Colony Growth of Peripheral Blood Cells from Patients with Acute Lymphocytic Leukemia

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SUMMARY

The colony-forming potential of peripheral blood cells from ten patients with acute lymphocytic leukemia, as well as their urinary colony-stimulating activity levels, has been studied. Peripheral white blood cells from 4 of 10 patients with acute lymphocytic leukemia formed significant numbers of colonies when grown on feeder layers of normal human white blood cells. The colonies were composed of peroxidase-positive cells with lobulated nuclei. Urinary colony-stimulating activity levels were variable and appeared to be related to the total absolute granulocyte count in both treated and untreated patients.

INTRODUCTION

With the use of human peripheral white blood cell feeder layers or conditioned media prepared from human spleen or white blood cells, it has been shown that peripheral blood and bone marrow cells from patients with acute granulocytic leukemia can be stimulated to divide and form colonies in vitro in semisolid culture systems. The colonies formed arise from single cells which, on the basis of recent evidence, appear to represent a leukemic cellular population and not a remaining normal cell line. The colonies formed are generally smaller than those grown from normal human bone marrow or peripheral blood cells, but they appear to go through a process of morphological maturation that is similar to that of mature granulocytes. The colony growth of peripheral blood cells and bone marrow cells from patients with acute lymphocytic leukemia has been studied. While some growth has been observed, the activity has been correlated with the ability of the patient's peripheral blood cells to form colonies in the systems used.

MATERIALS AND METHODS

All of the patients studied were seen (between September 1971 and January 1972) at the University of Colorado Medical Center and at Denver Children's Hospital, Denver, Colo. All of the cases were newly diagnosed or were in clear-cut relapse when the studies were done. No chemotherapy had been given during the 24 hr preceding the studies.

The culture method utilized here has been described previously in detail. Feeder layers of peripheral white blood cells were prepared as follows. Heparinized peripheral blood collected from normal individuals was allowed to sediment by gravity at room temperature for 1 to 2 hr. The plasma containing the white blood cells was removed and mixed with a 9:1 mixture of McCoy's 5A medium and 5% agar in a concentration of 1 X 10⁶ cells/ml. One-mi aliquots were then plated into 35-mm Falcon Petri dishes, and the plates were stored at 37° for up to 14 days prior to use.

Peripheral white blood cells from patients were collected and prepared in the same way. Once the cells had been collected in plasma, one-half of the sample was removed, and the cells were centrifuged out and washed 3 times with McCoy's 5A medium. Washed and unwashed cells were then plated on feeder layers in concentrations of 12,500, 25,000, 50,000, 100,000, and 200,000 cells/plate in 0.3% agar in McCoy's 5A medium as previously described. Plates were incubated at 37° in a fully humidified incubator with a constant flow of CO₂ in air. Colony counts were done at Day 18 to 20 of incubation.

For microscopic studies, colonies were removed from the agar with a finely drawn Pasteur pipet and were either stained with aceto-orcein or spread on glass slides and stained with Giemsa.

Dialyzed, sedimented, sterile urine specimens, prepared as previously described, were tested for colony-stimulating activity with C57BL mouse bone marrow cells. Plates were incubated for 7 days at 37°, and colony counts were done as an index of the levels of colony-stimulating activity present.

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Peripheral blood cells from 8 of 10 patients with acute lymphocytic leukemia grew 3 or more colonies (range, 3.3 to 122) when plated at 200,000 cells. This compares with the 0 to 2 colony-forming cells that can be detected in normal human peripheral blood (8). These data, as well as the clinical parameters and differential counts, are shown in Table 1. Colony growth was observed most frequently in the younger age groups, in which patients had high peripheral blast cell counts. Only 1 adult patient with acute lymphocytic leukemia, M. M., had peripheral blood cells that gave rise to a large number of colonies.

