Isolation of Myeloid Progenitor Cells From Peripheral Blood of Chronic Myelogenous Leukemia Patients

By James D. Griffin, Richard P. Beveridge, and Stuart F. Schlossman

Myeloid progenitor cells (colony- and cluster-forming cells in semisolid medium, CFU-GM) were purified from the peripheral blood of chronic myelogenous leukemia (CML) patients. Lymphocytes, monocytes, and most immature myeloid cells were simultaneously depleted with specific monoclonal antibodies using an erythrocyte rosette technique for cell separation. Cells expressing Ia-like antigen were then selected from the residual cell population. Day 7 CFU-GM were enriched 44-116-fold in the Ia+ cell fraction, when compared to the unseparated cells, and up to 47% of the cells could form a myeloid colony or cluster in culture. This cell fraction contained up to 92% undifferentiated blasts, with the remainder mostly promyelocytes. The enriched CFU-GM cells were dependent on an exogenous supply of colony-stimulating factor for growth, and colony formation was linear with cell concentration over a large range (10^6-10^7 cells/ml). This technique of rosette depletion and enrichment with specific monoclonal antibodies provides a unique method for purifying a homogeneous population of myeloid precursor cells with defined surface antigen characteristics.

STUDIES OF MYELOID differentiation have been greatly facilitated by the use of in vitro assays for committed progenitor cells capable of forming colonies of mature granulocytes or monocytes (CFU-GM) in semisolid medium.1-3 Using these assays, it has been demonstrated that myeloid colony growth requires the presence of colony-stimulating factors that can be supplied by monocytes,4 activated T cells,5-6 or conditioned media.7-10 Although some of these regulatory factors have been highly purified, studies of their molecular mechanism of action have been hampered by the lack of purified populations of progenitor cells.11 These progenitor cells have been difficult to purify because of low frequency (0.05%-0.1% in bone marrow) and lack of distinguishing physical characteristics. However, cell separation on the basis of size or density has resulted in substantial enrichment of CFU-GM.12-15

In addition to size or density separation techniques, differences in surface antigens have been used to purify progenitor cells. Depletion of cells bearing surface immunoglobulin, receptors for sheep erythrocytes or complement receptors has led to enrichment of CFU-GM cells from peripheral blood.16 The fluorescence-activated cell sorter (FACS) is ideally suited for the purification of populations of cells that have a distinctive surface antigen phenotype and has been used to enrich for CFU-GM by selecting for cells on the basis of light scatter properties,17-20 binding of antibodies,17,18,27 or binding of fluoresceinated lectins.19,20,22 However, the yield of purified progenitor cells obtained by FACS is necessarily limited by the rate of which cells can be sorted, and obtaining large numbers of such cells may be difficult.19 Methods for CFU-GM purification that allow for processing of large numbers of cells would therefore be of value.

The peripheral blood of chronic myelogenous leukemia (CML) patients contains an increased number of CFU-GM cells compared to normal peripheral blood and represents a readily available source of these cells.23 The progeny of these CML CFU-GM are functionally similar in many respects to normal myeloid cells.24 We have developed a two-step technique to prepare large numbers (≥10^8) of CFU-GM cells from CML peripheral blood using a panel of murine monoclonal antibodies reactive with specific subpopulations of human peripheral blood cells. In the first step, T cells, B cells, "null" cells, monocytes, and most immature myeloid cells are depleted using antibodies reactive with each subpopulation. Cells that bind monoclonal antibody are detected by their ability to form rosettes with erythrocytes coated with anti-murine immunoglobulin.25 Cell separation of positive and negative cells is then achieved by density sedimentation. After this depletion step, CFU-GM are then further purified from the negative cell fraction in a second rosetting step, selecting those cells that bear Ia-like antigen. Using this method, a population of cells with the morphological appearance of undifferentiated blasts can be prepared with a very high colony-forming efficiency in agar (30%-50%), with a yield of over 90%. Initial studies on the characterization and growth requirements of these cells are presented.
CFU-GM PURIFICATION

