Abnormal Erythroid Progenitor Cells in Human Preleukemia

By David H. K. Chui and Bryan J. Clarke

Ten patients with preleukemia were studied by the erythroid cell clonal culture technique. In nine of these patients, erythroid colonies derived from peripheral blood BFU-E were not observed, while the other patient had markedly decreased peripheral blood BFU-E-derived erythroid colonies in vitro. In three patients, marrow cells were also cultured and no BFU-E-derived erythroid colonies were detected. These studies indicate that immature erythroid progenitor cells, BFU-E, in patients with preleukemia are either markedly decreased in number or grossly defective in their proliferative or differentiative capacities.

Preleukemia represents a heterogeneous group of hematopoietic disorders that are usually found in the elderly and are characterized by anemia unresponsive to the administration of the currently used hematinsics. In addition, varying degrees of neutropenia and/or thrombocytopenia are present in these patients. Bone marrow cellularity is usually increased or normal. Erythroid hyperplasia and dyserythropoiesis with megaloblastoid features, sometimes with ringed sideroblasts, are commonly found. The proportions of immature myeloid elements in the bone marrow of many of these patients are increased, and the myeloblasts and promyelocytes can account for 10%–30% of all marrow nucleated cells in some patients.

In two large prospective studies, it was reported that about 20% of patients with this syndrome ultimately developed acute nonlymphocytic leukemia. The etiology and pathogenesis of this primary marrow dysfunction are at present unknown.

Recently, it has become possible to study erythroid progenitor cells by the in vitro clonal culture technique. The mature erythroid progenitor cells, CFU-E, give rise to small erythroid colonies each consisting of less than 100 erythroblasts by the seventh day of culture. CFU-E are present in the bone marrow, but not normally in the peripheral blood. The immature erythroid progenitor cells, BFU-E, produce large and diffuse erythroid colonies, each consisting of thousands of erythroblasts by the twelfth day of culture. BFU-E are normally present both in the bone marrow and in the peripheral blood. We have used the erythroid cell clonal culture technique to study ten patients with preleukemia and have found that these patients either lack or have grossly defective immature erythroid progenitor cells, BFU-E. A preliminary report of this study has been published elsewhere.

Materials and Methods

Patients

Ten patients were studied. Their clinical and hematologic findings are summarized in Table 1. All of them presented with anemia, reticulocytopenia, and varying degrees of neutropenia and/or thrombocytopenia. There was no evidence of iron, vitamin B12, or folate deficiency. Marrow cellularity was usually increased or normal. Dyserythropoiesis was marked. The proportion of myeloblasts in the marrow often was increased, but the hematologic findings did not fulfill the usual criteria for the diagnosis of acute myeloid leukemia.

Healthy laboratory workers under age 40 served as normal controls in these studies. Five other elderly patients hospitalized at the McMaster University Medical Centre were also studied. Appropriate consent was obtained and up to 50 ml of venous blood was withdrawn from each person into a sterile plastic syringe containing 500–1000 U of heparin.

Bone marrow samples were obtained from aspiration performed for clinical diagnostic purposes. Up to 1.5 ml of three marrow samples were put in 2.0 ml of saline containing 100 U of heparin.

Erythroid Cell Culture

Peripheral blood and bone marrow mononuclear cells were obtained by the Ficoll/Hypaque cell separation technique and cultured in plasma clots at a concentration of 2–3 x 10^6 cells/ml, as previously described. Sheep plasma erythropoietin (Ep), step 3 (specific activity 3–14 U/mg of protein, Connaught Laboratories, Willowdale, Ontario) was added to some plasma clot cultures to make up 10% of the final culture volume. PHA-LCM was prepared according to the method of Messner.

PHA-LCM was prepared by the Ficoll/Hypaque cell separation technique and cultured in plasma clots at a concentration of 2–3 x 10^6 cells/ml, as previously described. Sheep plasma erythropoietin (Ep), step 3 (specific activity 3–14 U/mg of protein, Connaught Laboratories, Willowdale, Ontario) was added to each plasma clot at a final concentration of 2–10 U/ml. The cultures were terminated on days 6–8 and on day 12, and stained with benzidine. Erythroid colonies derived from mature erythroid progenitor cells (CFU-E), and immature erythroid progenitor cells (BFU-E) were scored on days 6–8 and on day 12, respectively, according to published criteria.

