Monoclonal Antibodies Detecting Antigenic Determinants With Restricted Expression on Erythroid Cells: From the Erythroid Committed Progenitor Level to the Mature Erythroblast

By Takashi Yokochi, Martha Brice, Peter S. Rabinovitch, Thalia Papayannopoulou, and George Stamatoyannopoulos

Two new cell surface antigens specific for the erythroid lineage were defined with cytotoxic IgM monoclonal antibodies (McAb) (EP-1; EP-2) that were produced using BFU-E-derived colonies as immunogens. These two antigens are expressed on in vivo and in vitro derived adult and fetal erythroblasts, but not on erythrocytes. They are not detectable on resting lymphocytes, concanavalin-A (Con-A) activated lymphoblasts, granulocytes, and monocytes or granulocytic cells or macrophages present in peripheral blood or harvested from CFU-GM cultures. Cell line and tissue distributions distinguish McAb EP-1 and EP-2 from all previously described monoclonal antibodies. McAb EP-1 (for erythroblastic antigen-1) inhibits the formation of BFU-E and CFU-E, but not CFU-GM, colonies in complement-dependent cytotoxicity assays. By cell sorting analysis, about 90% of erythroid progenitors (CFU-E, BFU-E) were recovered in the antigen-positive fraction. Seven percent of the cells in this fraction were progenitors (versus 0.1% in the negative fraction). The expression of EP-1 antigen is greatly enhanced in K562 cells, using inducers of hemoglobin synthesis. McAb EP-2 fails to inhibit BFU-E and CFU-E colony formation in complement-dependent cytotoxicity assays. EP-2 antigen is predominantly expressed on in vitro derived immature erythroblasts, and it is weakly expressed on mature erythroblasts. The findings with McAb EP-1 provide evidence that erythroid progenitors (BFU-E and CFU-E) express determinants that fail to be expressed on other progenitor cells and hence appear to be unique to the erythroblast lineage. McAb EP-1 and EP-2 are potentially useful for studies of erythroid differentiation and progenitor cell isolation.

MONOCLONAL ANTIBODIES (McAb) addressed to cell surface antigens with a differential expression during erythroid differentiation are of potential value for studies of erythropoiesis. Although erythroid-specific cell surface markers exist (i.e., glycophorin), these do not appear to be expressed on erythroid progenitors.1,2 In addition, none of the surface markers that were previously shown to be present on erythroid progenitors, i.e., HLA-A, B, and C antigens, HLA-DR antigens, transferrin receptors, or i/i antigens, are exclusively or selectively expressed in erythroid progenitors;3 to our knowledge, no marker specific only for erythroid progenitors has been reported.

The purpose of this study was to attempt to generate monoclonal antibodies specific for erythroid progenitors. In contrast to all previous studies, in the present study we used BFU-E-derived immature erythroblasts as antigens. We generated two monoclonal antibodies that appear to be specific for the erythroid series. One, designated as McAb EP-1 (for erythroblastic antigen-1) recognizes a determinant that is expressed on BFU-E, CFU-E, and erythroblasts, but it is absent in erythrocytes. The second antibody, McAb EP-2, detects a determinant (EP-2) that is present on immature erythroblasts, but not on BFU-E, CFU-E, or erythrocytes.

MATERIALS AND METHODS

Cells

Cells used for immunization were generated from fetal liver clonal erythroid cultures. Livers from first trimester abortuses were used because of the high number of BFU-E progenitors present in this tissue and the relatively low number of coexpressing granulocytic progenitors. Fetal liver samples were prepared for culture as previously described,4 and an appropriate concentration of cells (10⁵/ml) was inoculated in methylcellulose media using previously described methodology.5,6 The cultures were monitored for growth by inspection of plates under a dissecting microscope.