MATERIALS AND METHODS

Source of Peripheral Blood Cells

Peripheral blood samples (5–10 ml) were obtained from CML patients undergoing initial diagnostic testing. All patients had white cell counts greater than 10⁴/µl, and ≤1% blasts on a differential count. Blood was collected into sterile heparinized syringes, and mononuclear cells separated by sedimentation on Ficoll-diатrizoate gradients. Interface cells were washed 2 times in minimal essential medium (GIBCO, Grand Island, N.Y.) containing 2.5% pooled human AB serum (MEM-AB wash). In some cases, cells were cryopreserved in the vapor phase of liquid nitrogen in 10% dimethylsulfoxide and 20% heat-inactivated fetal bovine serum (FBS) until use. These samples were thawed in the presence of deoxyribonuclease I, 100 µg/ml (Worthington Biochemicals, Freehold, N.J.) to minimize cell agglutination. Peripheral blood was also obtained from normal volunteers and similarly prepared.

Monoclonal Antibodies

The production and characterization of monoclonal antibodies anti-MY8,7 anti-Mol,28 anti-B1,29 anti-T11,30 and anti-I231 have been previously reported. Anti-I2 detects a nonpolyorphic region of the human Ia-like antigen (p29, 34).31 The antibodies were obtained in ascitic fluid of mice injected intraperitoneally with hybridoma cells.

CFU-GM Assay

CFU-GM cells were analyzed by plating 10¹–10⁴ mononuclear cells/ml in Iscove’s modified Dulbecco’s MEM (IMDM), 20% FBS, 0.3% agar over a feeder layer of 10⁴ normal peripheral blood leukocytes in 0.5% agar. After 7 and 14 days of culture (37°C, 5% CO₂), colonies (>40 cells) and clusters (8–40 cells) were enumerated using an inverted microscope. Colony morphology was determined by a modification of the method of Kubota et al.32 The agar overlayer plug was separated from the underlayer and dried onto a glass slide. The slide was then fixed in acetone-methanol fixative (60% acetone:10% methanol, 30 mM citrate, pH 5.4) for 1 min at room temperature, washed in tap water, and then stained sequentially for naphthol acetate esterase (nonspecific esterase, NSE), and naphthol AS-D chloroacetate esterase (specific esterase, SE). Staining was done at 37°C for 0.5 and 1 hr, respectively. After washing, cells were counterstained with hematoxylin (Gill Formulation, Fisher Scientific) for 2 min. Monocytes stained heavily with NSE, while granulocytes stained brightly with SE and weakly with NSE.33 Eosinophil colonies were detected separately by staining fixed agar overlayers with Luxol Fast Blue.32 Slides were stained for 2 hr at room temperature in Luxol Fast Blue (Eastman Kodak, Rochester, N.Y.), 100 mg in 100 ml 70% ethanol saturated with urea, and counterstained with hematoxylin. Eosinophil colonies were identified by the characteristic bright green cytoplasmic staining. Cell populations enriched for CFU-GM cells were routinely plated at several dilutions (10¹–10⁴/ml).

Rosette Cell Separation Technique

Preparation of antisera. Goats were immunized with rabbit immunoglobulin (Ig), and immune serum subjected to ammonium sulfate precipitation, and pepsin digestion to produce goat anti-rabbit F(ab')₂, as previously described.34 Antibody was stored in 10 mg/ml 0.16 M borate-buffered saline (BBS), pH 7.4, at −20°C until use. Rabbits were immunized with mouse Ig and immune serum sequentially passed over a human Ig affinity column (to remove cross-reacting antibodies) and a mouse Ig affinity column. Purified rabbit anti-mouse Ig was then eluted from the second column with 1 M glycine buffer, pH 2.0, extensively dialyzed against BBS, and stored at −20°C until use.

