A Monoclonal Antibody Against an Erythrocyte Ontogenic Antigen Identifies Fetal and Adult Erythroid Progenitors


A murine monoclonal antibody (MoAb) designated FA6-152 has been obtained by immunizing mice with fetal erythrocytes. This antibody agglutinates fetal but not adult erythrocytes. Among blood cells, this antibody bound to both adult and fetal monocytes, platelets, and reticulocytes, but did not react with lymphocytes and granulocytes. Fluorescent labeling of marrow cells and of in vitro BFU-E, CFU-GM, and CFU-MK–derived colonies has shown that the antigen defined by FA6-152 MoAb was absent from the granulocytic precursors and was detected on the megakaryocytic lineage at a later stage of differentiation than the platelet-specific markers. In contrast, the antigen appeared as a very early marker of the erythrocyte differentiation since all erythroblasts, including proerythroblasts, were labeled even before the expression of glycoporphin A. Cells from adult marrow and fetal liver were sorted with the FA6-152 MoAb and studied by electron microscopy and cell culture. The negative fraction contained granulocytic, monocytic, and megakaryocytic precursors, whereas the positive fraction was devoid of these precursors and contained monocytes, erythroblasts at all stages of maturation, and a homogenous population of blasts. Cultures have shown that the only hematopoietic progenitors present in this positive fraction were CFU-E and some BFU-E. The antigenic density was related to the differentiation stage of the erythroid progenitors. In conclusion, this antibody is similar to the previously described SF1 MoAb (Bernstein and Andrews, J Immunol 128:876, 1982; and Andrews et al, Blood 62:124, 1983) and provides a useful probe for studies leading to improved understanding of normal and malignant erythroid differentiation.

MATERIALS AND METHODS

Immunization protocol. Balb/c mice (IFCA Credo, Lyon, France) were immunized using a suspension of 20-week-old fetal red cells (RBC). These hepaticized RBC were washed three times in saline (0.15 mol/L NaCl), and 20 × 10⁶ cells were injected on day 1 and day 18 intraperitoneally. Three days after the last immunization, spleen cells were prepared, and fusion was performed as previously reported.¹,²

Supernatant screening. Culture supernatants were tested by a hemagglutination test using papain- and bromelain-treated adult and fetal RBC. The subsequent reactions were performed in microtubes by mixing 20 µL of the aforementioned RBC suspension and 20 µL of supernatants. The mixture was incubated for 30 minutes at 37°C and then read with a microscope for agglutination.

Cloning. The cells secreting an antibody of interest were cloned by the limiting dilution technique. The cells taken from one well were brought into suspension with a feeder layer (thymocytes from Balb/c mice) and distributed in 96-well plates so as to obtain an average cell density of 5, 1, and 0.5 cells per well. Two successive clonings were performed. For further studies, the culture supernatant or ascitic fluid was used as sources for the MoAb.

Characterization. Identification of the MoAb was carried out using a panel of adult different erythrocyte phenotypes; agglutination tests were performed by adding one volume of a 10%-treated RBC suspension to an equal volume of culture supernatant or ascitic fluid. Both macroscopic and microscopic readings were made after one hour of incubation at 22°C. Immunoglobulin class and subclass of the monoclonal antibody were determined by immunodiffusion of ascitic fluid against subclass-specific antiimmunoglobulin antisera (Meloy Laboratories, Springfield, Va).

Samples. Adult RBC of known phenotype or fetal blood samples obtained from therapeutic abortion or fetoscopy for antenatal diagnosis were used. The blood cells were collected into heparin, washed three times in saline (0.15 mol/L NaCl), mixed in a glycerol (17.5%), mannitol (1.44%), and NaCl (0.32%) solution, and frozen in liquid nitrogen. Adult RBC with rare phenotypes were thawed and bromelin treated. Liver and thymus cells were removed one hour after delivery from therapeutic abortion, placed into Dulbecco's medium containing 10% foetal calf serum, and subsequently frozen.