In order to obtain a population of cells that was enriched in progenitor cells, cultures containing young erythroblast clones, composed of an average of 100-200 cells, were used. This stage was reached at approximately the end of the first week in culture. Because the fetal liver erythroblast colonies usually reach, at 12-14 days, an average size of 5 x 10⁵-10⁶ cells, it was expected that the 100-200 cell colonies would be enriched in progenitor cells. This suggestion is based on previous observations in which young colonies (composed of less than 100 cells), following dispersion and replating to secondary plates, produced CFU-E-like colonies with a 70%-80% regenerating efficiency (unpublished data from this laboratory). However, typically, in erythroid cultures, initiation of maturation is asynchronous both between colonies and within each colony; thus, in addition to the mostly immature or colorless colonies, others with evidence of hemoglobinization (pink color), either over the entire colony or in a portion of a colony, were present in the harvested samples. Hence, the populations of cells used as antigens and for primary screening were composed of immature erythroblasts, erythroblasts of intermediate degree of maturity, and, presumably, progenitor cells of BFU-E and CFU-E type.
To obtain the cells required for immunization and screening, the methylcellulose plates were inspected, and the small number of morphologically identifiable granulocytic colonies present in these plates were selectively plucked out. Subsequently, the plates were harvested and pooled. The harvested semisolod cultures were washed 2 times, in phosphate-buffered saline (PBS) with 2.5% fetal calf serum, to remove the majority of methylcellulose particles and once in PBS; the PBS cell suspension was used in the studies described here.

**Immunization and Production of Hybridomas**

BALB/c mice were inoculated intravenously once with $10^7$ culture-derived erythroid cells of the above composition. Three days following immunization, spleen cells were fused with myeloma cell line P3/NS-1/1-Ag4-1 (NS-1), as previously described.

**Primary Screening**

For initial screening, a $^{51}$Cr release cytotoxic assay was used to select hybridomas secreting cytotoxic antibodies against erythroid cells from culture. The latter were harvested from cultures similar to the ones used for immunization. The washed cells were incubated with $^{51}$Cr (100 $\mu$Ci/10$^6$ cells) at 37°C for 1 hr. An aliquot of labeled cells (10$^5$ cells/100 $\mu$l), 50 $\mu$l of hybridoma supernatant, and then 50 $\mu$l of appropriately diluted rabbit complement (Pel-Freeze Biologicals, Rogers, AR) were added sequentially to each well of a round-bottom microplate. The plates were incubated at 37°C for 30 min, and then centrifuged for 1 min at 150 g. To achieve maximum $^{51}$Cr release, a control sample of cells was lysed by adding 0.5% NP-40 as a substitute for complement. Nonspecific cytotoxicity was measured by incubating radiolabeled cells and complement without the hybridoma supernatant. One hundred microliters of supernatant from each well was assayed for radioactivity in a gamma scintillation counter.

**Secondary Screening**

The purpose of secondary screening was to exclude McAb directed against antigens present on nonproliferating mature cells and McAb directed against nonspecific activation antigens present on actively proliferating cells. This screening was done by reacting the supernatant of hybridomas found positive upon the primary screening with peripheral blood mononuclear cells and cells of the 8226 human myeloma cell line. Reactivity with these two cell types was assessed with the cytotoxicity assay, described above, and with indirect immunofluorescence. Only hybridomas negative for antibodies to peripheral blood mononuclear cells and myeloma cells, but positive against erythroid cells, were selected for further characterization.

**Cells Used for McAb Characterization**

**Normal cells.** Human peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density gradient (Pharmacia Fine Chemicals, Piscataway, NJ). The unfractionated peripheral blood mononuclear cells, collected as above, were separated into sheep erythrocyte rosette negative (E-) and positive (E+) fractions. E- and E+ cell fractions contained about 5% and 60% HLA-DR antigen positive cells, respectively. Monocytes were obtained by adhering peripheral blood mononuclear cells to plastic dishes, washing away nonadherent cells, then scraping off the adherent population with a rubber policeman. More than 80% of the adherent cells were HLA-DR positive. Granulocytes and erythrocytes were purified from peripheral blood buffy coat by layering on Histopaque 1119 (Sigma Chemical Co., St. Louis, MO). The granulocyte fraction was contaminated with very few other cells (<5%). Platelets were isolated by centrifugation of platelet-rich citrated plasma. Nucleated bone marrow (BM) cells were obtained by treating buffy coats of normal bone marrow aspirates with a hemolytic buffer (0.17 M Tris, 0.16 M ammonium chloride). Fetal liver cells, fetal bone marrow cells, and thymocytes were obtained after fine mincing of tissue fragments with scissors, passing the cell suspensions through a fine nylon mesh, and finally treating them with a hemolytic buffer.