Preparation of sheep erythrocytes coated with anti-mouse immunoglobulin. Goat anti-rabbit F(ab')₂ was attached to erythrocytes as follows: sheep erythrocytes (Microbiological Associates, Walkersville, N.C.) were washed 5 times with 0.9% NaCl, and 0.5 ml packed erythrocytes were added to 0.5 ml chromium chloride (Fischer Scientific, 1 mg/ml in 0.9% NaCl) and 0.5 ml goat anti-rabbit F(ab')₂ (1 mg/ml in BBS) and 25 All solutions were passed through a 0.45-µm filter unit (Millipore Corp., Bedford, Mass.) prior to use. The cell suspension was gently mixed by shaking for 7 min at 23°C. The reaction was terminated by addition of 10 ml cold phosphate-buffered saline, and after centrifugation, the Ig-coated erythrocytes were washed 5 times with 0.9% NaCl. These cells were stable when stored as a 10% suspension at 4°C for up to 4 days. Prior to use, coated erythrocytes were pelleted and suspended in an equal volume of rabbit anti-mouse Ig (1 mg/ml). After incubation at 4°C for 30 min, nonbound antibody was removed by washing the erythrocytes 2 times in MEM-AB wash, and the erythrocytes used as a 10% suspension. Direct coupling of rabbit anti-mouse Ig or monoclonal antibody to the erythrocytes gave less reproducible cell separation than the method described.

Cell preparation. A quantity of 0.5–1 x 10⁶ CML peripheral blood mononuclear cells was incubated with 1 ml of MEM-AB wash containing 1:100 dilutions (as ascites) of one monoclonal antibody or a mixture of several monoclonal antibodies for 30 min at 4°C. Unbound antibody was removed by 2 wash steps. To form immune rosettes, 10⁵ monoclonal antibody-treated cells were added to 0.75 ml of a 10% Ig-coated erythrocyte suspension, and the mixture was pelleted (300 g, 10 min) and then incubated at 4°C for 30 min. The red cell-erythrocyte mixture was then vigorously suspended with a Pasteur pipette, and the mixture fractionated by addition of 10 ml cold phosphate-buffered saline, and after centrifugation, the Ig-coated erythrocytes were washed 5 times with 0.9% NaCl. These cells were stable when stored as a 10% suspension at 4°C for up to 4 days. Prior to use, coated erythrocytes were pelleted and suspended in an equal volume of rabbit anti-mouse Ig (1 mg/ml). After incubation at 4°C for 30 min, nonbound antibody was removed by washing the erythrocytes 2 times in MEM-AB wash, and the erythrocytes used as a 10% suspension. Direct coupling of rabbit anti-mouse Ig or monoclonal antibody to the erythrocytes gave less reproducible cell separation than the method described.

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ground fluorescence was determined by staining unseparated peripheral blood cells with a control ascites similarly diluted. It was determined that rosette separation of peripheral blood cells using individual antibodies depleted 85%–99% of the expected cell population with ≥5% loss of antigen negative cells. In experiments with normal bone marrow cells, a variable number of monocytes, mature myeloid cells, and nucleated erythrocytes, often agglutinated, could be found in the Ficoll pellet, regardless of the antibody used.

Screening of antibodies for reactivity with the CFU-GM cell

Antibodies were screened individually for reactivity with the CFU-GM cells of CML peripheral blood by incubating 10⁶ mononuclear cells with anti-MY8, Mol, B1, T11, or I2 as described above. After rosette separation of positive and negative cells, CFU-GM cells were assayed, plating cells at 10⁵ cells/ml in agar.

Terminal Deoxynucleotidyl Transferase Assay

Terminal deoxynucleotidyl transferase (TdT) was assayed by indirect immunofluorescence as described by Bollum. Rabbit anti-calf TdT and fluoresceinated goat anti-rabbit Ig were from Bethesda Research Laboratories.