Dexamethasone disodium phosphate (Merck, Sharp and Dohme Canada Limited, Kirkland, P.Q.) was added to some cultures at a final concentration of 10^{-8} M.

Leukocyte Conditioned Medium (PHA-LCM)

PHA-LCM was prepared according to the method of Messner. Briefly, peripheral blood mononuclear cells from healthy individuals were incubated at 37°C at a concentration of 10^6 cells/ml of medium consisting of 1% phytohemagglutinin (GIBCO, Grand Island, N.Y.), 20% fetal calf serum (Flow Laboratory, Rockville, Md.) and alpha modified Eagle medium (GIBCO). After 7 days, the supernatant was harvested, millipored, and stored frozen at −18°C until use. PHA-LCM was added to some plasma clot cultures to make up 10% of the final culture volume.
ABNORMAL BFU-E IN PRELEUKEMIA

Table 1. Clinical and Hematologic Findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Hb (g/dl)</th>
<th>Neutrophils (10⁹/liter)</th>
<th>Platelets (10⁹/liter)</th>
<th>Cellularity</th>
<th>Morphology</th>
<th>Clinical Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.M.</td>
<td>73</td>
<td>M</td>
<td>5.1</td>
<td>2.4</td>
<td>250</td>
<td>Increased</td>
<td>10% Myeloblasts</td>
<td>Died of chronic myelomonocytic leukemia 2 yr after diagnosis.</td>
</tr>
<tr>
<td>R.P.</td>
<td>59</td>
<td>M</td>
<td>6.7</td>
<td>0.6</td>
<td>101</td>
<td>Decreased</td>
<td>20% Myeloblasts</td>
<td>Died of septicemia 3 yr after diagnosis.</td>
</tr>
<tr>
<td>V.A.</td>
<td>79</td>
<td>F</td>
<td>7.9</td>
<td>0.4</td>
<td>84</td>
<td>Increased</td>
<td>Erythroid hyperplasia. Some ringed sideroblasts. Left shift in granulopoiesis.</td>
<td>Died of septicemia 1 yr after diagnosis.</td>
</tr>
<tr>
<td>E.E.</td>
<td>61</td>
<td>M</td>
<td>9.8</td>
<td>0.1</td>
<td>479</td>
<td>Decreased</td>
<td>15% Myeloblasts</td>
<td>Died of septicemia 3 mo after diagnosis.</td>
</tr>
<tr>
<td>M.G.</td>
<td>73</td>
<td>F</td>
<td>4.9</td>
<td>0.7</td>
<td>35</td>
<td>Increased</td>
<td>10% Myeloblasts</td>
<td>Died of acute leukemia 3 yr after diagnosis.</td>
</tr>
<tr>
<td>B.G.</td>
<td>44</td>
<td>M</td>
<td>11.2</td>
<td>2.4</td>
<td>Normal</td>
<td>Increased</td>
<td>10% Myeloblasts</td>
<td>Died of acute leukemia 13 yr after diagnosis.</td>
</tr>
<tr>
<td>A.K.</td>
<td>74</td>
<td>M</td>
<td>11.7</td>
<td>0.6</td>
<td>77</td>
<td>Normal</td>
<td>Erythroid hyperplasia. Some ringed sideroblasts. Increased myeloblasts.</td>
<td>Has remained pancytopenic for 3 yr.</td>
</tr>
<tr>
<td>M.S.</td>
<td>68</td>
<td>F</td>
<td>8.3</td>
<td>3.6</td>
<td>206</td>
<td>Increased</td>
<td>Erythroid hyperplasia. Numerous ringed sideroblasts.</td>
<td>Has remained anemic for 7 yr.</td>
</tr>
<tr>
<td>C.T.</td>
<td>83</td>
<td>M</td>
<td>10.9</td>
<td>0.3</td>
<td>79</td>
<td>Normal</td>
<td>Increased myeloblasts.</td>
<td>Has remained pancytopenic for 1.5 yr.</td>
</tr>
<tr>
<td>B.T.</td>
<td>77</td>
<td>M</td>
<td>8.5</td>
<td>0.8</td>
<td>28</td>
<td>Increased</td>
<td>Dyserythropoiesis. Some ringed sideroblasts.</td>
<td>Has remained pancytopenic for 3 yr.</td>
</tr>
</tbody>
</table>