**Activated normal lymphocytes.** A quantity of 2 x $10^7$ peripheral blood mononuclear cells was cultured in 2 ml RPMI containing 15% fetal calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate, antibiotics, and 5 x $10^{-5}$ M 2-mercaptoethanol in 24-well plates with or without concanavalin-A (Con-A, Millipore, Freehold, NJ). After 2 days, cultures were pooled and washed, then treated with test monoclonal antibodies in an indirect immunofluorescence assay. Control samples from Con-A-treated lymphocytes were tested with an antitransferrin receptor monoclonal antibody (5E9), which labeled approximately 80% of these cells, although it was negative on the cells before culture.

**Cell lines.** Table 6 lists the cell lines used in this study and their properties. Lines K562, HEL, KG-1, EM-2, HL-60, U937 are maintained in our laboratory. Lympoid cell lines, MOLT-4, Nalm 6, Ramos, Raji, T-51, 8226, and U266 were obtained from Dr. E. Clark; 2102 EP human teratocarcinoma cells were kindly provided by Dr. S. Hakomori; MPC pancreatic carcinoma cells by Dr. J. Adamson; human diploid fibroblasts by Dr. G. Martin; A549 human lung carcinoma cells by Dr. L. Altman.

**Cytotoxicity of Progenitors (BFU-E, CFU-E and CFU-GM)**

Cells from BM and fetal livers were incubated at room temperature in monoclonal antibody-rich supernatant or in a 1:100–1:500 dilution of ascites for 30 min, washed twice, and then treated with a 1:2 final dilution of pretreated rabbit complement (Pel-Freeze Biologicals, Rogers, AR) for an additional 60 min at room temperature. The cells were then washed twice and resuspended to the original volume. Antitransferrin receptor or anti-HLA-DR monoclonal antibodies were used as positive controls. An irrelevant monoclonal antibody (anti-gamma chain McAb) was also used as negative control. The treated cell suspensions were inoculated in clonal assay cultures in semisolid media (methylcellulose for BFU-E or CFU-GM and plasma clots for CFU-E), as described previously.

**Indirect Immunofluorescence and Cell Sorting**

Cell populations for testing (1–2 x $10^5$ cells in 100 $\mu$l) were treated with 100 $\mu$l of hybridoma supernatant or a 1:200 dilution of ascites fluid for 30 min at 4°C. The cells were washed 3 times and then incubated with 50 $\mu$l of an appropriately diluted fluorescein-conjugated goat anti-mouse IgM (Tago Inc., Burlingame, CA). After a 30-min incubation at 4°C and 3 washings, the cells were scored for surface immunofluorescence-positive cells under a fluorescence microscope or with the aid of a fluorescence-activated cell sorter (Ortho System 50H Cytofluorograf with a 2150 computer, Westwood, MA), as described previously.

**Immunoprecipitation and Western Blotting**

Immunoprecipitation and western blotting were carried out according to previously described methodology. Briefly, exponentially growing KG-1 cells (which react positively with EP-1, see below) were metabolically labeled with $^{35}$S-methionine (200 $\mu$Ci/ml) in methionine-free medium overnight. External proteins in KG-1 cells were also labeled with $^{35}$S using the lactic acid oxidase.
cells were reacted with McAb EP-1 or EP-2, as analyzed by flow cytometry in a fluorescence-activated cell sorter (FACS) (Fig. 1A). Both EP-1 and EP-2 hybridomas or ascites fluids showed that results were obtained when these cell populations were nontants detected by both antibodies (Table 1).

