ABSTRACT

The treatment of acute nonlymphocytic leukemia results in predictable bone marrow hypoplasia and eventual cellular repopulation. In order to study this postchemotherapy repopulation, assays for hematopoietic progenitor cells were performed on bone marrow samples obtained from seven patients with acute nonlymphocytic leukemia who had received similar chemotherapeutic induction regimens. Burst-forming units (erythrocyte), colony-forming units (megakaryocyte), colony-forming units (granulocyte-macrophage), and colony-forming units (granulocyte-erythrocyte-megakaryocyte-macrophage) were cloned from human bone marrow mononuclear cells 5 and/or 10 days following completion of chemotherapy. All patients were pancytopenic and had hypocellular marrows when studied. Assays were performed 7 to 30 days prior to complete remission. Colony-forming units (granulocyte-macrophage) were equivalent to control values 5 days following chemotherapy, while burst-forming units (erythrocyte) and colony-forming units (granulocyte-erythrocyte-megakaryocyte-macrophage) were not assayed at that time. Ten days following chemotherapy, colony-forming units (granulocyte-macrophage) were equivalent to control values 5 days following chemotherapy, while burst-forming units (erythrocyte) were 29% of control values. Colony-forming units (macrophage) were 10 to 15 times normal values 10 days following chemotherapy. In contrast to colonies from normal individuals, those grown from marrow obtained following chemotherapy were frequently macroscopic and were composed of thousands of cells. Patient marrow had larger proportions of progenitor cells in S phase of the cell cycle than did normal controls. These studies suggest the presence of a stem cell in human bone marrow which is resistant to chemotherapeutic agents and has a high capacity to regenerate hematopoietic progenitor cells. The period following completion of chemotherapy for acute nonlymphocytic leukemia appears suitable for the study of the hierarchical nature of human hematopoiesis.

INTRODUCTION

Since the 1950s, it has been recognized that cells which reside in the bone marrow or spleens of mice are able to salvage syngeneic animals from hematological death following lethal irradiation (1–3). In 1961, Till and McCulloch (4), using an in vivo assay system, demonstrated the presence of such a hematopoietic cell, the CFU-S.³ Progeny of the CFU-S include cells of multiple lineages (5–7), as well as additional multipotential CFU-S (5, 8). In 1976, Rosendaal et al. (9) demonstrated that bone marrow from mice pretreated with 5-FUra or hydroxyurea contains a higher proportion of CFU-S than does normal, untreated marrow. These stem cells have a high capacity to generate other stem cells (self-renewal) as well as the progenitors of granulocytes, erythrocytes, macrophages, and megakaryocytes (10). The noncycling, marrow-repopulating stem cells which survive 5-FUra treatment are associated with a special class of progenitors (high proliferative potential colony-forming cells) which can form very large colonies in vitro (11). Post-5-FUra bone marrow has a greater capacity to repopulate lethally irradiated mice and is thought to be an enriched source of pluripotential stem cells (10). In 1979, Fauser and Messner (12) described a human multipotential stem cell, the CFU-GEMM, the progeny of which included cells of granulocyte, erythrocyte, monocyte-macrophage, and megakaryocyte lineages. Replating experiments showed that these cells have a limited ability to self-renew (13), thus suggesting that CFU-GEMM may represent a human counterpart of the murine day 9 CFU-S.

We hypothesized that human bone marrow recovering from the effects of chemotherapy with cycle-active agents might also serve as an enriched source of hematopoietic stem cells. Due to insurmountable ethical considerations, the administration of potentially lethal chemotherapeutic agents to normal volunteers cannot be pursued, but these agents are routinely administered to patients with cancer. Although such patients are not normal, their marrows provide unique opportunities to study the effects of chemotherapy on human stem cells and progenitor cells.