NUMEROUS ONTOGENIC changes are observed in the biology of the erythrocyte. The most striking ontogenic event concerns hemoglobin switching. Nevertheless, blood group antigens also exhibit numerous important developmental changes. The antigens of the A, B, and H systems are slightly expressed at the third week of the intranarto life.¹ As early as the 40th day of fetal life, the antigens of the ABH, Duffy, JK, and rhesus blood group systems are fully expressed on the surface of the erythrocytes. However, fetal erythrocytes differ from adult erythrocytes by the arborized pattern of the carbohydrate structure of their glycolipids and glycoproteins.²³ This difference is now considered to be the basis of i-to-I antigen conversion.⁴⁶ The i/I expression during adult erythroid differentiation mimics the ontogenic changes. Indeed, i antigen is preferentially detected on immature erythroblasts whereas increasing expression of i antigen is observed during terminal erythroid differentiation.¹³ However, at the level of adult erythroid progenitors, ie, CFU-E and BFU-E, i antigen predominates over i antigen.⁸ Therefore, ontogenic erythrocyte antigens can be helpful markers in studies of cellular differentiation and may be also useful for identification of fetal erythrocytes in maternal blood during pregnancy.

Our purpose was to define new ontogenic erythrocyte antigens with monoclonal antibody (MoAb) obtained by immunizing mice with fetal erythrocytes and to investigate the expression of such antigens during the erythroid differentiation.

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Submitted Nov 21, 1984; accepted July 3, 1985.

Supported in part by Groupe des entreprises francaises dans la lutte contre le cancer grants.

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Isolation of the cells. Normal mononuclear cells from heparinized peripheral blood or bone marrow were separated by Ficoll-Metrizate gradient density centrifugation (Lymphoprep, Nye-gaard, Sweden; d1.077). Adherent and nonadherent peripheral blood mononuclear cells were separated by a two-hour adherence on plastic Petri dishes. The granulocytes from the pellet of the Ficoll-Metrizate gradient density centrifugation were separated from RBC by dextran sedimentation. Platelets were obtained from platelet-rich plasma. Reticulocytes were separated on a pthalhate oil density gradient from a patient with autoimmune hemolytic anemia.

Bone marrow cells were isolated from aspirates obtained from donors for bone marrow transplantation procedures. Nucleated cells were either obtained from a buffy coat preparation or after Ficoll-Metrizate gradient density centrifugation. Blood cells from 40 patients with acute leukemia were also tested.

Cell lines. Five lymphoblastoid cell lines, five Burkitt cell lines (including Daude and P3HR1), three T cell lines (Jurkat, Molt 4, HSB 2), HL60 (a promyelocytic cell line), U 937 (a monoblastic cell line), two erythroleukemic cell lines (HEL and K 562), and two hybrid cell lines9 between K 562 and PHA-LCM and K 562 and DAUDI (Putko and Dutko, respectively) were investigated. In addition, HL 60 and U 937 were studied before and after 12-0-tetradecanol phorbol-13-acetate (TPA) and 1.23 dihydroxy-vitamin D3 induction. All cell lines were cultured in RPMI medium (Boehringer, Mannheim, West Germany) with 10% fetal calf serum (Boehringer).

Fluorescent labeling on separated cells. Supernatant or ascitic fluid was used undiluted or 100-fold diluted in sterile phosphate-buffered saline (PBS), respectively. Unfixed cells were incubated at 4°C for 30 minutes with the antibody. After three washes in cold PBS, cells were incubated with a 10-1-diluted fluorescein-conjugated goat F(ab')2 fragment directed against mouse IgGs (Cappel Laboratories, Cochranville, Pa) for 30 additional minutes, washed, smeared, and fixed for one minute with pure methanol. They were examined under a Zeiss episcopc equipped with appropriate filters for fluorescein and rhodamine and for phase contrast.

Cell sorting. Fluorocytometric analysis and cell sorting were performed with an Ortho 50 H cytofluorograf (Ortho Diagnostics, Westwood, Mass) as previously described.9 Light-density marrow cells from bone marrow transplantation donors were frozen, thawed just before the cell sorting experiment, labeled with an antibody by an indirect fluorescent technique (see the previous section), and kept in ice until cell sorting. Collectcd cells from the two fractions were used for clonogenic assays, cyto centrifuge preparations, and electron microscopic (EM) studies (see the following section).