Platelets, and erythrocytes were negative for determinants detected by monoclonal rat anti-mouse $\kappa$-chain antibody (kindly provided by Dr. T. Cotner) was also used for immunoprecipitation. The resulting immunoprecipitate was dissolved in SDS sample buffer under reducing conditions and separated electrophoretically using 10% acrylamide gels (SDS-PAGE). Molecular weights were calibrated through the use of known protein markers (myosin 200,000, phosphorylase B 94,000, bovine serum albumin 69,000, ovalbumin 43,000, carbonic anhydrase 30,000, soybean trypsin inhibitor 21,100, $\alpha$-lactalbumin 14,400). After staining, the gels were subjected to fluorography.

For western blotting, unlabeled cell lysates were electrophoresed under the above conditions and then transferred to nitrocellulose sheets by electroblotting. Antigen bands were then detected by radiiodinated protein A binding assay and autoradiography.

RESULTS

Screening of Hybridomas

In the primary screening, 400 hybridomas were tested and 15% of these were found to react with culture-derived erythroid cells. These positive hybridomas were submitted to secondary screening, as described in Materials and Methods, and five were selected by their failure to react with peripheral blood mononuclear cells and myeloma cells. Of these, two independent clones secreting IgM antibodies, designated as McAb EP-1 and McAb EP-2, were further cloned three times by limiting dilution and further characterized. Ascites fluid generated from EP-1 and EP-2 hybridoma inoculation were found to have cytotoxic titers in excess of 1:100,000 when tested against unfractionated adult BM samples (Table 1); this percentage was approximately the same as the percentage of benzidine-positive nucleated erythroid cells in smears prepared from these aspirates. EP-2 antigen was detectable in less than 5% of adult BM in two of ten samples (Table 1). In two experiments, flow cytometry described in Materials and Methods, and positive cells were counted under the fluorescent microscope.

EP-1 antigen was detectable in 5%–20% of unfractionated adult BM samples (Table 1); this percentage was approximately the same as the percentage of benzidine-positive nucleated erythroid cells in smears prepared from these aspirates. EP-2 antigen was detectable in less than 5% of adult BM in two of ten samples (Table 1). In two experiments, flow cytometry described in Materials and Methods, and positive cells were counted under the fluorescent microscope.

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Reactivity With Peripheral Blood Cells

Detailed testing using supernatants of cloned EP-1 and EP-2 hybridomas or ascites fluids showed that neither EP-1 nor EP-2 antigen was detected on unfractionated peripheral blood mononuclear cells by indirect immunofluorescence (Table 1). Fractionated populations of lymphocytes, granulocytes, monocytes, platelets, and erythrocytes were negative for determinants detected by both antibodies (Table 1). The same results were obtained when these cell populations were analyzed by flow cytometry in a fluorescence-activated cell sorter (FACS) (Fig. 1A). Both EP-1 and EP-2 antigens were not detectable on thymocytes isolated from freshly removed thymus and on Con-A-activated lymphoblasts from peripheral blood (Table 1).

Reactivity With Adult Bone Marrow Cells

In ten experiments, unfractionated bone marrow cells were reacted with McAb EP-1 or EP-2, as...
ric analysis of normal adult bone marrow cells reacted with McAb EP-1 or McAb EP-2 showed a significant number of EP-1 positive cells but no EP-2 positive cells (Fig. 1B). However, when bone marrow cells from a patient with sickle cell anemia were used, 40% and 10% of the cells were positive for EP-1 and EP-2, respectively (Table I and Fig. 1C).

