A Comparison of Granulopoiesis in Culture From Blood and Marrow Cells of Nonleukemic Individuals and Patients With Acute Leukemia


The objective of this study was to compare the concentration of committed granulopoietic progenitor cells (CFU-C) in marrow and blood. For individuals without leukemia, a highly significant correlation was observed between the concentration of CFU-C obtained from the two sites. However, CFU-C in blood had a slower sedimentation velocity than that reported for marrow and were found not to be in the DNA synthetic phase of the cell cycle using the tritiated thymidine suicide technique. In patients with acute leukemia, no correlation was observed between concentrations of CFU-C in marrow and peripheral blood, regardless of whether the patients were newly diagnosed, in remission, or in relapse. We concluded that studies of the peripheral blood do not yield the same information in respect to granulopoietic progenitor cells as do studies of the marrow.

EXTENSIVE REPORTS have appeared on the colony-forming capacity of cells from the marrow of normal individuals and patients with leukemia. While data from different laboratories has shown some variation, there is general agreement that colony formation by marrow cells is abnormal in patients with preleukemia,1,2 newly diagnosed leukemia, or recurrent leukemia,3-5 while remission is usually associated with findings similar to those obtained from normal marrow.5,6 Less information is available for colony formation by cells obtained from the peripheral blood;7,8 yet this source is more readily available than marrow and could be monitored more frequently and with less discomfort to the patient. Use of peripheral blood might also avoid problems in expressing the colony-forming efficiency of marrow where such problems arise from varying contamination of marrow specimens with blood. Recently, we reported a method of overcoming the major disadvantage of blood as a source of granulopoietic progenitors, that is, their scarcity; in this technique, all the cells are enriched by a Ficoll-Hypaque density gradient procedure that yields reproducible results, not only in the same sample of blood, but also as a function of time in individual normal donors.9 The purpose of the present study was to determine whether or not studies of the peripheral blood would yield information equivalent to that obtained from marrow.

We found a close correlation between colony formation by cells from blood and marrow from nonleukemic individuals, although differences in cycle state were detected. In contrast, in patients with acute leukemia, colony formation by...
peripheral blood cells was not correlated with the marrow results. This lack of correlation persisted in remission. We concluded that, in spite of its convenience for repeated sampling, peripheral blood could not substitute for marrow in studies of granulopoiesis in acute leukemia.

MATERIALS AND METHODS

Patients

Peripheral blood and/or marrow was obtained from 21 nonleukemic individuals; of these, two were normal transplant donors, and the others were patients undergoing hematologic investigation. The latter group consisted largely of patients with various forms of anemia, infection, or nonhematologic malignant disease. This patient population was similar to that used as nonleukemic controls in other studies from this laboratory. Blood and marrow were obtained from six patients with persistent acquired neutropenia of unknown origin. Samples were obtained from 17 patients with acute myeloblastic leukemia (AML), 1 patient with blast crisis in chronic myelogenous leukemia, and 9 patients with acute lymphoblastic leukemia (ALL). Of these, seven patients with AML were assessed prior to treatment. Multiple assessments were carried out on these and the other patients during the course of treatment. The criteria for classification of the leukemic patients were those described by Hasselback et al., and patients were considered to be in remission when their marrow contained less than 5% blast cells in association with normal peripheral granulocyte and platelet numbers.

Chemotherapy for AML consisted of induction with a combination of cyclophosphamide, arabinosyl cytosine, and vincristine, as previously described. A combination of BCNU and cyclophosphamide was used in maintenance. The treatment of patients with ALL was less uniform but usually employed vincristine-prednisone for remission induction and methotrexate for consolidation and maintenance (for details see ref. 5).

Collection of Cells

Between 20 and 60 ml of venous blood were collected using heparin as an anticoagulant. Marrow was aspirated from sternum or posterior iliac spines in heparinized syringes.