RESULTS

Screening of Monoclonal Antibodies for Reactivity With CFU-GM Cells

The reactivity of monoclonal antibodies anti-MY8, Mol, T11, B1, and I2 with normal and CML peripheral blood is shown in Table 1. Anti-MY8 Mol, T11, and B1 were selected to provide a panel of antibodies reactive with most peripheral blood cells, excluding CFU-GM. These antibodies were tested individually in an immune rosette separation assay to determine binding to the CFU-GM cells of CML peripheral blood. As shown in Table 2, CFU-GM cells were found exclusively in the MY8, Mol, T11, and B1 negative cell fractions. In contrast, >90% of CFU-GM (day 7 and day 14, all morphological colony types) were found in the I2+ cell fraction.

Immune Rosette Isolation of CFU-GM Cells

CML peripheral blood mononuclear cells were separated by immune rosettes into MY8, Mol, T11, and B1 positive and negative cell fractions using all four antibodies simultaneously. The MY8, Mol, B1, T11 negative fraction was further separated into I2 positive and negative fractions. Aliquots of each cell fraction were assayed for CFU-GM and cytocentrifuge smears examined for cell morphology (Table 3). Total cell recovery in the 4 experiments shown was 51.4% ± 19.4% (range 28%–72%). The MY8, Mol, B1, T11 positive cell fraction contained 40.1% ± 11.8% of the starting cells; the I2− fraction contained 8.9% ± 7.6%; and the I2+ fraction contained 1.9% ± 1.6% of the starting cells.

Cell differentials of the separated cell fractions were performed on Wright stained cytocentrifuge smears (Table 3). As shown, the unseparated cell fraction was typical of chronic phase CML peripheral blood in each case. The MY8, Mol, B1, T11+ fraction contained primarily promyelocytes and more mature myeloid cells and most of the morphologically recognizable lymphocytes. In contrast, the I2+ fraction consisted largely of undifferentiated blasts (mean 73%, range 52%–92%). These cells were large (approximately 15μ), basophilic, and had a high nuclear:cytoplasmic ratio, multiple nucleoli, and few or no cytoplasmic granules.

Table 1. Monoclonal Antibodies Used in the Purification of CML CFU-GM

<table>
<thead>
<tr>
<th>Antigen Designation</th>
<th>Normal Peripheral Blood</th>
<th>CML Immature</th>
<th>CML Myeloid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Granulocytes</td>
<td>Monocytes</td>
<td>T</td>
</tr>
<tr>
<td>MY8</td>
<td>+ ++</td>
<td>+ +</td>
<td>−</td>
</tr>
<tr>
<td>Mol</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>B1</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>T11</td>
<td>+ / −</td>
<td>+ ++</td>
<td>−</td>
</tr>
<tr>
<td>I2</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

* Determined by an indirect immunofluorescence assay. +++, >80% cell fluorescent; +, 20%–80% cells fluorescent; + / −, <20% cells fluorescent; −, <1% cells fluorescent.

Table 2. Reactivity of Monoclonal Antibodies With CFU-GM Cells

<table>
<thead>
<tr>
<th>Cell Fraction</th>
<th>CFU-GM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unseparated†</td>
<td>112 ± 11</td>
</tr>
<tr>
<td>MY8 + ‡</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>MY8−</td>
<td>152 ± 31</td>
</tr>
<tr>
<td>Mol +</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Mol−</td>
<td>122 ± 12</td>
</tr>
<tr>
<td>B1 +</td>
<td>0</td>
</tr>
<tr>
<td>B1−</td>
<td>119 ± 16</td>
</tr>
<tr>
<td>T11 +</td>
<td>3 ± 4</td>
</tr>
<tr>
<td>T11−</td>
<td>141 ± 4</td>
</tr>
<tr>
<td>I2 +</td>
<td>212 ± 15</td>
</tr>
<tr>
<td>I2−</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

* Day 7 colonies per 10⁵ mononuclear cells.
† Ficoll-Hypaque prepared mononuclear cells from CML peripheral blood.
‡ Cell fractions prepared by the immune rosette technique described in Materials and Methods.

