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 (2, 5, 7, 11, 14). 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 (3, 9). 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 from patients with acute lymphocytic leukemia has been studied (1, 13). While some growth has been observed, the number and size of the colonies were definitely smaller than those from the cells of patients with acute granulocytic leukemia.

The following studies were undertaken to determine the colony-forming potential of peripheral blood cells from patients with acute lymphocytic leukemia when those cells were grown over feeder layers of normal human peripheral white blood cells. Ten patients with acute lymphocytic leukemia have been studied; the peripheral blood cells from 8 of these patients formed colonies that appeared to be granulocytic. The serum and urinary colony-stimulating activity from these patients also has been studied, and this 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 (12, 14). 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 10^6 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 CO_2 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 (15). Plates were incubated for 7 days at 37°, and colony counts then were done as an index of the levels of colony-stimulating activity present.
RESULTS

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.

The levels of urinary colony-stimulating activity in 4 patients (Table 1) were variable and did not appear to correlate with colony growth of peripheral blood cells, total white blood cell counts, or clinical course. There did, however, appear to be a correlation between absolute numbers of mature granulocytes in the peripheral blood and urinary colony-stimulating activity. The highest levels of urinary colony-stimulating activity were found in patients with the highest granulocyte counts. This correlation was also noted in patients undergoing treatment (Chart 1). Despite a very high initial white blood cell count, urinary colony-stimulating activity and peripheral granulocyte counts were very low in the patient shown. With treatment, both values rose with a concomitant fall in total white blood cell count.

DISCUSSION

These studies have indicated that peripheral white blood cells from some relapsed acute lymphocytic leukemia patients can be stimulated to divide and form colonies in vitro in semisolid culture systems. The nature of the colony-forming cell and its origin and lineage have yet to be determined. The finding that the colonies formed appeared to be largely granulocytic suggests that the colony-forming cell is not of the leukemic population. This would indicate that, in some patients with acute lymphocytic leukemia, large numbers of granulocyte precursors remain that circulate in the peripheral blood. However, this question is complicated by the inability, thus far, to identify and characterize the colony-forming cell in such culture systems. While the bulk of evidence indicates that these cells are committed granulocyte precursors, recent data suggest that some cells may be capable of multipotential differentiation (4, 6, 16, 18). Thus it is conceivable that the colony-forming cell in the studies reported here may be an undifferentiated cell line with the appearance of a lymphoblast.

The finding that no colony growth was obtained from the

<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></td>
<td></td>
<td></td>
<td>Blasts</td>
<td>Neutrophils</td>
<td>Lymphs</td>
</tr>
<tr>
<td>C. H.</td>
<td>23 M</td>
<td>16,500</td>
<td>90</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>R. M.</td>
<td>6 F</td>
<td>345,000</td>
<td>95</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>A. S.</td>
<td>53 M</td>
<td>8,900</td>
<td>61</td>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>D. P.</td>
<td>11 M</td>
<td>254,000</td>
<td>83</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>R. K.</td>
<td>24 M</td>
<td>48,300</td>
<td>71</td>
<td>10 segmented 6 unsegmented</td>
<td>13</td>
</tr>
<tr>
<td>C. J.</td>
<td>7 M</td>
<td>3,900</td>
<td>12</td>
<td>678</td>
<td>5</td>
</tr>
<tr>
<td>A. P.</td>
<td>4 M</td>
<td>4,700</td>
<td>11</td>
<td>60</td>
<td>26</td>
</tr>
<tr>
<td>M. M.</td>
<td>30 F</td>
<td>72,000</td>
<td>24</td>
<td>50</td>
<td>26</td>
</tr>
<tr>
<td>G. R.</td>
<td>14 M</td>
<td>1,300</td>
<td>36</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>S. R.</td>
<td>14 F</td>
<td>3,700</td>
<td>86</td>
<td>1</td>
<td>13</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).
peripheral blood cells of 2 of the 10 patients studied suggests either that granulocyte colony-forming cells had been reduced as the result of the disease process or that these patients represent a distinct disease entity undistinguishable by morphological and clinical criteria. The only clear-cut difference in these 2 patients was a lower total white blood cell and blast cell counts.

Brown and Carbone (1), who studied the colony-forming potential of bone marrow cells from patients with acute lymphocytic leukemia, found an increase in such cells with treatment and remission. Such studies (over a prolonged period of time) have not been done on peripheral blood cells. However, we would predict that the opposite would occur (that, with treatment, the number of colony-forming cells would decrease in the peripheral blood). These studies are now under way and are being correlated with changes in colony-forming cell numbers in the bone marrow.

The finding, noted previously (15), that these patients have variable levels of urinary colony-stimulating activity is at present unexplained. Further work is essential to determine the relationship between total granulocyte numbers and colony-stimulating activity. Previous data have suggested that these cells may be a major source of colony-stimulating activity in vitro and in vivo (2, 10, 14, 17). This concept is in part supported by the information presented here, which shows a close correlation between rises in colony-stimulating activity and peripheral granulocyte counts, a finding that has also been noted in hematologically normal humans undergoing elective operative procedures (17).

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.

ACKNOWLEDGMENTS

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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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