In order to morphologically identify the EP-1 antigen positive cells, a bone marrow sample (that had 17% EP-1 positive cells by FACS analysis) was sorted into an EP-1 positive fraction (15% of sorted cells) and a negative fraction (82% of sorted cells); a minor portion (3% of sorted cells) of marginally fluorescing cells, residing between the gates separating the collected populations, was excluded. Crosscontamination was estimated at 0.6% and 0.2% for the positive and negative fractions, respectively. Slides were prepared from the collected cell populations in a cytocentrifuge and subjected to staining with benzidine and Wright-Giemsa. Differential cell counts in the positive and negative fractions appear in Table 2. The positive cell fraction consisted almost exclusively of aggregates of erythroblasts at all stages of maturation, such as proerythroblasts, basophilic erythroblasts, and orthochromatophilic erythroblasts (Fig. 2A). Few reticuloendothelial cells surrounded by erythroblasts were also found, whereas other cell types were extremely rare. The negative fraction consisted largely of myeloid and lymphoid cells and few erythrocytes (the majority of red cells were lysed by a hemolytic buffer prior to antibody labeling) (Fig. 2B).

Reactivity With Fetal Erythroid Cells

The reactivity of McAb EP-1 and McAb EP-2 with fetal bone marrow and fetal liver cells was assessed in several experiments (Table 1).

EP-1 and EP-2 antigens were expressed in a significant proportion of fetal bone marrow cells (Table 1 and Fig. 1D); the frequencies of positive cells were similar to the proportion of erythroblasts (mainly proerythroblasts) in these samples.

EP-1 antigen was detected on more than 50% of fetal liver cells from early (45–60 days) and late (90–150 days) gestation fetuses (Table I and Fig. 1, E and F). EP-2 antigen was detectable on a few (2%–7%) cells in fetal livers from late gestation fetuses, whereas 60%–80% of cells from livers of early gestation fetuses were positive (Table 1 and Fig. 1, E and F).

Small numbers of embryonic (yolk sac) erythroblasts, recognized by their characteristic morphology (i.e., large cytoplasmic volume and small nucleus) were present in two early fetal livers we studied (gestational days: 38 and 48). These embryonic cells failed to react with McAb EP-1 or McAb EP-2.

Expression on BFU-E-Derived Erythroblasts

In 13 experiments, erythroid colonies were plucked from BFU-E cultures (of adult peripheral blood or adult bone marrow, or of fetal liver cells), and the dispersed cells were labeled with McAb EP-1 or McAb
EP-2. Consistently, McAb EP-1 strongly stained and agglutinated over 80% of BFU-E-derived erythroblasts. The number of EP-2 antigen positive cells varied from experiment to experiment (from 10% to 80%). Neither EP-1 nor EP-2 antigen determinant was expressed on granulocytic cells and macrophages harvested from the same plates.

**Relationship of EP-1 and EP-2 Antigen Expression to Erythroid Maturation**

To test whether the expression of EP-1 or EP-2 antigens was related to the degree of maturity of erythroblasts, immature and mature erythroblasts were reacted with McAb EP-1 and McAb EP-2. The source of immature erythroblasts were “immature” BFU-E colonies composed of morphologically early, nonhemoglobinized cells; these colonies were identified as such by their “white” (not colored) appearance and characteristic colony morphology under the dissecting microscope. The source of mature erythroblasts were the fully hemoglobinized (red) BFU-E colonies.

As shown in Table 3, McAb EP-1 reacted strongly with both immature and mature erythroblasts. Similar results were obtained when erythroblasts from peripheral blood, bone marrow, or fetal liver cultures were used. The FACS-obtained profiles of reactivity of McAb EP-1 with either immature or mature erythroblasts showed a strong shift in the positive direction compared to the negative control (Fig. 3, A and B).

McAb EP-2 reacted mainly with immature erythroblasts (Table 3), suggesting that EP-2 antigen is predominantly expressed on immature cells; only a minority of the mature erythroblasts were labeled by McAb EP-2 (Table 3). The FACS-derived profile of mature erythroblasts labeled with McAb EP-2 did not significantly shift in the positive direction (Fig. 3B), whereas that of immature erythroblasts showed a definite shift (Fig. 3A). The preferential expression of EP-2 antigen on immature erythroblasts may explain the varied frequency of EP-2 positive cells in bone marrow samples or erythroid colonies described in the previous sections.