Ultrastructural studies. The two fractions of cells (positive and negative) collected with the cell sorter were fixed by 1.25% glutaraldehyde in Gey's basic salts for ten minutes, washed and incubated in 2 mg/mL diaminobenzidine medium (Sigma Chemical Co, St Louis). The cells were then washed, postfixed, dehydrated, and embedded in epon. Thin sections were examined with a Philips electron microscope. This technique permits monitoring of different peroxidase activities, ie, myeloperoxidase, platelet peroxidase, and the peroxidase activity of hemoglobin. Cells were also studied by the immunogold technique with the FA6-152 MoAb.

Reactivity with erythrocytes. Two weeks after fusion, 320 wells out of 1,024 contained hybrid cells. Twelve were secreting antibodies against adult and fetal human RBC. Only one well, ie, FA6-152, produced an antibody that agglutinated only fetal RBC. The supernatant was tested against a panel of 69 adult RBC typed in the ABO, rhesus, Kell, Duffy, Kidd, P, MNSs, Lutheran, Colton, Cartwright, Xg, Sid, and Dombrock systems, a panel of 16, 18, 20, 22, 24, 27, 30, and 32-week-old fetal RBC and a panel of 23 cord blood RBC. Only fetal RBC up to 32 weeks old were agglutinated by the FA6-152 MoAb (Table 1). This antibody was characterized as an IgG1 antibody. Even adult RBC with a rare phenotype such as Tj-α-, Cartwright-, Gerbich-, Vel-, Colton-, kpb-, U-, i, PK, Bombay, and Celano were not agglutinated by the MoAb.

By fluorescent technique, adult erythrocytes were unlabeled, but some adult reticulocytes were faintly stained. About 50% of fetal RBC were labeled, whereas 10% to 20% cord blood RBC were stained. Cell sorting of artificial mixtures of fetal and adult erythrocytes was performed. For an initial mixture of 10% fetal RBC, 95% fetal RBC (labeled by an anti-γ-globulin chain) were present in the positive fraction whereas for a mixture containing 1% fetal RBC, 60% fetal RBC were present in the positive fraction.

Reactivity with other blood cells. In the adult, 30% to 40% of the blood mononuclear cells were labeled. These cells were larger than the others and exhibited some granulation in phase-contrast microscopy. More than 90% of the adherent cells were stained, whereas less than 1% were labeled in the nonadherent population. Granulocytes were unlabeled. All the platelets from several donors were labeled by the antibody in a double fluorescent procedure using a polyclonal
antifactor vWF to identify the platelets. EM studies by the immunogold technique have clearly confirmed that all platelets and monocytes bind the antibody whereas lymphocytes and granulocytes were devoid of the antigen.

**Reactivity with hematopoietic precursors—Labeling of blood cell precursors.** Ten percent to 35% of the marrow cells were labeled by the antibody. When examined by phase contrast, they consisted of erythroblasts at all stages of maturation, megakaryocytes, and monocytes. Granulocytic precursors were unlabeled. Similar results were obtained at the EM level. To determine more precisely the stage of maturation of the cells labeled by FA6-152, fluorescent labeling was performed on BFU-E-, CFU-GM-, and CFU-MK-derived colonies. All the erythroblasts composing a BFU-E colony were stained by the antibody at the early or late days of culture. Blood BFU-E colonies were stained as early as day 5 of culture. In similar experiments, MoAb directed against glycophorin A labeled the BFU-E-derived colonies, but not before day 8 or 9 of culture. Pure granulocytic colonies remained entirely unlabeled by the MoAb. Only large macrophages were intensively stained in the CFU-GM assay. Large mature megakaryocytes shedding platelets were intensively stained by the FA6-152 MoAb. At day 7 of culture when a large number of immature megakaryocytes were present, only 60% of the megakaryocyte labeled by antifactor vWF were reactive with FA6-152 MoAb. In particular, small megakaryocyte precursors were weakly labeled or unstained. Results were similar in colonies cultured from fetal progenitors, but with a more intense staining on fetal erythroblasts and megakaryocytes than on their adult counterparts.

