of the blast cells, the mitotic figure, and the megakaryocyte in this field (63× oil objective).

TABLE I

Differential blood counts[a] of MY-10-antigen-positive vs-negative marrow cells

Marrow Cells	Unsorted[b]	My-10-Dull	My-10-Bright
Blast cells	5.8	6.2	86.6
Promyelocytes	13.0	7.2	2.8
Myelocytes	9.0	4.0	0.2
Metamyelocytes	10.8	5.8	0.6
Band Forms	5.4	5.4	0.2
Segmented neutrophils	2.6	4.2	1.0
Basophils	0.8	0.8	0.0
Eosinophils	0.0	0.2	0.0
Monocytes	10.4	5.8	6.2
Lymphocytes	27.0	38.2	1.8
Plasmacytes	1.0	0.8	0.2
Erythroblasts:			
Orthochromatophilic	10.8	20.0	0.4
Polychromatophilic	0.6	1.8	0.0
Basophilic	1.2	1.2	0.0
Proerythroblasts	1.0	1.8	0.0
Megakaryocytes	1.0	0.0	0.0

[a] Percent of >500 Wright's-stained cells counted.

[b] Cells were anti-My-10-treated and were passed through FACS laser but not sorted. The results are from the same experiment as Tables II and III and Figures 4 and 5.

TABLE II

Cytochemical analysis of FACS-separated My-10-antigen-positive primitive cells[a]

Cytochemical Stain	Percent Primitive Cells Cytochemically Positive[b]
Peroxidase	14
Sudan black	10
Periodic acid schiff	16
NASD chloroacetate esterase	8
Nonspecific esterase: diffusely stained	29[c]
Focally stained	1[c]

[a] Mature cells were not scored in this analysis of the "primitive blast" cells (67%, all morphologically immature with a fine, open chromatin pattern) and promyelocytes (3%).

[b] Two hundred cells counted; each cytochemical test was done on a separate slide, except for the esterases, which were done on the same slide.

[c] Values were zero with NaF added (NaF inhibits nonspecific esterase of monocytes).

patients found to have leukemia with ≥80% marrow blast cells were tested with these antibodies by indirect immunofluorescence. Specimens that contained ≥20% fluorescent cells (over background) were counted as "positive" for that antigen (41). The My-10 antigen was expressed on blast cells from approximately 30% of the acute leukemia specimens, both lymphocytic and nonlymphocytic, but on none of the few chronic leukemia specimens tested, including two specimens of chronic myelogenous leukemia in "myeloid" blast crisis or other specimens tested (Table IV).

TABLE III

Colonies in methylcellulose culture after FACS experiment

	Unsorted[a]	My-10-Dull	My-10-Bright
Recovered viable cells:	(100%)	97%[b]	2%[b]
CFC-GM colonies per 10^5 cells	197 (±62)	2 (±1)	11,900 (±2324)
Recovered CFC-GM[c]	9,850	32	4,760
BFU-E colonies per 10^5 cells	61 (±9)	4 (±0)	5,200 (±1188)
Recovered BFU-E[c]	3,050	90	2,080

[a] Cells were anti-My-10 treated and were passed through FACS laser but not sorted.

[b] These values were percents of the sum of the cells recovered from the FACS in both fractions. This sum represented only 41% of all cells delivered into the FACS, a routine yield with this instrument (necessary to reduce overlapping sorted populations and to reduce the incidence of >1 cell per sorted per droplet).

[c] Product of (CFC/10^5 cells) × (number of viable cells in fraction).

DISCUSSION

Human KG-1a undifferentiated leukemia cells were used as an immunogen to raise monoclonal antibodies against potential blast cell-surface antigens. Initial hybridoma supernatants were screened for binding to granulocytes as well as to KG-1a cells to identify and exclude antibodies that recognized antigens (such as HLA or My-1 (5, 20)) shared with mature granulocytes. By using this strategy, we selected hybridomas that were specific for the primitive KG-1a cell line, and after cloning we investigated the binding specificities of these monoclonal antibodies. Of these antibodies, anti-My-10 appeared to have the most narrow cellular specificity in that it bound only to the KG-1a and KG-1 cell lines of the nine human leukemia cell lines tested. We were able to show that the My-10 antigen is a KG-1a cell-surface protein of approximately 115 kD. The expression of My-10 on a variety of nonmyeloid cell types (including the many malignant and minor normal lymphoid cell sub-

TABLE IV

Reactivity of patients' marrow[a] leukemic blast cells with Anti-My-10

Disease[b]	Percent Positive Specimens[c]
Acute nonlymphocytic leukemia	28% (18/65)
Acute lymphocytic leukemia	32% (10/31)
cALLa positive (6/23)	
HLA-DR positive, cALLa negative (2/3)	
T cell (Leu-1 or T11 positive) (0/5)	
Chronic Lymphocytic Leukemia	0% (0/10)
Chronic Myelogenous Leukemia	0% (0/3)
Myeloblastic crisis (0/1)	
Basophilic blast crisis (0/1)	
Untreated chronic phase (0/1)	
Mycosis fungoides[a]	0% (0/1)
Lymphoma	0% (0/2)
Non-T, non-B	
B cell	
Undifferentiated carcinoma (marrow involvement)	0% (0/1)

[a] Peripheral blood (>80% leukemic mononuclear cells) was studied instead of bone marrow in nine chronic lymphocytic and three acute lymphocytic leukemia specimens, as well as in the one mycosis fungoides specimen. Aspirate cells or mechanically dissociated cells from lymphomatous nodes were studied in the two patients with lymphoma.

