New Hematopoietic Differentiation Antigens Detected by Anti-K562 Monoclonal Antibodies

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ABSTRACT

Following immunizations of BALB/c mice with K562 cells, we have obtained seven original monoclonal antibodies (MoAbs): (a) One MoAb, GA3, defines an antigen essentially restricted to the red cell series. This antigen is expressed on immature erythroblasts but is not detectable on the surface of early and late erythroid progenitors. GA3 MoAb immunoprecipitates a M, 105,000 glycoprotein on K562 cells. (b) Two MoAbs, 14B6 and 12B1, react with cells of the monocytic series. MoAb 14B6, which also faintly stains platelets, is reactive with immature myeloid cells and the majority of hematopoietic progenitors. The 14B6 antigen has been immunoprecipitated from 12-O-tetradecanoylphorbol-13-acetate treated K562 cells as a M, 130,000-100,000 protein. Antigen 12B1 is expressed only on cultured monocyte/macrophages and is restricted to a subpopulation of monocytes and to follicular dendritic cells. It is not detected on hematopoietic progenitors. Immunoprecipitation experiments performed on 12-O-tetradecanoylphorbol-13-acetate treated K562 cells revealed a glycoprotein with a molecular weight of 93,000-86,000. (c) Two anti-K562 MoAbs, CF4 and HE10, recognize a myeloid differentiation antigen expressed from the granulomonocytic colony forming unit stage to polymorphonuclear neutrophils. These MoAbs detect an apparently original glycolipid moiety distinct from LeX. (d) Two MoAbs recognize antigens expressed on the granulomonocytic series. 2E1 recognizes the monocyte low affinity Fc receptor (M, 40,000) and defines a new cluster of myeloid differentiation (CDw32). The antigen is expressed on a small portion of immature hematopoietic progenitors. 8F5 identifies a M, 95,000 protein which is also present on plasma cells. In some experiments, it is detected on erythroid colony forming unit analysis. Immunizations with K562 cells thus resulted in the production of antibodies recognizing antigens of the monocytic, granulocytic, as well as erythroid series. However, three of them are also detected on hematopoietic progenitors.

INTRODUCTION

The K562 cell line (1) was considered originally as a granulocytic cell line on the basis of (a) positive staining with human antisera directed against granulocytic antigens and (b) the absence of lymphoid markers (2, 3). The presence of hapten X (CD15), an antigen which is mostly associated with granulocytic differentiation, has been demonstrated on the surface of K562 cells (4, 5). However, it was shown later that K562 cells exhibit markers of the erythroid series, including hemoglobin and glycoporphin A (6-8). Exposure to hemin or sodium butyrate favors hemoglobin synthesis (9-11) but does not induce terminal erythroid differentiation. A peroxidase activity cytochemically identical to that of platelet peroxidase has been detected in rare cells of the K562 cell line (12). After TPA3 or sodium butyrate induction, the number of cells exhibiting this peroxidase activity increased with concomitant appearance of platelet glycoprotein IIIa (13, 14). Therefore, whether K562 is a pure erythroleukemic cell line simultaneously expressing early and late markers of erythroid differentiation or an abnormal pluripotent cell line is still a matter of debate.

The purpose of this study was to analyze a panel of monoclonal antibodies obtained after immunization with K562 cells, assuming that characterization of the antibodies would provide further elements concerning cell lineage specific antigens present on these cells. We show here that immunization with K562 cells resulted in the production of antibodies recognizing new antigens specific for the monocytic series as well as other original antibodies directed against granulocytic and erythroid antigens.

MATERIALS AND METHODS

Isolation of Monoclonal Antibodies

Isolation of Monoclonal Antibodies

Female BALB/c mice, 4 months old, were given injections of K562 cells (107 cells by injection). Three days after the last immunization, the spleen was removed and gently teased. Splenocytes were fused with the mouse myeloma cell line NS1 according to the technique of Köhler and Milstein (15). Briefly, spleen cells from the immunized mice were added to myeloma cells in a ratio of 4 to 8 lymphocytes per myeloma cell and fused in the presence of 50% polyethylene glycol. Cells were then grown in microplates (Falcon, Oxnard, CA) containing RPMI 1640 (Gibco, Grant Island, NY), supplemented with hypoxanthine-aminopterin-thymidine, 15% heat inactivated horse serum (Gibco), L-glutamine (2 mM), and gentamicin (50 mg/liter). Supernatants from wells containing growing hybrids were screened by ELISA assay on K562 cells. Growing hybridomas secreting specific antibodies were subcloned by the limiting dilution technique.