Removal of T Lymphocytes From Mononuclear Cells

T lymphocytes were removed from peripheral blood mononuclear cells by the technique of sheep erythrocyte rosetting. A quantity of 10⁷ mononuclear cells in 1 ml of alpha-modified Eagle medium was added to 1 ml of 5% sheep erythrocytes (Qualicum Scientific, Ottawa, Ontario) in normal saline. This cell suspension was centrifuged at 20°C for 10 min at 300 g. The cell pellet was incubated at 20°C for 60 min and was then resuspended. The cell suspension was placed on Ficoll/Hypaque and centrifuged at 20°C for 30 min at 400 g. The mononuclear cells, depleted of T lymphocytes, were present at the interface. These cells were collected, washed, and plated in plasma clot cultures at a concentration of 10⁶ cells/ml of plasma clot. Under the present culture conditions, normal peripheral blood mononuclear cells depleted of T lymphocytes by the aforementioned technique, when plated at 10⁶ cells/ml, regularly gave rise to many BFU-E-derived large erythroid colonies on day 12 of cultures.

RESULTS

Clinical Picture

The hematologic data of the 10 patients at the time of this investigation are presented in Table 1. All but one of these patients were older than 59 yr. Seven of them were males. All 10 patients were anemic with hemoglobin levels varying between 5.1 and 11.7 g/dl with a mean of 8.5 g/dl. Varying degrees of poikilocytosis and anisocytosis were noted in all 10 patients. Macrocytosis, with a mean cell volume of 95 fl or above, was present in 6 patients. Seven patients had peripheral blood neutrophil counts of less than 10⁹/liter. Immature granulocytic cells were found in peripheral blood in 7 patients. Six patients had platelet counts of 101 × 10⁹/liter or less. Significant numbers of megathrombocytes were noted in the peripheral blood in all but 2 patients.

The marrow cellularity at the time of the present study was determined to be increased in 6 patients, normal in 2, and decreased in 2. Increased myeloblasts in the bone marrow were found in all but 2 patients. Dyserythropoiesis was commonly found and ringed sideroblasts were present in the marrow of 4 patients.

At the time of this communication, 6 of the 10 patients have died, 2 from acute myeloid leukemia, 1 from chronic myelomonocytic leukemia, and 3 from septicemia.

Erythroid Progenitor Cells in Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells obtained from the patients were cultured in vitro in plasma clots with erythropoietin. These cultures were harvested after 6–8 days in vitro, and the number of CFU-E-derived colonies was determined. In 9 patients, there were 16 ± 26 CFU-E colonies (range 0–80) per 10⁶ mononuclear
The BFU-E data are expressed as mean ± SD. All the cultures were done in the presence of erythropoietin except for the cultures related to J.H. in which both erythropoietin and dexamethasone were added.

Table 2. BFU-E-Derived Colonies in Cultures of Peripheral Blood Mononuclear Cells