**Hematopoietic cell lines and leukemic cell.** Among the cell lines tested, only HEL, Putko, and Dutko expressed the antigen defined by FA6-152 on a majority of cells. This antigen was also detected on a minority of K562 and U937 cells. (Table 2) Fifteen cases of acute lymphoblastic leukemia were investigated, and all cells were negative. In 25 cases of acute myeloid leukemia, only the cases containing a mature or immature monocytic component (M4 and M5) according to the French-American-British (FAB) classification were stained; M1, M2, and M3 cases were always negative.

**Morphologic features of the marrow cell fraction collected by cell sorting.** These experiments were performed on bone marrow aspirates obtained from normal donors for bone marrow transplantation. These samples were heavily

### Table 1. Reactivity of FA6-152 With Erythrocytes

<table>
<thead>
<tr>
<th>Adult RBC</th>
<th>No.</th>
<th>Agglutinated</th>
<th>Cord RBC</th>
<th>No.</th>
<th>Agglutinated</th>
<th>Fetal RBC (Age)</th>
<th>No.</th>
<th>Agglutinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>10</td>
<td>—</td>
<td>A1</td>
<td>15</td>
<td>—</td>
<td>A (18 wk)</td>
<td>4</td>
<td>++</td>
</tr>
<tr>
<td>A2</td>
<td>5</td>
<td>—</td>
<td>A2</td>
<td>5</td>
<td>—</td>
<td>O (20 wk)</td>
<td>5</td>
<td>++</td>
</tr>
<tr>
<td>A3</td>
<td>3</td>
<td>—</td>
<td>B</td>
<td>6</td>
<td>—</td>
<td>O (22 wk)</td>
<td>7</td>
<td>++</td>
</tr>
<tr>
<td>A4</td>
<td>3</td>
<td>—</td>
<td>0</td>
<td>8</td>
<td>—</td>
<td>A (24 wk)</td>
<td>5</td>
<td>++</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td>O (27 wk)</td>
<td>4</td>
<td>++</td>
</tr>
<tr>
<td>0</td>
<td>41</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td>O (30 wk)</td>
<td>4</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>O (32 wk)</td>
<td>3</td>
<td>++</td>
</tr>
</tbody>
</table>

### Table 2. Reactivity of FA6-152 With Several Hematopoietic Cell Lines

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Lymphoblastoid (five cell lines)</th>
<th>Burkitt's lymphoma (five cell lines)</th>
<th>T-ALL (three cell lines)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U937 3.5 ± 2</td>
<td>TPA induced 2 ± 1</td>
<td>Dehydroxy-vitamin D3 3.5 ± 2</td>
</tr>
<tr>
<td></td>
<td>HLA 60</td>
<td>TPA induced</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HEL 75 ± 10</td>
<td>K562 2 ± 2</td>
<td>Putko (hybrid between K562 and P3 HR-1) &gt;90</td>
</tr>
<tr>
<td></td>
<td>Dutko (hybrid between K562 and Daudi) &gt;90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: T-ALL, T cell acute lymphocytic leukemia.
contaminated by cells of blood origin, thus explaining the high percentage of lymphocytes and monocytes.

Thirty percent to 40% of the examined cells were labeled. This positive fraction comprised 79% to 85% monocytes; 1% to 2% additional immature monocytes were also present. The positive fraction included all the erythroblasts and proerythroblasts. No identifiable myeloid precursors were present. This fraction also included 3% to 8% blast cells that were not labeled by polyclonal antibodies against factor vWF or myeloperoxidase. These cells contained carbonic anhydrase I, another early erythroid marker.

The two fractions collected were subsequently examined by EM. The negative fraction contained all the neutrophil, eosinophil, and basophil precursors. Promonocytes synthesizing myeloperoxidase (i.e., presence of a peroxidase activity in the Golgi apparatus and the endoplasmic reticulum) or having terminated this synthesis and rare promegakaryoblasts identified by the typical platelet peroxidase were also present. In the positive fraction, the majority of cells examined were monocytes that were rather mature since no synthesis of myeloperoxidase was detected. Proerythroblasts were clearly identified by the presence of ferritin in rhophecytosis vesicles and in small clusters in the cytoplasm. A homogenous population of blast cells without any cytotoxic or morphologic criteria for identification were present (Fig 1). They differed from the blast cells of the negative fraction by their large size, abundant cytoplasm rich in free ribosomes, mitochondria of large size, and granules located in the Golgi apparatus zone. The slightly indented nucleus contained a large and multiple nucleolus.