Assay for Granulopoietic Colony Formation

The assay for granulopoietic colony formation by peripheral blood cells was that described by Rubin et al., in which a modified Ficoll-Hypaque separation technique was used to obtain a reproducible and representative enriched sample of mononuclear cells. Cells from this sample were plated at concentrations from 2 x 10⁵ to 10⁶ cells/plate in 0.8% methylcellulose, 20% fetal calf serum, 20% α medium, and unless otherwise specified, with 20% media conditioned by normal peripheral leukocytes (LCM) as a source of colony-stimulating activity (CSA). For marrow, buffy coat cells were obtained and cultured as described by Iscove et al. For both sources, the cultures were incubated from 10 to 14 days at 37°C in 7.5% CO₂ with air. Granulocytic colonies containing in excess of 20 cells were scored using an inverted microscope. The counting convention was that described by Messner et al. This convention is standard in our laboratory. The results were expressed as colony-forming units responsive to CSA (CFU-C) per 10⁸ nucleated cells from peripheral blood and 10⁷ nucleated cells from marrow.

Preparation of Adherent, Nonadherent, and Nonphagocytic Populations

Ficoll-Hypaque-separated peripheral blood cells were further separated into adherent and nonadherent populations by the method of Messner et al. Periperal leukocytes obtained by the Ficoll-Hypaque separation procedure were washed three times and then allowed to adhere for 30 min to glass petri dishes in α medium with 20% fetal calf serum at 37°C. The supernatant was decanted and the adherence procedure repeated for a further 2 hr to provide nonadherent populations. Adherent cells were obtained by washing the original petri dishes twice and then removing remaining adherent cells by scraping the dishes gently with a rubber policeman.
Nonphagocytic populations were obtained by placing the Ficoll-Hypaque-separated cells from peripheral blood in roller tubes with 40 mg of carbonyl iron powder (Domtar, Montreal, P.Q., Canada) and incubating the mixture in a water bath at 37°C. The mixture was agitated frequently and after 30 min the iron powder and iron-laden cells were attracted to the bottom of roller tubes by the use of a magnet. Supernatant was poured off and this step was repeated as often as necessary to remove all the iron powder and iron-laden phagocytic cells. The remaining cells were considered nonphagocytic (NP cells).

**Cell Separation by Velocity Sedimentation**

Cell suspensions were separated by velocity sedimentation at unit gravity using the method described by Miller and Phillips. This technique separates cells principally on the basis of cell size.

**Destination of the Cells in DNA Synthesis by Tritiated Thymidine Killing**

The method of Becker et al., as modified by Iscove et al., was employed to measure the proportion of peripheral CFU-C in the S phase of the cell cycle. The method depends upon exposing cell suspensions briefly to high specific activity ³HTdR and measuring the loss of colony-forming ability. The principle is that cells in DNA synthesis incorporate the radioactive precursor, and their proliferative potential is destroyed by it. The procedure is controlled by demonstrating that the addition of cold thymidine to the incubation mixture prevents the lethal effects of the ³HTdR.

**RESULTS**

**Interacting Populations Affecting Granulopoiesis in Culture of Cells From the Peripheral Blood**

Cell populations in nonleukemic peripheral blood responsible for granulopoiesis in culture resemble those in marrow; in both cases, granulopoietic progenitors coexist with cells that produce colony stimulating activity (CSA). Thus, addition of CSA to peripheral blood cells separated only by Ficoll-Hypaque has little effect on colony formation. A typical experiment is shown in Fig. 1, which depicts a linear relationship between nucleated cells plated and colonies observed in the presence and absence of added CSA. Similar results were obtained in 15 other experiments; these yielded a mean of 60 ± 23 colonies/10⁶ cells in the absence of CSA and 63 ± 25 colonies/10⁶ cells in the presence of CSA. Depletion of leukocyte suspensions of either adherent or phagocytic, or both adherent and phagocytic cells reduced colony formation.

![Fig. 1. Linear relationship between colony formation and number of Ficoll-Hypaque-separated leukocytes plated with added CSA (○) and without CSA (△).](image-url)
the addition of either CSA or adherent cells restored colony-forming capacity (Table 1).