Control Monoclonal Antibodies

14B6 (anti-LeX, CD15) reacts with granulocytes, their precursors, and monoblasts (16); C17 is an anti-platelet GP IIa MoAb (17); LICR/LON/R10 is an anti-glycophorin A MoAb (18); and OKM1 is an anti-C3bi receptor MoAb (CD11), which stains monocytes, granulocytes, and null cells (19).

Cell Lines

K562, HL60 (20), and U937 (21) cells were routinely cultured in RPMI 1640 supplemented with 10% heat inactivated fetal calf serum, L-glutamine (2 mM), and gentamicin (50 mg/liter).

Differentiation Induction with Hemin, TPA, and DMSO. K562 cells were treated with 0.1 mM hemin (type III; Sigma) for 5 days. TPA (Sigma, St. Louis, MO) induction of K562, HL60, and U937 cells was performed by treating the cells with 160 nM TPA for 48 h. DMSO (3 Supported by a Catalan Institution (CIRIT). 4 The abbreviations used are: TPA, 12-O-tetradecanoylphorbol-13-acetate; MoAb, monoclonal antibody; BFU-E, erythroid burst forming unit; CFU-E, erythroid colony forming unit; CFU-GM, granulomonocytic colony forming unit; CFU-MK, megakaryocytic colony forming unit; DMSO, dimethyl sulfoxide; PBS, phosphate buffered saline; ELISA, enzyme linked immunosorbent assay.
Normal Hematopoietic Cell Isolation

Blood Cells. Erythrocytes were obtained from the sedimented pellet of blood samples after 3 washings. Lymphocytes were prepared by centrifugation of blood samples on Ficoll-metrizoate density gradient (Lymphoprep, Oslo, Norway) and made free of monocytes by adherence (45 min at 37°C) on Petri culture dishes. Erythrocyte resetting T-lymphocytes were obtained by using previously described methods (22). Monocytes were recovered by scraping the dishes used for adherence. Granulocytes were prepared after elimination of erythrocytes at 37°C with Plasmagel (R. Bellon, Paris, France) and of mononuclear cells on a Ficoll-metrizoate gradient. Platelets were obtained from platelet concentrates provided by the Centre Départemental de Transfusion Sanguine du Val de Marne (Creteil, France), washed 3 times, and stored in 2 mM EDTA-PBS buffer.

Bone Marrow Cells. Bone marrow cells were prepared by centrifugation of bone marrow samples on a Ficoll-metrizoate gradient to eliminate most of the erythrocytes and mature granulocytes.

Cells Derived from CFU-GM, CFU-E, BFU-E, and CFU-MK Assay. Light density marrow cells were separated by Ficoll gradient as above. The plasma clot technique was used as reported previously (23). The stimulatory factors used were either 10% crude supernatant from the MO-cell line, plus 1 unit/ml porcine erythropoietin (60 units/mg of protein; Centre National de Recherche Scientifique, Paris, France), or 2.5% phytohemagglutinin leukocyte conditioned medium for CFU-GM, erythroid progenitor, and CFU-MK assay, respectively. Cells were cultured for 7 days for CFU-E and from 12 to 14 days for the other progenitors.

ELISA. The ELISA technique was used for the screening of supernatants from the hybrid cells. Briefly, 5 × 10^5 cells previously washed in PBS were incubated for 1 h at 37°C with 0.1 ml of the hybridoma supernatants. Cells were then washed in PBS and again incubated for 1 h at 37°C in 0.05 ml diluted goat anti-mouse immunoglobulin peroxidase conjugate (Amersham, Les Ulis, France), for 1 h at 37°C. After washing, 2, 2'-azinobis(3-ethylbenzathionil) sulfonate (Amerham) was added to perform a colorimetric reaction, measured on Titertek Multiscan MC (Flow, Paris, France) at 410 nm.