Hematopoietic progenitor content of the adult bone marrow cell fractions collected by cell sorting. After indirect fluorescent labeling with FA6-152 MoAb, marrow cells were analyzed and sorted on the basis of fluorescent intensity (Tables 3 and 4). A typical histogram is shown in Fig 2. It was characterized by a clear population of negative cells and a marked shoulder preceding the FA6-152 bright cells. We could determine that this shoulder was contaminated by 2% to 10% negative cells when control IgG1-labeled cells were studied. In contrast, the FA6-152 bright cells contained less than 0.05% negative cells.

In two experiments, the positive fraction (positive I) only contained the FA6-152 bright cells, whereas the negative fraction also included the shoulder of fluorescence (Fig 2A). Results are shown in Table 3. More than 50% of the CFU-Es were grown from the positive I fraction, largely in the absence of all the other hematopoietic progenitors. The CFU-Es giving rise to eight to 32 colony were predominantly found in this fraction, whereas most of the CFU-Es giving rise to 32 or more cells were detected in the negative fraction containing both truly negative cells and cells expressing a low antigenic density of the antigen (Fig 3).

In two additional experiments (Table 4), FA6-152 bright cells and a part (experiment 3) or all (experiment 4) of the cells in the shoulder of fluorescence and therefore corresponding to cells exhibiting a low antigenic density were included in the positive fraction (positive II fraction). Most of all CFU-Es and a part of the day 12 BFU-E were grown from the positive II fraction, whereas all the other hematopoietic progenitors including the day 16 BFU-E were absent from this fraction. The positive sorting of cells expressing a low antigenic density in only experiment 4 may explain the differences between experiments 3 and 4.

Finally, cells with a high antigenic density (positive I) were separated from the cells with a low antigenic density (intermediate) (Table 4, experiment 5, Fig 2B). The positive I fraction contained only a part of the CFU-Es, whereas the intermediate fraction included the other CFU-Es and a

### Table 3. Progenitor Cell Growth From the Two Fractions Collected by Cell Sorting From Adult Bone Marrow

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>CF</th>
<th>Percentage of CF</th>
<th>CFU-E per 10^3 CF</th>
<th>Percentage*</th>
<th>Day 12 BFU-E per 10^3 CF</th>
<th>Percentage*</th>
<th>Day 16 BFU-E per 10^3 CF</th>
<th>Percentage*</th>
<th>CFU-GM per 10^3 CF</th>
<th>Percentage*</th>
<th>CFU-MK per 10^3 CF</th>
<th>Percentage*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Positive II</td>
<td>26</td>
<td>275 ± 24 52.5</td>
<td>8 ± 4 2</td>
<td>ND</td>
<td>4 ± 4 1.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>2 Negative</td>
<td>65</td>
<td>115 ± 11 37</td>
<td>134 ± 23 89.5</td>
<td>ND</td>
<td>126 ± 42 91.1</td>
<td>26 ± 5 93.3</td>
<td>26 ± 5 93.3</td>
<td>26 ± 5 93.3</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>3 Positive II</td>
<td>34</td>
<td>235 ± 30 53.5</td>
<td>3 ± 2 1.1</td>
<td>ND</td>
<td>28 ± 15 4.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>4 Negative</td>
<td>63</td>
<td>79 ± 10 33</td>
<td>148 ± 35 93</td>
<td>ND</td>
<td>308 ± 62 92.3</td>
<td>24 ± 8 80</td>
<td>24 ± 8 80</td>
<td>24 ± 8 80</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: CF, cell fraction; ND, not determined.

*The percentage is calculated as follows: CFU-E/10^3 sorted cells x percentage of viable cells in fraction/CFU-E/10^3 unsorted cells. In both cases, bone marrow from a marrow donor for marrow transplantation was separated by Peculiar density centrifugation, stored frozen with 10% dimethylsulfoxide in liquid nitrogen, and thawed for sorting.