<table>
<thead>
<tr>
<th></th>
<th>EP Added</th>
<th>Control</th>
<th>EP and PHA-LCM Added</th>
<th>Control</th>
<th>EP and Dexamethasone Added</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.M.</td>
<td>0</td>
<td>65 ± 19</td>
<td>0</td>
<td>59 ± 15</td>
<td>0</td>
<td>78 ± 10</td>
</tr>
<tr>
<td>R.P.</td>
<td>3 ± 4</td>
<td>67 ± 35</td>
<td>7 ± 7</td>
<td>100 ± 67</td>
<td>8 ± 9</td>
<td>94 ± 89</td>
</tr>
<tr>
<td>V.A.</td>
<td>3 ± 4</td>
<td>72 ± 19</td>
<td>1 ± 1</td>
<td>90 ± 30</td>
<td>1 ± 2</td>
<td>80 ± 7</td>
</tr>
<tr>
<td>E.E.</td>
<td>0</td>
<td>55 ± 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.G.</td>
<td>0</td>
<td>37 ± 5</td>
<td>0</td>
<td>62 ± 12</td>
<td>0</td>
<td>30 ± 10</td>
</tr>
<tr>
<td>B.G.</td>
<td>1 ± 2</td>
<td>111 ± 28</td>
<td>0</td>
<td>128 ± 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.K.</td>
<td>1 ± 2</td>
<td>142 ± 20</td>
<td>0</td>
<td>57 ± 45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.S.</td>
<td>6 ± 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.T.</td>
<td>4 ± 3</td>
<td>12 ± 9</td>
<td>19 ± 14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.T.</td>
<td>22 ± 6</td>
<td>82 ± 28</td>
<td>3 ± 4</td>
<td>44 ± 38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4 ± 7</td>
<td>79 ± 33</td>
<td>3 ± 4</td>
<td>77 ± 30</td>
<td>6 ± 8</td>
<td>70 ± 28</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD of number of BFU-E-derived colonies/10⁶ cells plated. For each point, a mean of 7 ± 4 cultures (range 3–17) were done.

cells plated. In the studies of 6 of these patients, normal peripheral blood mononuclear cells were also cultured at the same time and no CFU-E colony was observed.

In 9 patients, extremely few BFU-E-derived erythroid colonies were observed on day 12 of cultures (Table 2). In the other patient, BFU-E-derived erythroid colonies were present but markedly decreased in number when compared to those from the normal individual. In most instances, peripheral blood mononuclear cells obtained from a normal individual were also cultured at the same time as the patient’s cells were cultured, in order to ascertain that the absence of BFU-E-derived colonies in cultures of patients’ cells was not due to aberrant culture conditions.

PHA-LCM as well as dexamethasone were added to a number of cultures. No significant augmentation of BFU-E-derived erythroid colony growth in patients’ cultures was observed with either substance (Table 2).

Five other patients, aged 59–82, were also studied in order to determine if peripheral blood BFU-E are present in elderly individuals, particularly those with anemias. Four of these five patients had either secondary or iron deficiency anemias. Their peripheral blood mononuclear cells were cultured in plasma clots, and there were 80 ± 52 (range 46–173) BFU-E-derived large erythroid colonies per 10⁶ cells plated (Table 3).

BFU-E in Peripheral Blood Mononuclear Cells Depleted of T Lymphocytes

In order to rule out the possibility that failure of growth of BFU-E-derived erythroid colonies in vitro may be due to the presence of inhibitory T lymphocytes, the technique of sheep erythrocyte rosetting was employed to remove T lymphocytes from the peripheral blood mononuclear cells obtained from four patients. Peripheral blood mononuclear cells from normal individuals were similarly treated. The T-lymphocyte-depleted mononuclear cells were then cultured in vitro in the presence of erythropoietin with or without PHA-LCM and dexamethasone. In all instances, the

Table 3. BFU-E-Derived Colonies in Cultures of Peripheral Blood Mononuclear Cells Obtained From Elderly Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Clinical Condition</th>
<th>Hb (g/dl)</th>
<th>Peripheral Blood Mononuclear Cells Plated</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.H.</td>
<td>59</td>
<td>M</td>
<td>Multiple intraabdominal abscesses secondary to hemorrhagic pancreatitis and abdominal surgery</td>
<td>10.1</td>
<td>173 ± 25</td>
</tr>
<tr>
<td>C.A.</td>
<td>62</td>
<td>M</td>
<td>Recovery from anemia of undetermined etiology</td>
<td>15.8</td>
<td>59 ± 13</td>
</tr>
<tr>
<td>G.S.</td>
<td>73</td>
<td>M</td>
<td>Severe malnutrition and large decubitus ulcer</td>
<td>7.5</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>T.G.</td>
<td>74</td>
<td>M</td>
<td>Iron deficiency</td>
<td>10.0</td>
<td>57 ± 11</td>
</tr>
<tr>
<td>J.H.</td>
<td>82</td>
<td>M</td>
<td>Iron deficiency</td>
<td>5.4</td>
<td>67 ± 4</td>
</tr>
</tbody>
</table>

The BFU-E data are expressed as mean ± SD. All the cultures were done in the presence of erythropoietin except for the cultures related to J.H. in which both erythropoietin and dexamethasone were added.
cells from the patients failed to grow BFU-E-derived erythroid colonies, while the cells from normal individuals grew 210 ± 128 colonies/10⁶ cells plated (Table 4).