MATERIALS AND METHODS

Human Subjects

Following informed consent, bone marrow aspirations were obtained from normal volunteers and from seven patients with ANLL who had recently completed induction chemotherapy and were scheduled for bone marrow reevaluation according to treatment protocols. Patient marrows were sampled at 5 and/or 10 days following completion of the designated chemotherapeutic regimen (Chart 1). In all, four patient marrows were studied on day 5 following chemotherapy and five patients were evaluated 10 days after chemotherapy (Table 1). Three of the patients' marrows were studied on both day 5 and day 10.

³ The abbreviations used are: ANLL, acute nonlymphocytic leukemia; BFU-E, burst-forming unit, erythrocyte; CFU-M, colony-forming unit, megakaryocyte; CFU-GEMM, colony-forming unit, megakaryocyte-macrophage; CFU-GEMM, colony-forming unit, granulocyte-erythrocyte-megakaryocyte-macrophage; CFU-S, colony-forming unit, spleen; 5-FUra, 5-fluorouracil; PHA-LCM, phytohemagglutinin-stimulated leukocyte-conditioned medium; IMDM, Iscove's modified Dulbecco's medium; PBS, phosphate-buffered saline; PGP, platelet glycoprotein; CR, complete remission.
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Assays for Hematopoietic Progenitors

CFU-M-derived Colonies. Bone marrow mononuclear cells were obtained by Ficoll-Paque density centrifugation as described above, with the exception that α-medium minus nucleosides (Gibco Laboratories) was substituted for IMDM. The mononuclear cells thus obtained were suspended in α-medium minus nucleosides containing 2% FBS. Cells were cultured in 35-mm Petri dishes in 1-ml volumes containing 5 × 10⁶ cells. The plasma clot technique of McLeod et al. (14) was modified by the substitution of heat-inactivated human AB serum for FBS, α-medium minus nucleosides for NCTC-109 medium, and Eagle’s minimum essential medium for Hanks’ balanced salt solution. In the final 1-ml aliquot of each culture were the following supplements: nonessential amino acids (0.02 mmol/ml); l-glutamine (0.04 mmol/ml); and sodium pyruvate (0.02 mmol/ml). Varying concentrations (0–20% by volume) of aplastic anemia serum were included as a source of MEG-CSF. Each study was performed in duplicate. Culture dishes were incubated for 12 days at 37°C in a 100% humidified atmosphere of 5% CO₂ in air. Cultures were fixed in situ with methanol:acetone (1:3) for 20 min, washed with 0.01 M PBS (pH 7.2) and then distilled water, and allowed to air-dry. Plasma clots were stored frozen at −20°C until immunofluorescent staining was performed.

Purified human PGP was prepared by lithium diiodosalicylate phenol extraction of pooled human platelet concentrates as described by Marchesi and Chasis (15). New Zealand White rabbits were immunized by s.c. injections of 1 mg PGP in Freund’s complete adjuvant, followed by i.m. injections of 1 mg PGP in Freund’s incomplete adjuvant at 2 and 4 weeks. Serum was harvested at 6 weeks by cardiac puncture and stored in aliquots at −80°C.

Whole rabbit anti-PGP serum was diluted 1:200 with PBS, layered over the fixed plasma clot cultures, and incubated for 60 min at room temperature in 100% humidified air. After three washings with PBS, the specimens were reincubated for 60 min with fluorescein-conjugated goat anti-rabbit IgG (Meloy Laboratories, Inc., Springfield, VA) diluted with PBS (final concentration, 0.36 mg protein/ml). After being washed with PBS, the specimens were counterstained with 0.125% Evan’s blue for 2 min, washed with distilled water, and mounted in isotonic barbitral buffer (pH 8.6) in glycerol (1:3).

In vitro plasma clot cultures were scored in situ. The 35-mm Petri dishes were inverted, the base area was completely scanned, and fluorescein-positive colonies were enumerated at ×100 with a fluorescein microscope (Zeiss standard microscope 18 with IVFL vertical fluorescent illuminator; Carl Zeiss, Inc., NY). A megakaryocyte colony was defined as a cluster of three or more intensely fluorescent cells.