Immunofluorescence and Flow Cytometric Analysis. Cells were labeled in a standard double step immunofluorescence assay and analyzed by flow cytometry with an EPICS C analyzer (Coultronics, Hialeah, FL). Mouse immunoglobulins (10 μg/ml (Cappel, Cochranville, PA) were used as a negative control. The percentage of positive cells was determined by computer analysis using the Immuno EASY 88 program (Coultronics). Indirect immunofluorescence on in vitro cultures of hematopoietic colonies was directly performed in Petri dishes, after drying off the plasma clot culture. Cell staining was carried out using 0.4 ml antibody supernatant for each incubation plate. Cells incubated with PBS under the same conditions were used as negative controls. After labeling and washing, cells were fixed for 1 min with pure methanol. For colony examination, the Petri dish bottoms were cut, sealed on glass slides, and examined with an episcopic microscope. When CFU-MK colonies were identified, a second labeling was performed on methanol fixed cells using a polyclonal rabbit anti-IgG factor (Dakopatts, Copenhagen, Denmark).

Immunopanning. The ability of the five MoAbs, GA3, 12B1 14B6, CF4, and HE10, to bind to hematopoietic progenitors was tested by immunopanning (24). Briefly, nonadherent light density marrow cells were first incubated on immunoglobulin coated Petri dishes to deplete the Fc receptor positive cells. The nonadherent cells were recovered and separated by a 5 × 10^5 cells culture incubated with one of the previous MoAbs at a 2 × 10^3 dilution for 30 min at 4°C. Cells were extensively washed with RPMI medium containing 1% serum albumin and were incubated at 4°C on 100-mm Petri dishes previously coated with 50 μg of affinity purified goat anti-mouse IgG (Zymed Laboratories, San Francisco, CA) for GA3, 12B1, and 14B6 MoAbs and affinity purified goat anti-mouse chain for CF4 and HE10 MoAbs. Nonadherent cells were gently removed whereas adherent cells were recovered by a combination of vigorous pipetting and scraping with a rubber policeman. Both fractions were counted. A sample of each cell fraction was used to be cytoreinfused and stained with May-Grünwald-Giemsa. The remaining cells were cultured in methylcellulose at a concentration of 10^3-10^4/ml using as stimulating factor a combination of supernatant of the MO cell line and porcine erythropoietin, 1 unit/ml. CFU-E, BFU-E, and CFU-GM were screened on days 7, 12, and 14, respectively. Phytohemagglutinated lymphocyte conditioned medium was used for the growth of CFU-MK.

Cell Sorting. Cell sorting was performed on a fluorescence activated cell sorter, FACS IV (Becton Dickinson, Mountain View, CA). Light density fresh marrow cells were labeled by 8F5, 2E1, and HE10 MoAbs using the indirect immunofluorescence assay. The difference between the intermediate and the strongly positive fraction has been arbitrarily determined (see Table 1, legend). The strongly positive fraction was contaminated by less than 1% negative cells and the intermediate fraction by 7-8% negative cells. Cells were kept on ice until sorting. Positive cells were collected and cultured as described above using 10^4 or 2 × 10^5 cells/plate. Sorted cells were reanalyzed under a fluorescent microscope. The positive fraction for each antibody usually contains at least 90% of positive cells. Cytoreinjected preparations were also made for cytological examination.

Immunogold Labeling and Ultrastructural Cytochemical Studies. Marrow and blood Ficoll-metrizoate prepared cells were first stained by cell sorting and after washing. Gold coupled goat anti-mouse IgG (gold particles, 15 nm; Jansen Pharmaceutica, Olsen, Belgium). Cells were subsequently fixed by 1.25% glutaraldehyde in Gey's basic salts for 10 min (25) and incubated for 30 min in dianisobenzidine medium (2 mg/ml) (Sigma) (26). After inclusion in Epon, thin sections were examined under a CM10 Philips electron microscope after light lead citrate staining.

Immunoprecipitation. Steady state metabolic labeling of K562 cells (before and after TPA treatment), immunoprecipitation, and polycrylamide gel electrophoresis were performed as described previously (27). Briefly, [35S]methionine labeled cells were resuspended at 5 × 10^5 cells/ml in lysis buffer [10 μM Tris-HCl, pH 7.2, containing 1% (v/v) Triton X-100, 10 mM phenylmethylsulfonyl fluoride, and 0.15 mM NaCl]. After 15 min on ice, the samples were centrifuged at 1000 × g for 10 min and the supernatants were recovered. Radiolabeled protein samples, corresponding to 5 × 10^4 cells (50-μl fractions) were incubated at 4°C with 5 μl of MoAb ascites. After 1 h, 25 μl of protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) were added, and the incubation was continued for 1 h at 4°C. Protein A-Sepharose was then washed 3 times with lysis buffer containing 0.15 mM NaCl and in 6 successive washings in 0.2, 0.5, and 1 mM NaCl. The final pellet was resuspended in 50 μl of 10 mM Tris-HCl, pH 7.2, containing 2% (w/v) sodium dodecyl sulfate and when required 5% (v/v) 2-mercaptoethanol. Bound proteins were eluted by heating the suspension at 100°C for 5 min. The mixture was then centrifuged at 12,000 × g for 5 min, and the supernatant was analyzed by polycrylamide gel electrophoresis in the presence of SDS performed according to the method of Laemmli (28) using 7-12% acrylamide gradient slab gels for GA3, 14B6, and 12B1 MoAbs and 10% acrylamide slab gels for 2E1 and 8F5 MoAbs. Irrelevant murine IgG2a and IgG1 were used as negative controls in all these experiments.