### Table 4. Progenitor Cell Growth From the Two Fractions Collected by Cell Sorting From Adult Bone Marrow

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>CF</th>
<th>Percentage of CF</th>
<th>CFU-E per 10^3 CF</th>
<th>Percentage*</th>
<th>Day 12 BFU-E per 10^3 CF</th>
<th>Percentage*</th>
<th>Day 16 BFU-E per 10^3 CF</th>
<th>Percentage*</th>
<th>CFU-GM per 10^3 CF</th>
<th>Percentage*</th>
<th>CFU-MK per 10^3 CF</th>
<th>Percentage*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Positive II</td>
<td>34</td>
<td>402 ± 43 89</td>
<td>74 ± 12 29.8</td>
<td>0</td>
<td>91 ± 18 10.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4 Negative</td>
<td>63</td>
<td>24 ± 6 6.3</td>
<td>86 ± 23 84.3</td>
<td>73 ± 16 96</td>
<td>409 ± 41 87.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4 Positive II</td>
<td>34</td>
<td>480 ± 54 96</td>
<td>305 ± 46 71.5</td>
<td>48 ± 8 5.9</td>
<td>15 1</td>
<td>41 87.4</td>
<td>41 87.4</td>
<td>41 87.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5 Negative</td>
<td>60</td>
<td>0</td>
<td>68 ± 14 23.4</td>
<td>469 ± 52 87.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6 Positive I</td>
<td>34</td>
<td>228 ± 33 53.5</td>
<td>3 ± 2 1</td>
<td>3 ± 2 4.6</td>
<td>29 ± 15 8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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</table>

*The percentage was calculated as in Table 3.

In contrast to Table 3, the positive fraction also contained a part of the shoulder of the fluorescent histogram (experiment 3), or the whole shoulder (Experiment 4). In experiment 5, the intermediate fraction only contained the shoulder, whereas the positive fraction (see experiment 2) only contained the FA6-152 bright cells.
Fig 2. Fluorescence histograms of the bone marrow cells labeled with FA6-152 MoAb. The histogram was characterized by clearly negative and positive cells. However, a marked shoulder in the curve was constantly observed. (A) In experiments 1 and 2, the negative fraction contained a part of the cells located at the shoulder of the fluorescent intensity. The positive fraction contained only bright fluorescent cells. A window of cells between the two fractions was discarded. (B) In experiment 5 (identical bone marrow cells as in experiment 2), the positive fraction was collected as in (A). The other fraction, called intermediate, contained all the cells located at the shoulder of the fluorescence curve.

part of day 12 BFU-Es. The other hematopoietic progenitors were absent from these two fractions.

To demonstrate that the absence of BFU-E and CFU-GM in the positive fraction collected was not the consequence of the high concentration of monocytes as previously described,28 we studied the positive fraction after adherence (Table 5). No significant differences in the number of colonies were observed. In addition, the positive and negative fractions were remixed after sorting; the number of CFU-E and CFU-GM observed did not markedly differ from the sum of the number of colonies found in the two fractions, thereby indicating that no inhibitory cells were present in the two fractions.

Hematopoietic progenitor content of the fetal liver cell fractions collected by cell sorting. Concerning experiments in Table 1 on the adult marrow, we collected the bright stained cells as the positive fraction and the other as the negative (Table 6). The positive fraction contained more than 90% erythroblasts, 72.5% of the CFU-E, and 34% of the mature BFU-E; in contrast, the negative fraction contained most if not all the primitive BFU-E, CFU-GM, and CFU-MK.

DISCUSSION
In this report, we have described a MoAb obtained by immunizing mice with human fetal erythrocytes. The purpose of this immunization was to detect ontogenic antigens present on fetal erythrocytes but not on adult erythrocytes. The antigen detected by FA6-152 MoAb has this characteristic. Therefore, we have extensively studied the expression of this antigen on adult and fetal hematopoietic cells including

<table>
<thead>
<tr>
<th>Table 5. Adherence and Mixing Experiments</th>
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</thead>
<tbody>
<tr>
<td>CFU-E</td>
</tr>
<tr>
<td>Positive fraction before adherence</td>
</tr>
<tr>
<td>After adherence</td>
</tr>
<tr>
<td>Negative fraction</td>
</tr>
<tr>
<td>Positive fraction (before adherence) + negative fraction</td>
</tr>
</tbody>
</table>

