Peripheral Blood Blast Cell Progenitors in Human Preleukemia

By J. S. Senn, H. A. Messner, P. H. Pinkerton, L. Chang, B. Nitsch, E. A. McCulloch, and the Toronto Area Preleukemia Study Group

Progenitors of blast cell colonies have been identified in acute leukemia. The peripheral blood of 18 of 25 patients with preleukemic states yielded low numbers of blast cell colonies, and the colony-forming cells were in an active proliferative state when assessed using short-term exposure to tritiated thymidine. The clinical significance of blast cell colonies is uncertain, but we suggest that further analysis of this cultural abnormality may lead to a better understanding of mechanisms and management in preleukemia.

A number of hematologic disorders are designated preleukemic states since a high proportion of patients with these conditions will ultimately develop acute leukemia. Preleukemia could serve as a model to study the early stages and development of leukemia if an assay could be developed to determine the emergence of leukemic clones. A colony technique for measuring blast progenitor cells in acute leukemia has been described. The cells within the colonies have the morphological and cytogenetic properties of leukemic blast cells, and they proliferate actively when examined by short-term exposure to thymidine. In acute leukemia, correlation was observed between the efficiency of blast cell colony formation and the concentration of blast cells on the examined peripheral blood specimen; however, the plating efficiency varied from 1:100 to 1:10,000.

We report that colonies composed of blast cells can be identified in the peripheral blood of some preleukemic patients and that the colonies have high proliferative activity.

MATERIALS AND METHODS

Patients

Twenty-five adult patients with preleukemic states were examined on one or more occasions during the course of their illness (Table 1). The 14 male and 11 female patients ranged in age from 18 to 91 yr (median 67 yr). Diagnoses were idiopathic acquired sideroblastic anemia (IASA) (7 patients), preleukemia syndrome (PLS) (15 patients), and refractory anemia with excess myeloblasts (RAEM) (3 patients). PLS is defined by the presence of a cellular bone marrow with morphological abnormalities in two or more hematopoietic cell lines, and frequently associated with lowering of some peripheral blood counts. PLS is not characterized by a large spleen or other definable cause. Patients with IASA or PLS had no more than 6% blasts in the bone marrow, and patients with RAEM had no more than 15% marrow blast cells. Peripheral blood and bone marrow parameters shown in Table 1 were obtained at the time of initial evaluation for each patient. Five patients developed acute leukemia between 8 and 19 mo after diagnosis; the status of all the patients is indicated in Table 1.

Collection and Preparation of Peripheral Blood Cells

Blood cells were drawn in 50-ml syringes containing 1000 U of preservative-free heparin after verbal informed consent was obtained from each patient. Mononuclear cells of density less than 1.077 g/ml were prepared by centrifugation in LSM (Litton, Bionetics, Md.). The crude supernatant is referred to as PHA-LCM. The cell preparation was assessed for granulocyte (CFU-C), erythroid (BFU-E), pluripotent (CFU-GEMM), and T-cell colony-forming cells. Subsequently, the remaining cells were depleted of E-rosette-forming cells as previously described and plated for blast cell colonies. After two Ficoll-Hypaque separations, the residual cells prior to culture regularly contained less than 3% E-rosette-forming cells. In this assay, colonies of blast cell morphology contained no E-rosette-positive cells.

Preparation of Leukocyte Conditioned Medium (PHA-LCM)

Peripheral leukocytes from normal individuals and one patient with hemochromatosis were incubated for 7 days with alpha medium, 10% fetal calf serum, and 1% PHA (Burroughs-Wellcome, Research Triangle Park, N.C.). The crude supernatant is referred to as PHA-LCM.

Colony Formation by Granulocytic (CFU-C), Erythroid (BFU-E), and Pluripotent (CFU-GEMM) Progenitors

A quantity of 2 x 10⁴ mononuclear cells from peripheral blood specimens of preleukemic patients were cultured with alpha medium, 30% fetal calf serum, 5% PHA-LCM, and methylcellulose at a final concentration of 0.9%. After 4 days of incubation at 37°C in moist atmosphere containing 5% CO₂, 1 U of erythropoietin in a volume of 0.1 ml was added by micropipette. The culture plates were evaluated after 12–14 days for granulocytic colonies, erythroid bursts, and mixed hematopoietic colonies using an inverted microscope.
Assay for T-Cell Progenitors

Culture Assay for T-Cell Progenitors

T-lymphocyte progenitors were grown with 30% fetal calf serum, 0.5% PHA, 5 x 10^-2 M 2-mercaptoethanol, and 0.9% methylcellulose. Mononuclear cells (2 x 10^6) were cultured routinely under these conditions. The culture plates were scored for T-cell colonies after 5-7 days of incubation in humidified atmosphere of 5% CO_2 at 37°C. The proliferative state of the blast colony-forming cells was assessed using the method previously described. E-rosette-depleted mononuclear cells were exposed for 20 min to tritiated thymidine, prior to plating.