RESULTS

Monoclonal Antibody Production. MoAbs were generated following three different fusions using K562 cells as immunogen. Twelve hundred primary clones were screened by ELISA for the presence of antibodies reactive with K562 cells. Forty hybridomas consistently reactive were recloned. Immunofluorescence staining of a large panel of hematopoietic cells, including peripheral blood cells (monocytes, granulocytes, erythrocytes, platelets, erythrocyte rosetting T-lymphocytes and non-rosetting T-lymphocytes cells) and leukemia cell lines, such as HL60, U937, HEL, CEM, MOLT4, and Burkitt cells (RAJI, DAUDI), allowed us to eliminate MoAbs reacting with widely distributed markers and well characterized antigens. Following these restrictions, 7 original MoAbs (12B1, GA3, 14B6, 2E1,
8F5, CF4, and HE10) were selected for their possible lineage specificity, and their reactivities were analyzed in detail.

Monoclonal Antibody Reactivity with Peripheral Blood and Bone Marrow Cells. The 7 selected MoAbs were tested on peripheral blood cells by indirect immunofluorescence tests using an EPICS C flow cytometer (Table 1). The reactivity of the 7 MoAbs was also investigated on in vitro culture of hematopoietic colonies, i.e., BFU-E, CFU-E, and CFU-GM derived cells, by immunofluorescence and on marrow cells by the immunogold technique at the electron microscopic level. 12B1 MoAb did not react with any of the freshly isolated normal blood and marrow cells tested from six different donors but specifically reacted with the in vitro monocyte/macrophage colonies. The selective presence of 12B1 on cultured cells of the monocytic series has already been reported in detail (27).

GA3 MoAb slightly stained erythrocytes and a small fraction of T-cells (4%) which were CD3 and CD4 positive in double staining experiments. GA3 MoAb was negative with all other peripheral blood and bone marrow cells, except marrow erythroblasts. GA3 heavily labeled erythroid cells derived from BFU-E and CFU-E derived colonies and with the morphology of immature erythroblasts.

Two MoAbs, CF4 and HE10, were reactive only with granulocytes among blood cells. They labeled granulocyte colonies grown in vitro as well as the marrow granulocytic precursors from myeloblasts to polymorphonuclear neutrophils by electron microscopy with the immunogold technique. When blood cells were tested, 2E1 bound to monocytes, polymorphonuclear neutrophils from all series (mainly eosinophils and basophils), some lymphocytes, and platelets. In the marrow, when investigated by ultrastructural immunogold staining, it was reactive with a part of all hematopoietic precursors, especially monoblasts, myelocytes, and metamyelocytes, but did not label erythroblasts. Granulomonocytic colonies grown in vitro were also positive, as well as megakaryocytic colonies. Among peripheral blood cells, 8F5 MoAb stained monocytes and, very weakly, granulocytes. It was weakly reactive with a minority of marrow cells (6–15%) including some granulocytic precursors, plasma cells, and immature erythroblasts which were double labeled by 8F5 and an anti-carbonic anhydrase 1 antibody. Erythroid colonies were weakly stained. 14B6 MoAb strongly reacted with blood monocytes and faintly with platelets, in the absence of detectable labeling of granulocytes. Among in vitro cultured hematopoietic colonies, it weakly stained granulocytic ones whereas it strongly labeled the monocytic ones. 14B6 MoAb was also found to react with CFU-MK derived colonies, including large mature megakaryocytes and small immature cells identified by the presence of VWF. In the marrow, 14B6 was reactive with undifferentiated blasts and the granulocytic precursors from myeloblasts to myelocytes according to electron microscopic levels using the immunogold technique.