Both McAb EP-1 and McAb EP-2 failed to react with reticulocytes in the reticulocyte-rich red cell populations from a sickle cell anemia patient (15% reticulocytes) and cord blood (18% reticulocytes).

**Complement-Dependent Cytotoxicity of Progenitors**

McAb EP-1 produced a 60%–80% inhibition of BFU-E growth and 80%–100% inhibition of CFU-E growth in the complement-dependent cytotoxicity assay. CFU-GM colonies were unaffected (Table 4).

McAb EP-2 failed to significantly inhibit any type of colony formation (BFU-E, CFU-E, or CFU-GM; Table 4).

In the same experiments, we tested BFU-E and CFU-GM inhibition with an antitransferrin receptor cytotoxic McAb (CP-200; kindly provided by Dr. T. Cotner) or two McAb directed against HLA-DR antigen (B10a and 7.2). In contrast to EP-1 (which did not inhibit nonerythroid colony formation), the antitransferrin receptor and the anti-HLA-DR antibodies produced a complete inhibition (more than 90%) of colony formation from both BFU-E and CFU-GM (data not shown).

**Cell Sorting of Progenitors**

Since McAb EP-1 selectively inhibited BFU-E and CFU-E colonies in the complement-dependent cytotoxicity assay, we examined whether BFU-Es and CFU-Es could be positively enriched by cell sorting. Bone marrow cells from a sickle cell anemia patient were sorted into a positive cell fraction (10% of total cells), an intermediate fraction (20% of total cells), and a negative fraction (70% of total cells). Cells from each sorted fraction, as well as unsorted cells, were inoculated in semisolid media for erythroid and nonerythroid colony growth.

As shown in Table 5, in the cultures of cells from the positive fraction were 1,672 BFU-E colonies/10⁵ cells, whereas in the cultures of cells from the negative fraction, there were only 55.9 BFU-E colonies/10⁵ cells, yielding a 30-fold difference in BFU-E concentration. The corresponding numbers of CFU-E were

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<table>
<thead>
<tr>
<th>McAb</th>
<th>No. of Experiments</th>
<th>Percent Positive BFU-E-Derived Erythroblasts</th>
<th>Immature</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP-1</td>
<td>9</td>
<td>&gt;95</td>
<td>&gt;95</td>
<td></td>
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</tbody>
</table>

*The erythroid bursts used were from adult peripheral blood cultures (3 experiments), adult bone marrow cultures (3 experiments), and fetal liver cultures (3 experiments).†Percent of immunofluorescence-positive colony-derived erythroblasts is based on counting 300 cells.‡Immature: erythroblasts from virtually colorless erythroid bursts.$Mature: erythroblasts from red, well hemoglobinized erythroblasts.
5,609/10^5 cells in the positive fraction and 60.3/10^5 cells in the negative fraction, yielding a 93-fold enrichment in CFU-E. Thus, 7% of the cells of the positive fraction were erythroid progenitors.

There was a negative selection for CFU-GM progenitors in the EP-1 positive fraction (Table 5). There was a relative enrichment in CFU-GM colonies in the intermediate fraction. The possibility that these CFU-GM colonies of the positive and intermediate fractions were “mixed” colonies having a minor erythroid component was not tested.


The expression of these two antigens was tested by reacting McAb EP-1 and EP-2 with cells of 18 human cell lines (Table 6).

EP-1 antigen was expressed in five cell lines. Reactivities with McAb EP-1 were weak (K562, HEL, CEM, HL-60) to moderate (KG-1) in the absence of agglutination.

McAb EP-2 antigen was not detected on any cell line tested.

The pattern of reactivity of McAb EP-1 and EP-2 with cell lines indicated that antigens EP-1 and EP-2 are unrelated to HLA-A, B, C, or DR.


We examined whether the expression of EP-1 or EP-2 antigen on K562 cells could be enhanced with inducers of hemoglobin synthesis.