Materials and Methods
Table 3. Immune Rosette Purification of CFU-GM Cells From CML Peripheral Blood

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Cell Morphology*</th>
<th>Day 7†</th>
<th>Day 14</th>
<th>Percent Yield§</th>
<th>Fold</th>
<th>Enrichment</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Cell Fraction</td>
<td>Clusters</td>
<td>Colonies</td>
<td>Colonies</td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>1</td>
<td>Unseparated</td>
<td>1%</td>
<td>95</td>
<td>1</td>
<td>320</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td>My8, Mol, T11, B1+</td>
<td>2</td>
<td>8</td>
<td>14</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>I2</td>
<td>12</td>
<td>62</td>
<td>10</td>
<td>80</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>I2 +</td>
<td>76</td>
<td>14</td>
<td>10</td>
<td>19,220</td>
<td>4,840</td>
</tr>
<tr>
<td>2</td>
<td>Unseparated</td>
<td>1</td>
<td>8</td>
<td>88</td>
<td>3</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td>MY8, Mol, T11, B1+</td>
<td>0</td>
<td>21</td>
<td>73</td>
<td>6</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>I2</td>
<td>1</td>
<td>26</td>
<td>72</td>
<td>1</td>
<td>280</td>
</tr>
<tr>
<td></td>
<td>I2 +</td>
<td>52</td>
<td>31</td>
<td>17</td>
<td>0</td>
<td>35,500</td>
</tr>
<tr>
<td>3</td>
<td>Unseparated</td>
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<td>15</td>
<td>69</td>
<td>14</td>
<td>390</td>
</tr>
<tr>
<td></td>
<td>MY8, Mol, T11, B1+</td>
<td>0</td>
<td>12</td>
<td>57</td>
<td>31</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>I2</td>
<td>12</td>
<td>38</td>
<td>42</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>I2 +</td>
<td>72</td>
<td>21</td>
<td>6</td>
<td>1</td>
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<td>4</td>
<td>Unseparated</td>
<td>0</td>
<td>9</td>
<td>90</td>
<td>1</td>
<td>191</td>
</tr>
<tr>
<td></td>
<td>MY8, Mol, T11, B1+</td>
<td>0</td>
<td>3</td>
<td>95</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>I2</td>
<td>8</td>
<td>27</td>
<td>59</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>I2 +</td>
<td>92</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>21,100</td>
</tr>
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</table>

*Cell differential of a Wright's stained cytocentrifuge smear.
†Mycelocytes, metamyelocytes, bands, mature neutrophils, eosinophils, basophils, and monocytes.
§CFU-GM of each cell fraction compared to unseparated cells.

Proliferative Characteristics of Cell Fractions in Agar

The separated cell fractions were assayed for myeloid cluster- and colony-forming cells (Table 3). As shown, considerable enrichment of the CFU-GM cells was found in the I2+ MY8-, Mol-, B1-, T11- cell fraction, with 75.1%-104.5% of initial CFU-GM (day 7) recovered in this fraction. At day 7, a mean of 29.6% of cells plated from the I2+ cell fraction could form clusters or colonies (range 22.2%-47%), representing a mean enrichment of 79-fold in 4 experiments. Cells forming colonies at day 14 were similarly enriched (mean 46-fold). Thus, CFU-GM copurified with blasts and promyelocytes in these experiments. Other cell fractions contained very few CFU-GM cells. Interestingly, the I2-(MY8-, Mol-, B1-, T11-) cell fraction contained an increased number of blasts and promyelocytes (mean 35%), which were morphologically indistinguishable from I2+ blasts and promyelocytes, but lacked significant proliferative potential (0.11% based on day 7 clusters and colonies). I2- blasts may thus represent nonmyeloid progenitor cells or may not have the capacity to proliferate in an in vitro assay. Alternatively, an accessory cell necessary for CFU-GM growth could have been separated to the I2+ cell fraction. To test this hypothesis, varying numbers of I2+ cells were mixed with I2- cells, and a CFU-GM assay performed. If an I2+ accessory cell was present, an increased number of CFU-GM would be found in the mixed cells, as the accessory cell is added back to the I2- cell fraction. As shown in Table 4, no such increase was seen, suggesting that the I2- cell fraction had not been depleted of an accessory cell.