[b] Diagnosis defined by clinical features, blast cytomorphology and cytochemistry, and immunologic markers (1).

[c] Values represent percent of specimens with >20% (above MOPC 21 background) antibody-labeled cells (number positive specimens/number tested); see text.

types) remains to be determined. A subset of fresh acute leukemia (both lymphoid and nonlymphoid) blast cell specimens, however, expressed this antigen. It will be interesting to correlate My-10 antigen expression by patients' blast cells with the clinical features and outcomes of their leukemia (43–45); these ongoing studies will require large numbers of patients.

My-10 was expressed on few, if any, normal peripheral blood cells and few marrow leukocytes. The FACS was used to separate the rare My-10-positive normal human bone marrow cells from the predominant My-10-negative marrow cells. By this technique, approximately 1 to 2% of normal marrow cells were routinely obtained in the My-10-positive population. These cells were characterized by morphologic and cytochemical techniques, and for erythroid and myeloid hematopoietic progenitor cells by *in vitro* colony-forming assays. By morphologic analysis, the My-10 antigen is clearly a blast cell antigen, in that over 80% of the My-10-positive marrow cells were found to be blast cells. The My-10-positive fraction included blast cells of heterogeneous morphology. Morphologically and cytochemically definable myeloblasts and monoblasts were My-10 positive, but My-10 expression decreased in maturing myeloid cells, because only rare progranulocytes, promonocytes, and more mature granulocytic or monocytic cells were found in the My-10-positive cell fraction. Confirmatory results were obtained by using immune rosetting and immune adherence ("panning") to separate marrow cells by My-10 expression.

My-10-positive vs -negative FACS-separated marrow cell populations were assessed for myeloid progenitor cell content. Over 95% of all CFC-GM and BFU-E were in the My-10-positive fraction and were enriched many-fold (from normal human marrow in a single step). Thus, the My-10 antigen is expressed on CFC of all myeloid lineages tested to date. In fact, 18% of FACS separated My-10-positive cells formed day 14 colonies in a single assay system. Because in most experiments all initial CFC could be accounted for (and were found in the My-10-positive fractions), no evidence was found (in this system) for the existence of a My-10-negative helper or suppressor cell for hematopoiesis (46).

Because the human HLA-DR (Ia-like) antigen appears to be expressed on some if not all CFC (47–49), the hematopoietic progenitor cell expression of My-10 is similar to that of HLA-DR. However, monocytes and B cells are HLA-DR positive but My-10 negative. (In addition, the My-10 antigen is a 115 kD protein, whereas the HLA-DR glycoprotein is a 29/34 kD dimer.) Anti-HLA-DR monoclonals typically (50; unpublished results) detect greater than 10% of human marrow cells, whereas anti-My-10 labels only about 2%.

The T10 antigen reacts with terminal deoxynucleotidyl transferase-positive, bone marrow lymphoid cells, (T cell precursors) and CFC-GM, as well as a compartment of immature thymic lymphocytes (51). The phenotypes of other myeloid progenitor cells, with respect to T10, have not yet been published. Antibodies against the transferrin receptor (e.g., T9) react with proliferating but not resting progenitor cells, as well as with later marrow cells with only very limited proliferative potential, such as late erythroblasts (51).

Griffin's anti-My-7 monoclonal antibody reacts with some but not all CFC-GM, but no BFU-E. Anti-My-7 also reacts with more mature myeloid and blood cells (52). The AHN-7 monoclonal antibody also reacts with a subset of CFC-GM and with about 25% of marrow cells (41). Finally, Bodger's RFB-1 monoclonal antibody reacts with CFC-GM and marrow T (but not B) lymphocytes. In addition, it reacts weakly with mature blood T cells. Overall, about 30% of normal marrow cells express RFB-1 (53).

Of these antibodies that bind to human myeloid progenitor cells, anti-My-7 and RFB-1 react with nonprogenitor cells of a single lineage. RFB-1 reacts with progenitor cells (CFC-GM) that are outside of its mature cell-specific lineage (T cell) spectrum. The T10 antibody may fit into this category too, in that it reacts with early thymocytes as well as early myeloid cells. In contrast, the T9 antibody does not appear to have lineage specificity but reacts instead with all proliferating, transferrin receptor-positive cells. Thus, it appears to be a stage-specific rather than a lineage-specific antigen. Based upon our results, My-10 appears also to be a stage-specific rather than lineage-specific antigen.

Anti-My-10 appears to label the hematopoietic progenitor cell subset more specifically than any previously described monoclonal antibody, in that it reacts with the smallest percent of more mature marrow cells. This antibody has potential utility in hematopoietic research, in that it allows the isolation of a relatively pure population of immature hematopoietic cells, including progenitor cells for hematopoiesis, in a single step. My-10-positive marrow cells may be an appropriate normal control cell population to compare with leukemic blast cells and to use in studies on the mechanism of action of cells, factors, and genes that regulate hematopoietic cell proliferation and differentiation. For example, the near 100% recovery of most *in vitro* CFC in the My-10-positive marrow cell subpopulation indicates that My-10-negative accessory cells do not appear to be necessary for the growth or differentiation of these progenitor cells in this system.

Additional definition of the cell types contained in the My-10-positive marrow cell population is in progress. These studies will determine the utility of this antibody

for clinical research studies such as the assessment of progenitor cell numbers in patients with aplastic anemia, and potentially, the transplantation of hematopoietic stem cell-enriched, mature lymphocyte-depleted cell populations in human bone marrow transplantation.

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