K562 cells at an initial density of approximately 2 × 10^5/ml were cultured with 6-aminolevulinic acid (Porphyrin Products, West Logan, UT) at a concentration of 0.5 mM, or with hemin (Sigma Chemicals) at a concentration of 0.05 mM. Uninduced and induced cells were reacted with McAb EP-1 or EP-2, as

**Table 5. Cell Sorting of Adult Bone Marrow Cells Labeled With McAb EP-1: Enrichment of BFU-E and CFU-E Colonies in the EP-1 Antigen-Positive Fraction**

<table>
<thead>
<tr>
<th>Colonies</th>
<th>Number of Colonies/10^5 Cells Plated*</th>
</tr>
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<tbody>
<tr>
<td>BFU-E (day 7)</td>
<td>5,609.6 ± 681.4</td>
</tr>
<tr>
<td>BFU-E (day 13)</td>
<td>1,672.0 ± 130.3</td>
</tr>
<tr>
<td>BFU-GM (day 13)</td>
<td>13.0 ± 10.6</td>
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</table>

*Mean of colony counts/10^5 inoculated cells ± standard deviations of the mean.

†Numbers in parentheses indicate the proportion of cells in these fractions out of the total bone marrow cells analyzed through FACS.

<table>
<thead>
<tr>
<th>Cell Lines*</th>
<th>Origin or Phenotype</th>
<th>Reactivity† With EP-1</th>
<th>Reactivity† With EP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemopoietic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K562</td>
<td>Erythroleukemic</td>
<td>30-40</td>
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</tr>
<tr>
<td>HEL</td>
<td>Erythroleukemic</td>
<td>5-10</td>
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<tr>
<td>KG-1</td>
<td>Myeloblastic</td>
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<td>EM-2</td>
<td>Myeloblastic</td>
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</tr>
<tr>
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<td>Promyelocytic</td>
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<tr>
<td>U-937</td>
<td>Histioctic</td>
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<td>-</td>
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<td>CEM</td>
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<td>-</td>
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<td>Burkitt’s lymphoma</td>
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<td>Raj</td>
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<td>T-51</td>
<td>Epstein-Barr virus-transformed B cell line</td>
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<td>8226</td>
<td>Myeloma</td>
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</tr>
<tr>
<td>U266</td>
<td>Myeloma</td>
<td>-</td>
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</tr>
<tr>
<td>MPC</td>
<td>Pancreatic carcinoma</td>
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<tr>
<td>2102 Ep</td>
<td>Human teratocarcinoma cell line</td>
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<td>A-549</td>
<td>Human lung carcinoma cell line</td>
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<td>-</td>
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<tr>
<td>A-549 (ATCC-CCL 185)</td>
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<td></td>
</tr>
<tr>
<td>Human diploid fibroblasts</td>
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</table>

Species Specificity

McAb EP-1 and EP-2 failed to react with baboon (Papio cynocephalus) bone marrow erythroblasts or with baboon erythroid colonies or Rhesus monkeys (Macaca mulatta) erythroid colonies.

DISCUSSION

We have characterized two new monoclonal antibodies addressed to determinants present exclusively on erythroid cells of normal man. The reactivity of antibody EP-1 (Fig. 4), both against morphologically recognizable erythroid cells (erythroblasts) and erythroid-committed progenitors (CFU-E, BFU-E), appears to be unique and distinct from previously described antibodies directed against erythroid cell surface determinants.