**Erythroid Cell Cultures With Bone Marrow Cells**

As shown in Table 5, in three patients thus studied, bone marrow mononuclear cells grew CFU-E-derived small erythroid colonies by day 7 of culture but failed to give rise to any BFU-E-derived large erythroid colonies by day 12 of culture. Similar results were obtained when PHA-LCM or dexamethasone was added to these cultures.

**DISCUSSION**

Preleukemia is a syndrome characterized by primary marrow dysfunction. The hematologic pictures are varied, and the syndrome has been described under various terms such as refractory anemia, myelodysplastic syndromes, and smoldering leukemia. The present investigation shows that patients with this syndrome have either markedly deficient or grossly defective immature erythroid progenitor cells commonly referred to as BFU-E. In these studies, cultures of mononuclear cells obtained from healthy individuals were done at the same time as studies of the patients' cells. The number of BFU-E-derived colonies in cultures of peripheral blood mononuclear cells obtained from healthy individuals under age 40 is 79 ± 33/10⁶ cells plated (Table 2). The number of BFU-E-derived colonies from 5 other elderly patients, 4 with iron deficiency or secondary anemias, is 80 ± 52 (Table 3). On the other hand, in cultures of patients' cells, BFU-E-derived erythroid colonies were either absent or markedly decreased in number. These results show that the failure to observe erythroid colonies in cultures of patients' cells could not be ascribed to aberrant culture conditions or patients' old age per se. In addition to erythropoietin, the growth and differentiation of BFU-E requires another humoral factor commonly referred to as burst-promoting activity. It has also been shown that such activity is present in PHA-LCM. In the present experiments, PHA-LCM was added to the cultures without any significant enhancement of patients' BFU-E growth. These observations suggest that the failure of patients' BFU-E growth was not due to lack of burst-promoting activity.

Dexamethasone can stimulate human erythroid cell growth in vitro. It was recently reported that in about 10% of patients with preleukemic syndrome, glucocorticoid could enhance the granulocyte-macrophage colony growth in vitro. In the present study, dexamethasone failed to increase the BFU-E growth in vitro in any of the six patients thus studied. The failure of BFU-E growth in vitro may be due to T-lymphocyte-mediated suppression.
investigation employed the technique of sheep-erythrocyte rosetting in order to remove the T lymphocytes. In four patients thus studied, no BFU-E growth was observed, suggesting that T-lymphocyte suppression is unlikely to be a significant factor in this group of patients.

In three patients, bone marrow cells were cultured and no BFU-E-derived erythroid colonies were observed, identical to the results of the peripheral blood mononuclear cell cultures in these patients. Recently, it was reported that peripheral blood hematopoietic stem cells are in rapid equilibrium with bone marrow hematopoietic stem cells.29 Taken together, these observations indicate that the experimental results obtained by studying the peripheral blood BFU-E can reflect the nature and activity of the bone marrow BFU-E population.

Abnormal differentiation of granulocyte-macrophage progenitor cells in patients with preleukemia has been well documented.22 57 The present studies indicate that immature erythroid progenitor cells, BFU-E, in these patients are also defective. On the other hand, CFU-E growth in bone marrow cells is normal in one patient and decreased in the other two patients, as previously reported.25 28 A few CFU-E were present also in the peripheral blood of some of these patients.

Chromosomal abnormalities have been found in the marrow cells of approximately 35% of these patients.29 30 These observations support the hypothesis that preleukemia is a clonal disease of hematopoietic stem cells.7 The absence of BFU-E-derived erythroid colonies in cultures of patients’ peripheral blood and bone marrow mononuclear cells suggests that the abnormal clone of BFU-E is not capable of extensive proliferation and differentiation in the present culture conditions. Additional studies are necessary in order to further understand the pathogenesis of this group of primary marrow disorders.

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