BFU-E, CFU-GM, and CFU-GEMM-derived Colonies. Bone marrow mononuclear cells were obtained as described above for preparation of PHA-LCM and plated in 35-mm Petri dishes each containing 1 ml of a mixture of 30% FBS, 10% PHA-LCM, sheep erythropoietin (2 units/ml; Hyclone), 1% methylcellulose, and 6 × 10⁻⁴ M 2-mercaptoethanol (Eastman Kodak Co., Rochester, NY). Each study was performed in quadruplicate. Plates were incubated for 14 days at 37°C in 5% CO₂ in air with 100% humidity. Scoring was performed on an inverted microscope. Individual colonies composed of both hemoglobinized and nonhemoglobinized cells were classified as CFU-GEMM. Large, hemoglobinized colonies were scored as BFU-E. The remaining, nonhemoglobinized colonies were categorized as CFU-GM. Random colonies were plucked from plates, suspended in IMDM, prepared as cytospins (Cytospin 2; Shandon Southern Instruments, Inc., Sewickly, PA), stained with Wright’s stain, and scanned in order to verify the cell composition of colonies.

Tritiated Thymidine Suicide Technique

Bone marrow mononuclear cells were prepared as described above and suspended at a concentration of 10⁶ cells/ml in IMDM with 10% FBS. Aliquots (1 ml) were pulsed with 50 µCi tritiated thymidine (20.0 Ci/mmol; New England Nuclear, Boston, MA) for 20 min at 37°C and then

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Profile of patients studied

There were five males and two females. Median age was 31 years. Numbers in parentheses represent number of days of treatment received. Day of assay(s) indicates number of days elapsed between completion of chemotherapy and bone marrow sampling. Four patients attained complete remission, two attained partial remission.

Chart 1. Clinical course of a typical patient with ANLL during and following induction chemotherapy. Bone marrow aspirations (arrows, bottom line) were performed at diagnosis (day 3) and following chemotherapy (days 14 and 23). Note that bone marrow samples were obtained for culture prior to recovery from chemotherapy-induced myelosuppression. Ara-C, 1-β-D-arabinofuranosylcytosine.

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>FAB</th>
<th>Treatment</th>
<th>Day of assay(s)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. T.</td>
<td>F</td>
<td>56</td>
<td>AMSA (3) + ara-C (5)</td>
<td>5</td>
<td>CR</td>
<td></td>
</tr>
<tr>
<td>J. N.</td>
<td>F</td>
<td>20</td>
<td>Dauno (3) + ara-C (15)</td>
<td>5 and 10</td>
<td>CR</td>
<td></td>
</tr>
<tr>
<td>B. A.</td>
<td>M</td>
<td>31</td>
<td>Dauno (3) + ara-C (10)</td>
<td>10</td>
<td>CR</td>
<td></td>
</tr>
<tr>
<td>R. F.</td>
<td>M</td>
<td>64</td>
<td>Dauno (3) + ara-C (7)</td>
<td>10</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>D. O.</td>
<td>M</td>
<td>42</td>
<td>Dauno (3) + ara-C (10)</td>
<td>5 and 10</td>
<td>PR</td>
<td></td>
</tr>
<tr>
<td>J. H.</td>
<td>M</td>
<td>31</td>
<td>Dauno (3) + ara-C (10)</td>
<td>5</td>
<td>CR</td>
<td></td>
</tr>
<tr>
<td>C. H.</td>
<td>M</td>
<td>27</td>
<td>AMSA (3) + ara-C (5)</td>
<td>5</td>
<td>PR</td>
<td></td>
</tr>
</tbody>
</table>

* Classification of ANLL by French-American-British (FAB) guidelines.
* AMSA, amsacrine; ara-C, 1-β-D-arabinofuranosyl-cytosine; DAUNO, daunorubin; CR, complete remission; PR, partial remission.