The size of the colonies formed from the peripheral blood of these patients was intermediate, between that previously observed in peripheral blood cells of patients with acute granulocytic leukemia and that seen in normal human bone marrow. The average colony size at Days 18 to 20 of incubation was 400 cells. The low-power microscopic appearance of a typical colony is shown in Fig. 1A. The microscopic appearance of colony cells at Day 18 of incubation also is shown (Fig. 1B). The majority of colony cells at that time had slightly lobulated or segmented nuclei similar to those cells seen in normal human bone marrow cultures at earlier times of growth and incubation (12, 14). Few cells had the appearance of multilobulated mature granulocytes. By Day 18 of incubation, however, acute lymphocytic leukemia colony cells contained peroxidase-staining cytoplasmic granules (Fig. 1C). Occasional cells and colonies containing eosinophilic granules were observed. No colonies thought to be lymphoblasts were seen in these studies.

Table 1
Clinical data, number of colonies grown from the peripheral blood, and urinary colony-stimulating activity of patients with acute lymphocytic leukemia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years) and sex</th>
<th>Total WBC</th>
<th>Differential</th>
<th>No. of colonies/200,000 peripheral WBC</th>
<th>Urinary CSA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. H.</td>
<td>23 M</td>
<td>16,500</td>
<td>90 5</td>
<td>5</td>
<td>5.0</td>
</tr>
<tr>
<td>R. M.</td>
<td>6 F</td>
<td>345,000</td>
<td>95 5</td>
<td>32 1</td>
<td>13.5</td>
</tr>
<tr>
<td>A. S.</td>
<td>53 M</td>
<td>8,900</td>
<td>61 5</td>
<td>32 1</td>
<td>6.0</td>
</tr>
<tr>
<td>D. P.</td>
<td>11 M</td>
<td>254,000</td>
<td>83 5</td>
<td>9 3</td>
<td>122.0</td>
</tr>
<tr>
<td>R. K.</td>
<td>24 M</td>
<td>48,300</td>
<td>71 10 segmen-</td>
<td>13</td>
<td>3.3</td>
</tr>
<tr>
<td>C. J.</td>
<td>7 M</td>
<td>3,900</td>
<td>12 78</td>
<td>5</td>
<td>5 eosino-phil</td>
</tr>
<tr>
<td>A. P.</td>
<td>4 M</td>
<td>4,700</td>
<td>11 60</td>
<td>26 1</td>
<td>2 eosino-phil</td>
</tr>
<tr>
<td>M. M.</td>
<td>30 F</td>
<td>72,000</td>
<td>24 50</td>
<td>26</td>
<td>23.4</td>
</tr>
<tr>
<td>G. R.</td>
<td>14 M</td>
<td>1,300</td>
<td>36</td>
<td>64</td>
<td>3.6</td>
</tr>
<tr>
<td>S. R.</td>
<td>14 F</td>
<td>3,700</td>
<td>86 1</td>
<td>13</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*The abbreviation used is: CSA, colony-stimulating activity (mean number of colonies stimulated by 0.15 ml urine, with 75,000 bone marrow cells).
Chart 1. Urinary colony-stimulating activity levels and total peripheral white blood cell and absolute granulocyte counts in Patient R. M., who had acute lymphocytic leukemia. Therapy was instituted after the initial values (Day 1) were obtained and it resulted in marked hyperuricemia and 3 days of anuria, resulting in the lack of data during this period. Note the rise in urinary colony-stimulating activity during the period of increasing granulocyte count in the peripheral blood.

Further studies are now under way in an attempt to delineate further the colony-forming cell in peripheral blood of these patients and to determine whether differences in colony-forming potential and urinary colony-stimulating activity can be used as indicators of differential diagnosis, therapeutic responsiveness, and prognosis.

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REFERENCES

Colony Growth in Acute Lymphocytic Leukemia

Fig. 1. A, low-power microscopic appearance of a colony grown from the peripheral blood of Patient M. M., with acute lymphocytic leukemia, after 18 days of incubation. B, microscopic appearance of colony cells grown from the peripheral blood of the same patient, at Day 18 of incubation. Giemsa, x 800. C, peroxidase stain of colony cells from the same cultures, at Day 18 of incubation, showing cytoplasmic peroxidase-positive granules.
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