Monoclonal Antibody Reactivity with Cultured Human Cell Lines. The reactivity of the anti-K562 MoAbs with human myeloid cell lines (K562, HL60, U937) was analyzed before and after differentiation induction using TPA, DMSO, or hemin. Control MoAbs were used to assess differentiation induction of each cell line (16–19). Exposure of K562, HL60, and U937 cells to TPA induced an increased staining intensity with the MoAbs 12B1, 14B6, 8F5, and 2E1 (Table 2). Usually the rise observed in fluorescence did not concern the percentage of labeled cells, but the mean fluorescence, which increased about 30–60 channels with the logarithmic scale used here. This corresponds to a doubling in the amount of the recognized antigen. The reactivity of GA3 was markedly reduced after TPA treatment of K562 cells, which also abolished the reactivity of anti-glycophorin A MoAb, as reported previously (11). For CF4 and HE10 no significant change could be observed. No significant modification of the staining by any of the antibodies was observed on hemin treated K562 cells and on DMSO treated HL60 cells (data not shown).

Hematopoietic Progenitor Reactivity. All 7 MoAbs were first tested for their capacity to select cells by immune panning using K562 cells as the target. GA3, 12B1, 14B6, CF4, and HE10 MoAbs were quite effective since they were able to immobilize more than 90% K562 cells. In contrast, 2E1 and 8F5 MoAbs retained only 50% K562 cells. Therefore, in order to test the reactivity of these MoAbs with hematopoietic progenitors, the first five MoAbs were tested by the immune panning technique (Table 3), whereas the last two were used in cell sorting experiments (Table 4). Since the “negative fractions” obtained by immune panning were shown still to contain 8–10% positive cells, MoAbs unreactive with progenitors by this technique were therefore tested again in cell sorting experiments. In contrast, the “positive fractions” recovered by immune panning were shown to contain more than 95% positive cells. An anti-HLA-DR MoAb (K5, a generous gift from Dr. J. C. Brouet) which with this technique reacted with 80% of hematopoietic progenitors (except for CFU-E) was used as positive control. Three MoAbs, i.e., 14B6, 2E1, and 8F5, bound to a variable number of all types of hematopoietic progenitors. 14B6 was reactive with the majority of hematopoietic progenitors including BFU-E, CFU-GM, and also CFU-MK. In contrast, 2E1 labeled only a fraction of hematopoietic progenitors (Table 4). The reactivity of these MoAbs with hematopoietic progenitors was variable from one experiment to another, possibly as a consequence of the very faint staining on hematopoietic precursors. With 8F5, no progenitors was labeled except a weak staining of CFU-E (data not shown). CF4 or HE10 MoAbs which exhibited a very restricted reactivity to the granulocytic series were almost unreactive with day 7 or day 14 CFU-GM by immune panning, since only 1 to 7% of them were recovered in the positive fraction. HE10 MoAb was therefore tested in cell sorting experiments. In two experiments, HE10 antigen was clearly detected on day 7 CFU-GM, whereas the variability on day 14 CFU-GM (Table 4, Experiments B and C) reflected its very faint expression on these cells. All other progenitors were essentially negative with HE10. GA3 which reacted with marrow cells of the erythroid series and RBC, did not recognize BFU-E or CFU-E. The lack of reactivity of GA3 on hematopoietic progenitors was confirmed in cell sorting experiments (29). 12B1 MoAb was not detectable on any hematopoietic progenitors by immune panning or cell sorting (data not shown). The reactivity of the 7 MoAbs with hematopoietic cells is summarized in Fig. 1.

Table 1 Reactivity with normal peripheral blood cells
Staining of the cells was determined by an indirect immunofluorescence assay and was analyzed using an EPICS C flow cytometer. Results are shown by an arbitrary scale from + to +++. +, mean channel fluorescence 20 to mean 50; ++, mean channel fluorescence 50 to mean 100; ++++, mean channel fluorescence more than 100.