Chemotherapy

The seven patients included in this study met French-American-British criteria for the diagnosis of ANLL (Table 1). Patients were treated with daunorubicin, 50 µg/m² i.v. daily for 3 days, and with continuous infusion of 1-β-D-arabinofuranosylcytosine, 200 µg/m² for 7 days (age, >51 years) or 10 days (age, <50 years). Two patients were treated with 1-β-D-ara-C, 200 mg/m² for 7 days (age, >51 years) or 10 days (age, <50 years). Note that bone marrow samples were obtained for culture prior to recovery from chemotherapy-induced myelosuppression. Ara-C, 1-β-D-arabinofuranosylcytosine.

TREATMENT

Bone marrow aspirate

Table 1. Clinical course of a typical patient with ANLL during and following induction chemotherapy. Bone marrow aspirations (arrows, bottom line) were performed at diagnosis (day 3) and following chemotherapy (days 14 and 23). Note that bone marrow samples were obtained for culture prior to recovery from chemotherapy-induced myelosuppression. Ara-C, 1-β-D-arabinofuranosylcytosine; Dauno, daunorubicin; PR, partial remission.

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<th>Patient</th>
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<td>5</td>
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<td></td>
</tr>
<tr>
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<td>20</td>
<td>Dauno (3) + ara-C (15)</td>
<td>5 and 10</td>
<td>CR</td>
<td></td>
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<td>B. A.</td>
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<td>31</td>
<td>Dauno (3) + ara-C (10)</td>
<td>10</td>
<td>CR</td>
<td></td>
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<td></td>
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<td>M</td>
<td>42</td>
<td>Dauno (3) + ara-C (10)</td>
<td>5 and 10</td>
<td>PR</td>
<td></td>
</tr>
<tr>
<td>J. H.</td>
<td>M</td>
<td>31</td>
<td>Dauno (3) + ara-C (10)</td>
<td>5</td>
<td>CR</td>
<td></td>
</tr>
<tr>
<td>C. H.</td>
<td>M</td>
<td>27</td>
<td>AMSA (3) + ara-C (5)</td>
<td>5</td>
<td>PR</td>
<td></td>
</tr>
</tbody>
</table>

* Classification of ANLL by French-American-British (FAB) guidelines.
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PHA-LCM

Blood from normal donors was diluted 1:1 with IMDM (Gibco Laboratories, Grand Island, NY) containing preservative-free heparin (20 units/ml) and layered over equal volumes of Ficoll-Paque (specific gravity, 1.077 g/ml, Pharmacia Fine Chemicals, Inc., Piscataway, NJ). A mononuclear cell layer was obtained by centrifugation at 500 × g and 4°C, for 25 min in a Beckman model J-6B refrigerated centrifuge (Beckman Instruments, Inc., Fullerton, CA) and washed three times in IMDM. The cells were then resuspended at a concentration of 10⁶ cells/ml in IMDM containing 10% FBS (Hyclone Laboratories, Logan, UT) and 1% phytohemagglutinin (Wellcome Diagnostics, Dartford, England). Aliquots (25 ml) were placed into 125-ml culture flasks and incubated in 5% CO₂ in air at 37°C and 100% humidity for 7 days. Conditioned medium was harvested, filtered (Millipore-HA 0.45-µm filter unit; Millipore Corporation, Bedford, MA), and stored in aliquots at −20°C.
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saturated with ice cold thymidine and washed three times with cold IMDM. Cells were plated, incubated, and scored as described above for methylcellulose cultures. Control cells were incubated with medium alone and with 500 µg cold thymidine. Results were recorded as percentage of control.

RESULTS

Colonies cloned from bone marrow obtained following chemotherapy were frequently macroscopic and composed of thousands of cells of multiple lineages (Fig. 1). Normal bone marrow cells infrequently formed such large colonies.