Thus, in Ficoll-Hypaque-separated peripheral leukocytes granulopoietic progenitors interact with CSA-producing cells to yield colonies in culture. Separation of the nonadherent, nonphagocytic granulopoietic progenitors from the adherent, phagocytic CSA-producing cells abolishes colony formation. It is restored by reconstituting the cultures either with leukocyte conditioned media containing CSA, or with CSA-producing cells. This cell-cell interaction underlying granulopoiesis in cultures of peripheral blood cells is similar to that previously described for marrow cells.10

Comparison of Granulopoietic Colony Formation by Marrow Cells and Peripheral Blood Cells

Both marrow and peripheral blood samples were obtained from 21 non-leukemic individuals consisting of 15 patients or transplant donors without hematologic disorders and 6 patients with idiopathic neutropenia. In 12 of the 15 nonleukemic individuals, marrow and peripheral blood were obtained on the same day. The data are plotted in Fig. 2 as CFU-C/10^6 nucleated blood cells cultured only after the enrichment procedure described by Rubin et al.9 (see Materials and Methods) against CFU-C/10^6 marrow cells. Values from the two sources were correlated, regardless of whether or not the neutropenic patient data were included (correlation coefficient = 0.84, p < 0.001, excluding neutropenic patients; correlation coefficient = 0.77, p < 0.001, including neutropenic patients).
GRANULOPOIESIS IN ACUTE LEUKEMIA

Fig. 2. A comparison of colony-forming capacity of marrow and peripheral blood for 15 individuals without leukemia (e) and 6 idiopathic neutropenic patients (o). Their values are correlated significantly (p < 0.001).

Velocity Sedimentation of Cells From Peripheral Blood

The sedimentation velocities of populations in the peripheral blood were determined using the Stalup technique of Miller and Phillips. A representative experiment is depicted in Fig. 3: Fig. 3A shows the nucleated cell profile, showing two peaks; the majority of lymphocytes have a peak sedimentation velocity of approximately 3 mm/hr, while the larger granulocytes and monocytes have sedimentation velocities in excess of 6 mm/hr. These values are similar to those reported for cells obtained from marrow. Figure 3B shows the culture findings. Granulopoietic progenitors have a peak sedimentation velocity at 4 mm/hr; this is in contrast to the values between 5 and 6 mm/hr reported previously for marrow progenitors. Data similar to those of Fig. 3 were obtained from the peripheral blood samples of six donors.

The Proliferative State of Granulopoietic Progenitors in the Peripheral Blood

High specific \(^3\)HTdR was used to determine the percentage of peripheral granulopoietic progenitor cells in the DNA synthetic phase (S phase) of the cell cycle. The data are given in Table 2. Three patients (7, 8, and 9) also provided marrow cells on the same day. The data show that few if any granulopoietic progenitors incorporated sufficient radioisotope to destroy their colony-forming capacity. In contrast, the marrow specimens from patients 7, 8, and 9 showed greater reductions, similar to those reported previously, indicating a significant number of cells in the S phase.

Comparison of Granulopoietic Progenitors in Peripheral Blood and Marrow of Patients With Acute Leukemia

Fifty-one samples of marrow and peripheral blood were obtained on the same day from 17 patients with acute myeloblastic leukemia and 1 patient in blast crisis of chronic myelogenous leukemia. Of these, 27 samples were obtained during hematologic remission and 23 before treatment or in relapse. A comparison of the colony-forming efficiency of the marrow and peripheral blood samples is presented in Fig. 4. Neither in remission (Fig. 4A) nor in relapse (Fig. 4B) was a significant correlation observed (p > 0.1).
Fig. 3. Results of a typical sedimentation velocity separation of cells from nonleukemic peripheral blood. (A) Nucleated cell profile. (B) CFU-C profile.