The colony morphology in the I2+ cell fraction was determined by staining the agar layers for specific esterase, nonspecific esterase,32 and Luxol Fast Blue (for eosinophil colonies). There were no significant differences noted in the frequency of granulocyte, monocyte, mixed GM, or eosinophil colonies between unseparated cells and the I2+ cell fractions enriched.
for CFU-GM (data not shown). Also, mean colony size was similar in unseparated and 12+ cell fractions.

CFU-GM cells in the 12+ cell fraction were assayed over a large concentration range ($10^1-10^4$ cells/ml) using feeder layers of peripheral blood leukocytes. As shown in Fig. 1, proliferation of colony- and cluster-forming cells was nearly linear over the cell concentrations tested. However, in the absence of a source of colony-stimulating factor (feeder layers), virtually no growth of clusters or colonies was seen (Fig. 1), even at high cell concentrations ($10^4$/ml).

**Determination of TdT+ Cells in the 12+ Cell Fraction**

TdT+ cells were assayed by immunofluorescence in the 12+ cell fraction. Less than 2% of the cells showed nuclear fluorescence characteristic of TdT+ cells. The unseparated cell fractions contained less than 1% TdT-positive cells. Thus, the 12+ blasts (and, therefore, the majority of CFU-GM cells) were largely TdT−.

**DISCUSSION**

Human myeloid progenitor cells have been difficult to purify because of low frequency and overlap of cell size and density with mature cells.12-14 It has been possible to enrich for progenitor cells by taking advantage of differences in cell surface antigens16,18,21 or lectin receptors19,20,22 using the fluorescence activated cell sorter, especially with murine fetal liver or bone marrow cells. The peripheral blood of chronic myelogenous leukemia patients contains an increased number of myeloid progenitor cells capable of clonogenic growth in semisolid medium.23 In the experiments presented here, myeloid progenitor cells from CML patients have been successfully separated from the majority of other peripheral blood cells using an erythrocyte rosetting technique to separate cells on the basis of binding of monoclonal antibodies. A 44–116-fold enrichment of CFU-GM cells was obtained by simultaneous depletion of T cells, B cells, “null” cells, monocytes, and immature myeloid cells using monoclonal antibodies anti-T11, B1, Mol, and MY8, followed in a second step by selection of cells binding antibody anti-I2, which detects a nonpolymorphic region of the human Ia-like molecule.31 Seventy-five percent to >99% of myeloid precursor cells could be recovered in the 12+ fraction using this method, and up to 47% of the 12+ cells could form a cluster or colony at day 7 in a CFU-GM assay in soft agar.