The formation of CFU-GM-derived colonies by normal marrow

<table>
<thead>
<tr>
<th></th>
<th>Normal control</th>
<th>5 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>152.8 ± 5.4</td>
<td>202.0 ± 9.2</td>
<td>296.2 ± 17.4</td>
<td></td>
</tr>
<tr>
<td>173.0 ± 13.0</td>
<td>208.8 ± 8.6</td>
<td>286.2 ± 11.2</td>
<td></td>
</tr>
<tr>
<td>105.8 ± 12.6</td>
<td>88.5 ± 3.1</td>
<td>203.5 ± 32.3</td>
<td></td>
</tr>
<tr>
<td>104.8 ± 10.8</td>
<td>13.0 ± 1.7</td>
<td>282.5 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>95.7 ± 10.8</td>
<td>112.8 ± 4.4</td>
<td>117.0 ± 12.2</td>
<td></td>
</tr>
<tr>
<td>112.8 ± 4.4</td>
<td>47.8 ± 1.3</td>
<td>236.8 ± 22.8</td>
<td></td>
</tr>
<tr>
<td>47.8 ± 1.3</td>
<td>64.2 ± 4.2</td>
<td>219.5 ± 22.8</td>
<td></td>
</tr>
<tr>
<td>64.2 ± 4.2</td>
<td>54.2 ± 6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65.2 ± 3.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean: 97.7 ± 6.8</td>
<td>100.6 ± 15.8</td>
<td>234.7 ± 13.1</td>
<td></td>
</tr>
<tr>
<td>% of control: 100</td>
<td>103</td>
<td>240</td>
<td></td>
</tr>
</tbody>
</table>

*Average ± SE.

Table 3

Comparison of BFU-E-derived colony formation from normal bone marrow versus marrow obtained from patients following chemotherapy

<table>
<thead>
<tr>
<th></th>
<th>5 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANLL marrow obtained</td>
<td>0 ± 0</td>
<td>10.6 ± 3.4</td>
</tr>
<tr>
<td>following cessation of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>45.5 ± 3.6</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>58.8 ± 1.6</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>46.2 ± 8.7</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>60.5 ± 2.5</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>8.2 ± 2.1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>40.8 ± 4.3</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>35.0 ± 2.1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>35.3 ± 4.9</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>31.2 ± 3.4</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>3.0 ± 3.0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Mean: 37.1 ± 3.1</td>
<td>0 ± 0</td>
<td>10.6 ± 3.4</td>
</tr>
<tr>
<td>% of control: 100</td>
<td>100</td>
<td>29</td>
</tr>
</tbody>
</table>

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Fig. 1. Photomicrograph of colonies on day 14 of culture; parent cells were from marrow obtained postchemotherapy. No magnification used.

Table 2

Comparison of CFU-GM derived colony formation from normal bone marrow with that of marrow obtained from patients following the completion of induction chemotherapy

Values are expressed as the number of colonies per 10^6 cells plated. Each value represents the average ± SE for four replicate experiments using cells obtained from the same individual. Data are portrayed in a similar fashion in subsequent tables.

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The pattern of recovery of BFU-E derived colony formation was somewhat different (Table 3). No BFU-E were detected 5 days after chemotherapy and by 10 days following treatment...
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Table 4
Comparison of CFU-GEMM-derived colony formation from normal bone marrow versus patient marrow obtained following completion of chemotherapy

<table>
<thead>
<tr>
<th>Normal Controls</th>
<th>5 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean: 2.3 ± 0.3</td>
<td>0 ± 0</td>
<td>4.5 ± 1.3</td>
</tr>
<tr>
<td>% of control: 100</td>
<td>0</td>
<td>196</td>
</tr>
</tbody>
</table>

Table 5
Cell cycle analysis of bone marrow cells obtained following chemotherapy

Percentage of progenitor cells in S phase of the cell cycle for normal and postchemotherapy bone marrow as assessed by tritiated thymidine suicide. Numbers represent the average of five experiments for normal marrow and three experiments for patient marrow with the range given in parentheses. Each experiment consisted of four replicate plates using cells obtained from the same individual.