Cells from experiment 2 were grown in triplicate at 3 x 10⁴ cells per mL for each fraction. The positive fraction was studied before and after one-hour adherence to plastic. A total of 3 x 10⁴ positive and 3 x 10⁴ negative cells were also mixed.
GM, and CFU-MK differentiation were investigated. In the absence of neonatal RBC was not observed, whereas fluorescent labeling was detected on granulocytes and lymphocytes. A similar cellular antigen identified by the FA6-152 MoAb was synthesized at adult as in the fetus, FA6-152 MoAb labeled all erythroblasts as well as their counterparts obtained in vitro from BFU-E, CFU-GM, and CFU-MK differentiation markers.29

The antigen could not be biochemically characterized by immunoprecipitation and Western blotting on SDS-PAGE (Cartron, Centre National de Transfusion, Paris, 1985). The absence of destruction of the antigen by several proteolytic enzymes such as trypsin and pronase suggests that this antigen is a glycolipid. MoAbs against the carbohydrate structure expressed in megakaryocytic differentiation later or at the same time as the specific platelet proteins.19,30 In addition, investigation by EM of the positive and negative marrow cell fractions obtained by cell sorting as well as unsorted marrow cells has shown that promonocytes were devoid of the antigen identified by the FA6 152-MoAb, thereby indicating that this antigen is also a late marker of monocyte differentiation. All these results are summarized in Fig 4. Considering the results observed in the adult, the reactivity of FA6-152 is in many ways similar to two previously described MoAbs, ie, 5F131 and 20/3.32 Both of them are IgM MoAb obtained by immunization of mice with acute myelomonocytic leukemic cells or normal peripheral mononuclear cells. The reactivity of 5F1 and 20/3 on fetal cells has not been published. We recently found that 5F1 also binds to some fetal erythrocytes.

We investigated the reactivity of FA6-152 with hematopoietic progenitors. It clearly appeared that FA6-152 stained only the erythroid progenitors. Interestingly, there was a marked increase in the antigenic density from the BFU-E to the CFU-E in the adult as in the fetus. The primitive BFU-E33 did not bear the antigen identified by FA6-152, whereas the mature BFU-E33 expressed a low density of this antigen. Mature BFU-E and a large part of the CFU-E were found in the 5% marrow cells whose fluorescent intensity was just located at the threshold of positivity. In contrast, the CFU-E that gave rise to small-sized colonies were all included in the intensively stained fraction. Therefore, the

Antigenic determinant defined by FA6-152. It was not reticulocytes, most if not all monocytes, and all platelets bore this antigen as the SSEA-I antigen, which are also differentiated with a glycolipid. MoAbs against the carbohydrate structure present antigen was expressed in megakaryocytic differentiations and spaces of the vessels. Reactivity was therefore detected in the small intestine, kidney, liver, and thyroid.

Bone marrow, fetal liver hematopoietic precursors and also their counterparts obtained in vitro from BFU-E, CFU-GM, and CFU-MK differentiation were investigated. In the adult as in the fetus, FA6-152 MoAb labeled all erythroblasts including normoblasts. We could demonstrate that the antigen identified by the FA6-152 MoAb was synthesized at an earlier stage of erythroid differentiation than glycoporphin A since the colonies derived from blood BFU-E were labeled as early as day 5, whereas a MoAb against glycoporphin A did not stain the colonies before day 8. In contrast, the present antigen was expressed in megakaryocytic differentiation later or at the same time as the specific platelet proteins.19,30 In addition, investigation by EM of the positive and negative marrow cell fractions obtained by cell sorting as well as unsorted marrow cells has shown that promonocytes were devoid of the antigen identified by the FA6 152-MoAb, thereby indicating that this antigen is also a late marker of the monocyte differentiation. All these results are summarized in Fig 4. Considering the results observed in the adult, the reactivity of FA6-152 is in many ways similar to two previously described MoAbs, ie, 5F131 and 20/3.32 Both of them are IgM MoAb obtained by immunization of mice with acute myelomonocytic leukemic cells or normal peripheral mononuclear cells. The reactivity of 5F1 and 20/3 on fetal cells has not been published. We recently found that 5F1 also binds to some fetal erythrocytes.