Culture Assay for Blast Cell Progenitors (BCP)

Blast cell colonies were obtained in cultures of peripheral blood cells from 18 of 25 patients with preleukemic states; repeated assessments were performed on blood samples from 9 patients at different times during their clinical course. In general, a small number of blast cell colonies was observed. Blast cell colonies were identified by E-rosette formation with sheep red blood cells; all cells within the colonies were E-rosette positive.

Cycle State Analysis

The proliferative state of the blast colony-forming cells was assessed using the method previously described. E-rosette-depleted mononuclear cells were exposed for 20 min to tritiated thymidine, prior to plating.

RESULTS

Blast Cell Progenitor Assay (Table 2)

Blast cell colonies were obtained in cultures of peripheral blood cells from 18 of 25 patients with preleukemic states; repeated assessments were performed on blood samples from 9 patients at different times during their clinical course. In general, a small number of blast cell colonies was observed. Blast cell colonies were identified in subsequent studies on 4 patients who initially showed no evidence on these progenitors. Variation in colony number from patient to patient, or from sample to sample for individual patients, was not correlated with altered clinical or hematologic status.

Colony Assays for CFU-C, BFU-E, and CFU-GEMM (Table 2)

Granulopoietic colonies, erythroid bursts, and mixed hemopoietic colonies were usually observed in small numbers.

T-Cell Colony Formation (Table 2)

Precursors of T-cell colonies, identified by E-rosette formation with sheep red blood cells, were present in variable, but usually small numbers in the peripheral

Table 1. Preleukemia States—Patient Data and Morphology

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<th>Diagnosis</th>
<th>Patient No.</th>
<th>Hb (g/dl)</th>
<th>WBC (x 10^3/dl)</th>
<th>Percent Neutrophils</th>
<th>Platelet count (x 10^5/dl)</th>
<th>Percent Myeloblasts</th>
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* Marrows were hypercellular (20) or normocellular (5).
† A, alive; D, dead (without overt leukemia); AML, acute myeloid leukemia; number, months from diagnosis.
added. plates. number appears, assays were done at different times. observed. The fourth experiment performed on a patient with very low blast cell colony formation was assessed using state of blast cell colony-forming cells was a reduction of 36%, 43%, and 62% in the plating efficiency of blast cell colony-forming cells was obtained from three patients with preleukemia were also in active cell cycle. This observation points to a similarity between the blast cell precursors identified in preleukemia and overt acute leukemia.

DISCUSSION

Colonies of blast cell morphology were obtained from the blood of 18 of 25 patients with preleukemia. In general, the frequency with which these colonies could be observed was considerably lower than that frequently observed in acute myeloid leukemia. Two of the 5 patients who developed overt acute leukemia were studied again in the acute phase. Neither grew any blast cell colonies during the preleukemic phase and both showed growth of blast cell colonies at the time of overt disease. This limited observation does not permit conclusions about the possibility of using the blast colony assay in a predictive way to identify progression of the clinical status of preleukemic patients toward leukemia. However, the presence of blast cell colonies in significant numbers of preleukemic patients probably has biologic significance, since these colonies were not found in normal persons.

In acute myeloid leukemia, blast cell progenitors are actively proliferating, and blast cell progenitors obtained from three patients with preleukemia were also in active cell cycle. This observation points to a similarity between the blast cell precursors identified in preleukemia and overt acute leukemia.
In general, previous studies have indicated that granulopoietic progenitor cells are present in small but variable numbers in marrow specimens from patients with acute leukemia and preleukemia;\textsuperscript{11-14} thus, the finding of small but variable numbers of peripheral blood CFU-C is not surprising. It is of interest that other myeloid (BFU-E and mixed cell colonies) and lymphoid (T-cell colonies) progenitors are also present in low numbers.

Further follow-up of the remaining patients will be required to determine the significance of blast cell colonies in their peripheral blood. Evaluation of the different colony elements during the course of preleukemia and at the time of transformation to overt leukemia state is required. We suggest that blast cell colonies may be a marker for the leukemic state in preleukemia and that they may yield prognostic information as well as contributing to basic understanding of the biologic mechanism associated with disease progression to overt leukemia.

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**REFERENCES**

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