<table>
<thead>
<tr>
<th>CFU-GM</th>
<th>10 days post-chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.3</td>
<td>28.5 (16.0-29.5)</td>
</tr>
<tr>
<td>BFU-E</td>
<td>17.8 (7.4-59.5)</td>
</tr>
<tr>
<td></td>
<td>53.9 (0-88.5)</td>
</tr>
</tbody>
</table>

BFU-E cloning efficiency was only 29% of normal values.

CFU-M were assayed using bone marrow samples obtained 10 days following chemotherapy (Chart 2). In these studies, aplastic anemia serum was used as a source of MEG-CSF. Without an added source of MEG-CSF, the numbers of CFU-M-derived colonies from patients were roughly 10 times those cloned from untreated marrow. The dose-response curves of normal and postchemotherapy bone marrows remain parallel and plateau at concentrations of aplastic anemia serum greater than 20%. With maximal stimulation by MEG-CSF, CFU-M-derived colony formation from patient marrow was nearly twice that of normal marrow.

Table 4 depicts the formation of CFU-GEMM-derived colonies from normal and postchemotherapy bone marrow. Despite the fact that no CFU-GEMM-derived colonies were assayed from marrow obtained 5 days after chemotherapy, they were detected at levels approaching 200% of normal controls from marrow obtained 10 days postchemotherapy.

Tritiated thymidine suicide analysis of normal marrow and marrow obtained from 2 patients 10 days after chemotherapy is shown in Table 5. For the controls, an average of 12.3% CFU-GM and 17.8% BFU-E were found to be in S phase of the cell cycle, while, following chemotherapy, 28.5% of CFU-GM and 53.9% of patient BFU-E were in S phase.

DISCUSSION

Combination chemotherapy for acute leukemia predictably results in severe bone marrow hypoplasia that is eventually followed by reconstitution of normal hematopoiesis in 60–80% of treated patients (16). In this study, we examined hematopoiesis during the early postchemotherapy period of ANLL in order to determine the nature of the stem cells that survive exposure to chemotherapeutic agents. Marrow obtained 5–10 days postchemotherapy contained twice the numbers of CFU-GM and CFU-GEMM as did marrow from normal volunteers not exposed to chemotherapy. In addition, during this postchemotherapy period, there was a significantly greater proportion of progenitor cells in S phase of the cell cycle than was found in normal marrow. The progenitor cells cloned following chemotherapy often formed macroscopic colonies and thus appeared to have a higher proliferative potential than did cells from untreated marrow.

This study is limited by the necessity of utilizing different individuals for the patient and normal control populations. Ideally, the cloning efficiencies of patient marrows following chemotherapy would be compared to those obtained from the same individuals prior to the development of leukemia. This is impossible for obvious reasons. As can be seen from Tables 2–4, there is wide variation in the clonability of cells from different normal control marrows. However, the standard error of the mean for each is small, and the average values correlate well with those obtained by other investigators (17). These findings would lend support to the assumption that our normal controls are a representative group.

Quantitation of progenitor cells during this immediate postchemotherapy period is admittedly difficult because of inherent errors in utilizing samples obtained from hypocellular marrows. In the murine system, this problem is easily avoided by quantitating progenitor cells per limb. This is an alternative that is obviously not feasible in humans. With these reservations in mind, the data presented in this study clearly demonstrate the persistence of many hematological progenitor cells following chemotherapy. These progenitors superficially resemble the highly proliferative cells that survive 5-Flura treatment in mice, in that they form macroscopic colonies.

For cells to survive prolonged exposure to cycle-active agents they must be, on the whole, nondividing cells remaining in G0. In the present study, numerous cells surviving chemotherapy were in S phase of the cell cycle, which suggests that they either have been recruited from the G0 compartment or are the progeny of such quiescent cells. We hypothesize that, following the cessation of chemotherapy, these nondividing stem cells emerge from G0 and undergo rapid differentiation into the committed progenitor cells that were assayed in semisolid culture. These committed progenitor cells could then rapidly proliferate and result in hematological reconstitution. A more complete understanding of this repopulation process might provide information concerning the cellular events necessary for the attainment of CR.