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Liver cells from a 20-week-old fetus were frozen, then thawed for sorting experiments. Cells were labeled by FA6-152 and then separated as in Fig 2A in two fractions and cultured. Cultures were performed in triplicate at 3 x 10^6 cells per mL. Results are expressed per 10^6 cells in each fraction.

### Table 6. Cell Sorting of Fetal Liver Cells

<table>
<thead>
<tr>
<th></th>
<th>Day 5 CFU-E</th>
<th>Day 8 BFU-E</th>
<th>Day 12 BFU-E</th>
<th>Day 12 CFU-GM</th>
<th>Day 12 CFU-MK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF Percentage</td>
<td>CF Percentage</td>
<td>CF Percentage</td>
<td>CF Percentage</td>
<td>CF Percentage</td>
</tr>
<tr>
<td>Positive fraction</td>
<td>60% ± 0.7</td>
<td>40.7 ± 0.4</td>
<td>212 ± 28</td>
<td>32.26 ± 0.7</td>
<td>182 ± 24</td>
</tr>
<tr>
<td>Negative fraction</td>
<td>40% ± 0.4</td>
<td>26.7 ± 0.4</td>
<td>620 ± 34</td>
<td>62.8 ± 0.4</td>
<td>2.933 ± 0.4</td>
</tr>
</tbody>
</table>

Liver cells from a 20-week-old fetus were frozen, then thawed for sorting experiments. Cells were labeled by FA6-152 and then separated as in Fig 2A in two fractions and cultured. Cultures were performed in triplicate at 3 x 10^6 cells per mL. Results are expressed per 10^6 cells in each fraction.
presence of the antigen defined by FA6-152 on the erythroid progenitors was the reflection of their differentiation stage defining three or four classes of progenitors as previously described when using other differentiation markers such as their hormonal regulation, the size of the colonies, and the in vitro time course of the colonies.Primitive BFU-E whose differentiation depends upon another factor besides Epo were devoid of the antigen defined by FA6-152; mature BFU-E that have acquired an Epo sensitivity bore very low amounts of the antigen, and CFU-E were heterogenous with respect to their antigenic density. The majority of the colonies composed of 32 or more erythroblasts were derived from a progenitor with an antigenic density comparable to the mature BFU-E. In contrast, the colonies composed of eight (or four) to 32 erythroblasts were derived from a progenitor with a high antigenic density. Several authors have pointed out that the compartment of human CFU-E is heterogenous. In particular, the CFU-E that gave rise to small colonies with a peak at day 4 to 5 of culture were highly Epo sensitive; they appeared similar to the erythroid progenitor that is intensively stained by the FA6-152 MoAb. EM examination has shown the presence of a homogenous population of undifferentiated cells in the positive fraction. These cells stand as possible candidates for human CFU-E. They have morphologic features similar to the murine CFU-E isolated by a density gradient. In addition, they express another early erythroid marker, ie, carbonic anhydrase 1.

Most previously described MoAbs recognized either hematopoietic progenitors, irrespective of their cell lineages, or CFU-GM. Only one recently described MoAb, ie, EP-147 appears specific to the erythroid lineage from the erythroid progenitors (BFU-E and CFU-E) to the erythroblasts. The 5F1 MoAb has also been described as recognizing CFU-E. Therefore, some antibody competition was performed between 5F1 and FA6-152 MoAbs. Only a partial inhibition between the two MoAbs was observed, suggesting that they recognize different epitopes of the same antigen.

In conclusion, we have described a MoAb that recognizes an ontogenic antigen. This antigen is not detected on the surface of adult erythrocytes, but is present on their fetal counterparts. It is present on nucleated erythroblasts and the megakaryocytic and monocytic series. However, for these two hematopoietic cell lineages, FA6-152 is a late marker of differentiation; in contrast, the antigen recognized by FA6-152 is expressed on mature BFU-E with an antigenic density increasing until day 5 CFU-E. This antibody will permit an improved understanding of both normal and leukemic erythroid differentiation.

REFERENCES

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A monoclonal antibody against an erythrocyte ontogenic antigen identifies fetal and adult erythroid progenitors