Table 2. Effect of $^3$HTdR on the Colony-forming Ability of Human Peripheral Blood CFU-C

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Source of PB</th>
<th>Colonies/10⁶ PB Cells</th>
<th>Percentage Survival of Colony-forming Ability</th>
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<tr>
<td></td>
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<td>10 μCi/ml</td>
<td>200 μCi/ml</td>
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<td>1</td>
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<tr>
<td>9</td>
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Mean ± 1 SD (95% confidence limits)

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<th></th>
<th>Coloniess/10⁶ PB Cells</th>
<th>Percentage Survival of Colony-forming Ability</th>
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<tr>
<td></td>
<td>97 ± 45</td>
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*The per cent survival of colony-forming ability of bone marrow specimens obtained and assayed at the same time as the peripheral blood are 89%, 61%, and 76% for experiments 7, 8, and 9, respectively.
Similar data were obtained from nine patients with acute lymphoblastic leukemia. Figure 5A depicts a comparison between colony-forming efficiency in marrow and blood for 16 samples from four patients with ALL in remission, while Fig. 5B compares 11 samples from six patients in relapse. In neither instance was a significant correlation observed ($p < 0.5 > 0.1$ for patients in remission and $p > 0.1$ for patients in relapse).

CFU-C in the peripheral blood were assessed in seven patients with AML prior to treatment. Of these, five had achieved a complete remission. However, the initial values in these patients varied from very low levels of colony formation to confluent growth. The numbers were too small for a conclusion, but the
wide spread of values observed in patients who entered remission made it unlikely that a correlation would be obtained between initial colony-forming capacity by cells in the peripheral blood and treatment outcome.

**DISCUSSION**

The purpose of the present study was to compare granulopoietic progenitor cells from marrow and peripheral blood in order to determine whether or not the latter yielded information similar to the former. For patients without leukemia, a highly significant correlation was observed between the granulopoietic colony-forming capacity of marrow cells and peripheral leukocytes selected on the basis of their density in gradients of Ficoll-Hypaque. Since a venous sample is usually considered representative of peripheral blood, the correlation is consistent with the view that a random sample is representative of the granulopoietic capacity of the total marrow, at least to the extent that this capacity is displayed in cell culture. It would appear that contamination with blood is not a significant source of error in measuring marrow CFU-C in culture.

Granulopoietic progenitors in the blood have a slower sedimentation velocity than that reported for similar cells in marrow, indicating that progenitors in the blood may be smaller than those in marrow. The observation that few, if any, peripheral blood granulopoietic progenitors are in the DNA synthesis phase of the cycle is consistent with this observation, since resting cells may be smaller than those that have doubled their DNA content in preparation for division.

In striking contrast to the findings in nonleukemic individuals, granulopoietic progenitors from blood and marrow were not correlated in patients with acute leukemia, either myeloblastic or lymphoblastic. Nor did remission induction lead to the reestablishment of the correlation observed in samples from nonleukemic individuals. The explanation for this lack of correlation is unknown. It may be a consequence of the heterogeneity in leukemic marrow, with the result that a single sample is not representative. Many of the patients studied were receiving chemotherapy, either for remission induction or maintenance, and drug treatment may have affected the partition of cells between marrow and peripheral blood. A more interesting possibility is that the leukemic phenotype includes lesions in the mechanisms affecting the orderly release of cells from marrow to blood, and that this abnormality persists in remission. If any of the above explanations of the observations is correct, a complete cure of leukemia should be associated with a return to the correlation between granulopoietic progenitors in peripheral blood and marrow observed in nonleukemic individuals.

We have concluded that examination of granulopoietic progenitors in the more readily available peripheral blood does not provide the same information as that obtained from aspirations of the marrow. In nonleukemic individuals, the difference is *qualitative*, while in leukemia, the difference is *quantitative*. Whether or not assays of granulopoietic progenitors from the peripheral blood are useful in the diagnosis or monitoring of hematologic diseases requires further investigation.

**ACKNOWLEDGMENT**

The authors are grateful to Mr. Nazir Jamal for excellent technical assistance.
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