<table>
<thead>
<tr>
<th>MoAb Isotype</th>
<th>Erythrocytes</th>
<th>Platelets</th>
<th>Granulocytes</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>12B1</td>
<td>IgG2a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+*</td>
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<tr>
<td>GA3</td>
<td>IgG1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+*</td>
</tr>
<tr>
<td>14B6</td>
<td>IgG1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2E1</td>
<td>IgG2a</td>
<td>+</td>
<td>+</td>
<td>++*</td>
<td>+</td>
</tr>
<tr>
<td>8F5</td>
<td>IgG2a</td>
<td>-</td>
<td>+</td>
<td>+*</td>
<td>+</td>
</tr>
<tr>
<td>CF4</td>
<td>IgM</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
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<tr>
<td>HE10</td>
<td>IgM</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* 4% of erythrocyte rosetting T-cells.
Mainly eosinophils and basophils.
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Percentage of positive cells and mean fluorescence values are calculated after computer analysis including subtraction of values obtained for the negative controls (program ImmunoEasy 88, Coultronics). The averaged data and standard deviation of 3 independent experiments are shown. The screening on T- and B-lymphoid cell lines did not provide other elements and only experiments on myeloid cell lines are reported here in order to simplify the presentation of the data. Percentage of positive cells was determined by analysis on an EPICS C flow cytometer: 10^4 cells were accumulated. Mean fluorescence was determined on a logarithmic scale.

Table 2 Reactivity with K562, HL60, and U937 cells before and after treatment with differentiation inducers

<table>
<thead>
<tr>
<th>MoAbs</th>
<th>K562</th>
<th>HL60</th>
<th>U937</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>TPA</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>+cells</td>
<td>-cells</td>
<td>+cells</td>
</tr>
<tr>
<td>GA3</td>
<td>94 ± 4 (57 ± 4)</td>
<td>98 ± 2 (120 ± 11)</td>
<td>46 ± 3 (41 ± 9)</td>
</tr>
<tr>
<td>GA3</td>
<td>96 ± 2 (60 ± 7)</td>
<td>60 ± 2 (30 ± 6)</td>
<td>18 ± 4 (28 ± 5)</td>
</tr>
<tr>
<td>14B6</td>
<td>97 ± 3 (47 ± 8)</td>
<td>98 ± 2 (99 ± 8)</td>
<td>80 ± 9 (32 ± 9)</td>
</tr>
<tr>
<td>2E1</td>
<td>99 ± 1 (62 ± 10)</td>
<td>98 ± 2 (101 ± 8)</td>
<td>96 ± 2 (56 ± 7)</td>
</tr>
<tr>
<td>BF5</td>
<td>99 ± 1 (62 ± 7)</td>
<td>98 ± 2 (98 ± 9)</td>
<td>87 ± 8 (57 ± 9)</td>
</tr>
<tr>
<td>CF4</td>
<td>73 ± 8 (61 ± 8)</td>
<td>77 ± 7 (68 ± 8)</td>
<td>80 ± 6 (70 ± 9)</td>
</tr>
<tr>
<td>HE10</td>
<td>94 ± 5 (120 ± 10)</td>
<td>97 ± 3 (136 ± 11)</td>
<td>97 ± 2 (125 ± 15)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>TPA</td>
<td>None</td>
</tr>
<tr>
<td>Control</td>
<td>805H5</td>
<td>91 ± 4 (106 ± 7)</td>
<td>74 ± 6 (52 ± 9)</td>
</tr>
<tr>
<td>R10</td>
<td>93 ± 6 (68 ± 7)</td>
<td>15 ± 3 (13 ± 3)</td>
<td>98 ± 1 (64 ± 10)</td>
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<tr>
<td>C17</td>
<td>18 ± 3 (27 ± 5)</td>
<td>78 ± 5 (60 ± 7)</td>
<td>12 ± 4 (31 ± 7)</td>
</tr>
<tr>
<td>OKM1</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 3 Immune panning with GA3, 12B1, and 14B6 antibodies

Results are expressed as the number of colonies per 10^6 cells in each fraction. Numbers in parentheses represent the percentage of total number of progenitors in each fraction. A and B represent 2 independent experiments. The total recovery taking into account the total input of different progenitors was in the range of 90-150%, 70-90%, and 60-85%, respectively for CFU-E, CFU-GM, and BFU-E. In addition, 14B6 also binds to 55-70% CFU-MK.

Table 4 Cell sorting experiments with 2E1 and HE10 antibodies

A, B, and C are normal marrow specimens. Results are expressed as the number of colonies per 10^6 cells. Numbers in parentheses represent the relative percentage of progenitors in each fraction. Marrow specimen C was sorted with HE10 in 3 fractions: strongly positive (+), intermediate (+), and negative (−).