Recently To et al. (18) have reported increased numbers (25 times normal) of CFU-GM among peripheral blood cells obtained by leukapheresis from patients with ANLL in early (2–4 weeks following induction chemotherapy) CR. Their results have been interpreted as a "spillover" of these progenitors into peripheral blood due to intense activity in the bone marrow. They suggest that these progenitors are the result of proliferation of the pluripotential stem cell in response to marrow hypoplasia. Such intense proliferation could also explain our results, as outlined above. Some of the colonies observed when day 5 postchemotherapy marrow is cloned may also contain the primitive stem...
cells themselves. Future studies will include the replating of colonies obtained at this time to help resolve this issue.

The heritage (leukemic or normal) of the colonies grown from postchemotherapy marrow was not addressed in this study. Results reported by Vincent et al. (19) and Eridani et al. (20) would indicate that cloning efficiency of leukemic cells is subnormal. Vincent et al. cultured bone marrow cells from 43 adults with ANLL at first presentation. They describe two abnormal growth patterns, each seen in about one-half of their patients. Type 0 growth represents a failure to grow in culture; type B growth produces few colonies and large numbers of small clusters. Type 0 growth correlates with a favorable outcome. Eridani’s group performed clonogenic assays for CFU-GM and CFU-E using marrow from 27 patients with either acute lymphocytic leukemia or acute myelocytic leukemia. Marrows from all patients were evaluated at presentation and 15 patients were reevaluated during CR. Diminished cloning efficiency was found using marrow cells from patients at presentation, but increased cloning efficiency (approximately 150% normal colony formation) was noted during CR. However, no assays were performed soon after the completion of treatment and before CR, as in this study. The earliest evaluation was performed 1 month following CR, and at that time marrow cells appear to have significantly greater cloning efficiencies compared to those studied later in remission (10–80 months). The increased cloning efficiency following chemotherapy noted in the current paper is distinctly different from growth patterns described for untreated leukemic marrow cells and suggests that our colonies are not the progeny of leukemic progenitor cells. Utilization of cytogenetic and/or biochemical markers to identify the leukemic clones is planned in the future and could provide a definitive solution to this question. The increased cloning efficiency observed in Eridani’s (20) patients following remission is similar in degree to that noted in the current paper.

Wide variation was noted in the ability to clone marrow cells from various patients following chemotherapy. This observation suggests the possibility that cloning efficiency could be predictive of treatment outcome. Brownman et al. (21) attempted to establish the value of the clonogenic assay as a predictor of remission in patients with ANLL. All patients were studied either at first presentation or in relapse, prior to administration of chemotherapy; no patients were studied following treatment. This work has shown that poor in vitro growth of leukemic cells prior to treatment correlates with favorable treatment outcome and supports the earlier findings of Vincent et al. (19). The present work suggests that cloning efficiency following treatment may predict outcome, but we cannot establish any correlation between the two from our current data. Additionally no relationship can be found between cloning efficiency of an individual patient’s marrow cells and his/her pattern of myelosuppression following chemotherapy. Specifically no pattern is seen relating clonability of any of the three progenitor cell populations studied (CFU-GM, BFU-E, CFU-GEMM) with time to recovery of WBC, time to nadir WBC, or degree of the nadir. However, only seven individuals have been studied to date. A pattern may emerge as additional patients are added to the study.

The results presented in this paper suggest the presence of a stem cell that is relatively unaffected by chemotherapeutic agents and possesses a great capacity to rapidly regenerate hematopoietic progenitor cells. The period following the completion of induction chemotherapy for ANLL appears to be analogous to the murine 5-FUra model, which has been useful in the study of primitive hematopoietic stem cells.

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In Vitro Hematopoiesis following Induction Chemotherapy for Acute Leukemia

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