Although several antibodies have been found to react with erythroblasts and erythrocytes (i.e., antil,

\[ L5-1, L5-2, L5-3, L5-4 \]) only two types of antibodies have been found to be specific for erythroid cells: those directed against the glycoprotein molecule and the ones against the Rh blood group antigen. The latter two types of structures, however, have not been shown to be expressed on erythroid progenitors, and both appear to be expressed fully on mature erythrocytes; therefore, they seem to be distinct from the determinant detected by antibody EP-1, which is reacting directly with progenitor cells but not with mature red cells. On the other hand, none of the antibodies that have been described thus far to react with determinants present on erythroid progenitors (i.e., antitransferrin receptor antibodies, anti-HLA-A, B, C, or DR antibodies, anti-il antibodies, L4F3, 5F1, 53.6) are specific only for erythroid progenitors. This lack of identification of specific determinants present exclusively on erythroid progenitors has led to the prelimi-
nary impression among investigators that all nonlymphoid progenitors may have very similar cell surface phenotypes. The unique properties of antibody EP-1 demonstrate for the first time that distinct separation of erythroid progenitors from other types of progenitors is feasible.

The pattern of distribution of EP-1 antigen (a portion of BFU-E, the great majority of CFU-E, and virtually all erythroblasts) is of interest. It includes either actively cycling or proliferating erythroid cells and/or populations that are influenced by erythropoietin. This type of distribution invites speculation about the possible relationship of EP-1 antigen with either transferrin receptors or erythropoietin receptors. The possibility that we are dealing with a transferrin receptor-associated epitope is tenable only if one accepts that the transferrin receptor molecule, which is present in all actively proliferating cells, is sufficiently heterogeneous so that the one present on erythroid cells is characterized by unique (carbohydrate or glycolipid?) epitopes. The detection of a faint protein band by immunoprecipitation of a similar mobility as the one precipitated by a control antitransferrin receptor antibody (5E9), as well as the properties of the previously described L-51 antibody, may be cited to support this viewpoint. The second possibility, i.e., that EP-1 antigen is associated with the erythropoietin receptor, also remains an attractive one. Binding of EP-1 did not block subsequent erythropoietin action on progenitors (data not shown), but this finding does not exclude the above possibility, which can be pursued further in the future.

EP-1 antigen appears to be specific for human erythroid cells; it is present in fetal and adult human erythroblasts, but it was not detected in embryonic erythroblasts. This finding could be explained if the embryonic erythroblasts tested are equivalent in maturity to fetal or adult enucleated red cells, or if the membrane of embryonic erythroblasts truly lacks the EP-1 surface determinant.

The weak expression of EP-1 antigen in 4 of 18 cell lines tested is interesting. Whether this reactivity in the cell lines is directed against the same molecule as the one in erythroid cells or to a different molecule is unclear at present. The “induction” of EP-1 antigen in K562 cells treated with globin inducers represents, to our knowledge, the first demonstration of induction (or enhancement) of a nonglobin but erythroid-specific character in these cells.

Because of its unique properties (strong agglutination and specific reactivity with human erythroid cells), McAb EP-1 may be useful as a convenient and highly effective means of isolation of erythroid cells (from bone marrow samples, for example) for biochemical or molecular studies. In addition, because of its reactivity with erythroid progenitors (CFU-E, BFU-E), this antibody may be used for the detection and identification of “cryptic” erythroleukemias and, as such, may be superior to the ones previously used for that purpose.

The antigen detected by the second antibody (EP-2) described here appears to have a very restricted expression among maturing erythroid cells (Fig. 5). Because of the preferential expression on immature erythroblasts, and especially fetal erythroblasts, one is tempted to associate it with the i-antigenic determinants on erythroid cells. However, the lack of expression of EP-2 antigen in erythroid progenitors, which have been shown to express the i-determinants, and in other nonerythroid cells (i.e., lymphocytes) displaying the i-antigen on their surface, makes the above possibility unlikely. EP-2 may be related to other antigens that are modified during the maturation process of erythroid cells. In a previous report, we have identified biochemically 95 and 105 mol wt proteins present in erythroblasts but not in mature red cells; whether EP-2 antigen is related to these glycoproteins is unclear.

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Fig. 5. Diagrammatic representation of the phenotypic expression of EP-1 and EP-2 antigens during erythroid differentiation.


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T Yokochi, M Brice, PS Rabinovitch, T Papayannopoulou and G Stamatoyannopoulos