Ia antigen has been previously shown to be expressed by normal myeloblasts,21,36 leukemic myeloblasts,37 and CFU-GM.21,36,38 This antigen is acquired during differentiation of myeloid precursors from the pluripotent stem cell28,39 and is lost at about the level of the promyelocyte.21,36 Expression of Ia antigen on B cells, monocytes, and a subset of T cells may be important in cell–cell communication of these immunocompetent cells.40-44 However, the role of Ia antigens of nonimmune hematopoietic cells is unclear. In vitro proliferation of myeloid progenitors is dependent on myeloid colony-stimulating factors, and a requirement for cell contact with regulatory cells has not been established. However, observations in the continuous marrow culture system suggest that intimate associations of progenitor cells with cells of the microenvironment may be critical.41-44 The importance of Ia antigen in such associations remains to be determined. The experiments presented here have confirmed that progenitor cells of granulocytes, monocytes, and eosinophils express Ia-like antigens. Using monoclonal antibody anti-I2, it has been further possible to isolate a population of cells highly enriched for CFU-GM cells. The 12+ fraction consisted of up to 92% blasts, with the remainder mostly promyelocytes. The majority of the blast cells were morphologically undifferentiated, although some contained a few cytoplasmic granules, suggesting they were myeloblasts. Less than 2% of the cells in the 12+ fraction were TdT+, consistent with the hypothesis that TdT may be a marker for lymphocyte, but not myeloid, progenitor cells.35 Mature lymphocytes and monocytes were effectively separated from colony-forming cells. Thus, this technique pro-
vides a rapid method for the production of large numbers (>10^6) of myeloid progenitor cells from CML peripheral blood. Early attempts to morphologically identify myeloid progenitor cells from simian bone marrow suggested that CFU-GM cells are light density cells similar in size to lymphocytes. Recent experiments with FACS separation of human bone marrow cells, however, have indicated that myeloid colony- and cluster-forming cells are larger than lymphocytes. Beverly et al. sorted normal bone marrow cells into several fractions determined by reactivity with anti-HLe-1 and by cell size (low angle light scatter). CFU-GM were enriched primarily in the “large” cell fraction, which was comprised of 36% blasts, 3% maturing myeloid cells, 19% plasma cells, 3% lymphocytes, and 39% erythroid cells. Morstyn et al. purified human marrow progenitor cells by selecting cells simultaneously for low angle light scatter, high angle light scatter, and binding of a fluoresceinated lectin. In the most enriched fraction, 12% of the cells were blasts, 81% of the cells were promyelocytes, or myelocytes, and 3% were lymphocytes. The results presented here indicate that the myeloid progenitor cells of CML peripheral blood are highly enriched in a cell fraction that is composed primarily of blasts and promyelocytes. These experiments thus support the concept that morphologically recognizable early myeloid cells (myeloblasts and promyelocytes) have considerable proliferative capacity and may at least represent the majority of cells forming clusters in vitro.

Blast cells and promyelocytes were also somewhat enriched in the 12− cell fraction (mean 14-fold). However, the proliferative potential of the 12− cell fraction was actually decreased by a factor of 3.6-fold compared to unseparated cells (day 7 colonies plus clusters, Table 3). One explanation for this would be that growth of myeloid progenitor cells in vitro requires the presence of an accessory cell that is Ia+, and thus, colonies are observed only in the Ia+ fraction because of copurification of an accessory cell. In order to test that possibility, mixing experiments were performed in which 12+ cells were added at several ratios to 12− cells (Table 4). No increase in colony growth in the 12− fraction was detected, suggesting that loss of an accessory cell from the 12− fraction had not occurred. Also, there was no evidence that the 12− fraction contained an inhibitor cell, as even high ratios of 12− to 12+ cells did not suppress 12+ cell colony formation (Table 4). It is possible, however, that potential inhibitor cells present in unseparated CML cells were removed during the initial rosetting step. Thus, it appears that 12+ cells have a proliferative advantage in an in vitro assay over 12− cells, despite morphological similarities.

Purified myeloid progenitor cells as described here may facilitate the study of purified colony-stimulating factors. Proliferation of 12+ cells demonstrated an absolute requirement for exogenous colony-stimulating factors, as supplied by a peripheral blood leukocyte feeder layer, even when plated at high cell concentrations (Fig. 1). This suggests that endogenous cells capable of supplying these factors have been effectively depleted during the purification procedure. Also, the growth of 12+ cells was linear over a large concentration range. It should therefore be possible to study the mechanism of action of purified colony-stimulating factors on such cells.

The technique of cell enrichment described here may be useful for the purification of progenitor cells from normal peripheral blood and bone marrow, although modification of the monoclonal antibody panel may be required. The method should also be useful for enrichment of any cell present in low concentration, provided the appropriate antibodies are available for depletion and positive selection. This technique also provides a simple method for determining the surface antigen phenotype of progenitor cells by positive selection. Aliquots of bone marrow cells can be separated into antigen positive and negative fractions, and each assayed for the appropriate progenitor. This is particularly useful for nonlytic antibodies and for confirming results of complement lysis experiments.

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Isolation of myeloid progenitor cells from peripheral blood of chronic myelogenous leukemia patients

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