Biochemical Characterization of GA3, 12B1, 14B6, 2E1, and 8FS Antigens. Identification of membrane antigens detected by the antibodies GA3, 12B1, 14B6, 2E1, and 8FS was performed by immunoprecipitation experiments on biosynthetically labeled untreated or TPA treated K562 cells. When detergent solubilized lysates of untreated K562 cells were used, GA3 precipitated a radiolabeled band with an apparent molecular weight of 105,000 under reducing conditions (Fig. 2, Lane 2).
anti-K562 MoAbs: New Hematopoietic Differentiation Markers

The aim of the present study was to produce a number of new MoAbs raised against K562 cells in order (a) to further assess the phenotype of K562 cells and (b) to describe new markers for precise steps of normal hematopoietic cell differentiation. We selected 7 apparently original MoAbs. (a) MoAb GA3 detected on antigen expressed on the differentiated cells of the erythroid series, and particularly on erythroblasts. (b) MoAb 12B1 was restricted to the monocytic lineage and reacted with the more differentiated elements of these series (27). (c) Two other antibodies (CF4, HE10) specifically reacted with the cells of the granulocytic series. This reactivity resembled that of antibodies directed against the LeX determinant (CD 15) (5, 26). Two other antibodies (CF4, HE10) specifically reacted with the more differentiated elements of these series (27).

Fig. 1. Schematic representation of the reactivity of the 7 MoAbs with myeloid cells. Main reactivity. The minor reactivity of 2E1 and 8F5 with a subpopulation of hematopoietic progenitors is not illustrated as well as the reactivity of GA3, 2E1, and 8F5 with some lymphocytes or plasma cells.

Fig. 2. Fluorography pattern of immunoprecipitates obtained with GA3, 8F5, 2E1, 12B1, and 14B6 MoAbs from [*]S-methionine labeled K562 cells. Non-treated or TPA treated cells were metabolically labeled for 3 h, detergent solubilized, and immunoprecipitated as described in “Materials and Methods.” Four independent experiments are shown in A, B, C, and D. In A, C, and D, precipitates were analyzed under reducing conditions by 7-12% gradient polyacrylamide gel electrophoresis. In B, precipitates were analyzed under reducing conditions by 10% polyacrylamide gel electrophoresis. Lane 1, nonreducing background on TPA treated K562 cell lysate with an irrelevant IgG1 MoAb; Lane 2, K562 cell lysate treated with GA3; Lane 3, nonreducing background with an irrelevant IgG2a MoAb on K562 cell lysate; Lane 4, K562 cell lysate treated with 8F5; Lane 5, K562 cell lysate reacted with 2E1; Lane 6, K562 cell lysate reacted with 12B1; Lane 7, nonreducing background on TPA treated K562 cell lysate with an irrelevant IgG2a MoAb; Lane 8, TPA treated cell lysate reacted with 12B1. Lane 9, K562 cell lysate reacted with 14B6 MoAb. Lane 10, nonspecific background on TPA treated K562 cell lysate with an irrelevant IgG1 MoAb; Lane 11, TPA treated K562 cell lysate reacted with 14B6 MoAb. The migration of the [4C]-methylated protein molecular mass standards is shown: myosin, M, 200,000; phosphorylase, M, 92,500; bovine serum albumin, M, 69,000; ovalbumin, M, 46,000; carbonic anhydrase, M, 30,000; lysozyme, M, 14,300.

DISCUSSION

The aim of the present study was to produce a number of new MoAbs raised against K562 cells in order (a) to further assess the phenotype of K562 cells and (b) to describe new markers for precise steps of normal hematopoietic cell differentiation. We selected 7 apparently original MoAbs. (a) MoAb GA3 detected on antigen expressed on the differentiated cells of the erythroid series, and particularly on erythroblasts. (b) MoAb 12B1 was restricted to the monocytic lineage and reacted with the more differentiated elements of these series (27). (c) Two other antibodies (CF4, HE10) specifically reacted with the cells of the granulocytic series. This reactivity resembled that of antibodies directed against the LeX determinant (CD15) (5,
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16, 30), a highly immunogenic moiety expressed on K562 cells. Experiments with a panel of purified glycolipids have shown that CF4 and HE10 did not react with the LeX (data not shown) but recognize a long polar glycolipid chain in the ganglioside fraction. Purification and identification of these antigens will be reported elsewhere.3 The reactivity of CF4 and HE10 with a glycolipid moiety distinct from LeX has also been confirmed during the IIIrd international Myeloid Workshop (31). (d) MoAb 14B6 reacted with progenitors of all hematopoietic series. Among differentiated cells it was expressed only on monocytes and platelets. (e) The MoAb 2E1 recognized an antigen expressed on the granulomonocytic, platelet, and lymphoid series, which is a M, 40,000 protein. In the IIIrd International Workshop on Leucocyte Differentiation Antigens, it has been shown that 2E1 defines the new myeloid differentiation antigen CDw32, which corresponds to the low affinity Fc IgG receptor present on monocytes and platelets (31). (f) MoAb 8F5 detected a M, 95,000 protein expressed by monocytes, granulocytes, and plasma cells, and also present on a subset of epidermal cell. Additional data from the literature showed that 8F5 indeed reacted with the recombinant ICAM-1 molecule, the adhesion ligand of the LFA-1 molecule (31, 32).

The interest and the originality of these anti-K562 MoAbs has been further demonstrated during the IIIrd International Workshop on Human Leucocyte Differentiation Antigens (33). Among 150 new MoAbs tested in the myeloid panel of this workshop, MoAbs 12B1, 14B6, and 8F5 appeared to react with unique antigens. CF4 and HE10 were clustered in Group 11 with 2 other antibodies and, as already mentioned, 2E1 defined the CDw32 antigen with one other MoAb. GA3 was not submitted to the workshop.

The problem of the K562 cell origin has been widely debated. Since K562 cells coexpress markers of the myeloid, erythroid, and megakaryocytic series, they could be considered either as erythroid precursors presenting some features of lineage infidelity, or alternatively as pluripotent hematopoietic precursors.

The use of K562 cells as immunogen results in the obtainment of only one MoAb (GA3) directed against the erythroid lineage. Indeed, GA3 defines a M, 105,000 marker essentially restricted to the RBC series and preferentially expressed on immature erythroblasts. It was found only on K562 (and HEL) cells among the panel of human cultured cell lines tested (data not shown) as well as on some rare mature circulating T-cells.

GA3 antigen was strongly decreased on K562 cells after TPA treatment. Fukuda et al. have previously described the presence of a M, 105,000 glycoprotein in K562 (33) and immature erythroblasts (29), which is undetectable on RBC. This glycoprotein, now called leukosialin, has been identified among different hematopoietic cell series belonging to erythroid, myeloid, T-lymphoid, and B-lymphoid cell lineages (35). Among these series, the apparent molecular weight of the glycoprotein differs due to cell lineage restricted glycosylation. We have recently shown that GA3 indeed does recognize leukosialin and that the GA3 epitope is glycosylation dependent, explaining the relative specificity of GA3 for the erythroid lineage (29).

Interestingly, GA3 reacts with more differentiated elements of the erythroid series and even with RBC, although leukosialin is not expressed at this stage. An interesting possibility would be that the carbohydrate epitope recognized by GA3 on erythroblasts is carried by another moiety on more differentiated stages of the erythroid series.

Three antibodies, i.e., 14B6, 2E1, and 8F5, were found to be reactive with a variable fraction of hematopoietic progenitors including the erythroid ones, but the antigens defined by these antibodies disappeared from the erythroid series early during differentiation. Therefore, regarding these antigens, K562 cells behave as an erythroid cell with a major asynchrony since they coexpress differentiation markers of erythroid progenitors associated to late differentiation markers such as glycoporphin A.

The antigens recognized by CF4, HE10, and 12B1 could not be found on normal cells belonging to the erythroid lineage whatever their stage of differentiation. The presence of the 12B1 antigen at the surface of K562 cells, especially after TPA treatment, remains difficult to explain but is thus far the first monocytic marker detected on this cell line.

In conclusion, we show here that immunization with K562 cells resulted in the production of new antibodies reactive with distinct antigens of the erythroid, granulocytic, and monocytic series. No platelet specific MoAb was obtained, probably due to the low amount of platelet antigens expressed on K562 cells before TPA induction. Therefore, immunization with K562 cells resulted in the obtainment of several MoAbs which may define new clusters of the myeloid differentiation (31). Further studies are pursued to determine whether these markers could be useful for the definition of precise steps of hematopoietic differentiation and to understand the function of these newly defined antigens.

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New Hematopoietic Differentiation Antigens Detected by Anti-K562 Monoclonal Antibodies

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