Radiation Sensitivity of Human Bone Marrow Cells Measured by a Cell Culture Method

By J. S. Senn and E. A. McCulloch

A method is now available for testing the proliferative capacity of a class of human hemopoietic precursor cells. The technique is a modification of that developed by Bradley and Metcalf for the study of mouse marrow in culture, and depends upon the production of colonies from aspirated marrow cells in the presence of suitable "feeder cells." The precise significance of the human cells of origin of these colonies is unknown; however, it is likely that they play a significant role in granulopoiesis, since peroxidase positive granulocytes are among their progeny. Further, cells from mice that give rise to colonies under similar cultural conditions also contain granulocytes, and have been shown to be closely correlated with the mouse hemopoietic stem cells that give rise to spleen colonies. It seems reasonable, therefore, to use the culture method for the investigation of human hemopoiesis.

In the present paper, the culture method has been used to assess the radiation sensitivity of human bone marrow cells. Previous studies of the radiation sensitivity of marrow have been made either in rodents or in cell culture systems less obviously related to hemopoiesis than the one employed in our studies. These measurements of radiation sensitivity have all yielded values of the same order as that found for the proliferative integrity of mammalian cells in general. This finding led to the conclusion that human bone marrow cells might be expected to have a similar radiation sensitivity. The availability of a culture technique applicable to human marrow cells permitted us to test this view directly by comparing the radiation sensitivity of human and mouse marrow using the same technique to assess survival. The results indicate that a class of human marrow cells has a radiation sensitivity similar to that of a comparable class of mouse marrow cells.

Materials and Methods

Assay for Colony Formation

The assay for colony formation from human cells has been described previously. A two-layer culture system in 35-mm. plastic Petri dishes is used; the bottom layer contains...
4 x 10^4 mouse renal tubules immobilized in 0.5 per cent agar dissolved in CMRL 1066 and 10 per cent pooled human serum. The top layer contains the bone marrow cells under test in 0.3 per cent agar, CMRL 1066, and pooled human serum. Colonies containing in excess of 10 cells are counted after 10 days of growth. Two important modifications have been introduced. The first is in the method of obtaining cells; in previous experiments marrow was filtered through cotton. At present, marrow specimens are collected in 0.3 ml. of 1/1000 heparin (Connaught Medical Research Laboratories) and allowed to settle for two hours. Theuffy coat and fatty top layer are removed with a Pasteur pipette, mixed with CMRL 1066, and after counting the cells, appropriate dilutions are made for plating. This procedure regularly yields suspensions containing 10^7–10^8 nucleated cells per milliliter, a hundred-fold increase in yield over the previous method. The second modification is the addition of l-asparagine, 20 μg./ml. and B₁₂, 2 μg./ml. to the tissue culture medium throughout.

The culture technique for mouse cells has been described in detail and is similar to the method for human cells outlined above. Vitamin B₁₂ and l-asparagine were also added to cultures of mouse cells.

Radiation Procedures

Cells derived either from patients or from mice were irradiated in dilute suspension using a 182Cs radiation source designed by Cunningham, Bruce and Webb.¹²

Mice

Mice used as marrow donors in these experiments were F₁ hybrids between C₅7/Bl/He Oci and C₃H/He Oci, raised in the animal colony of the Ontario Cancer Institute. Renal tubules used in the cultures were obtained from eight-week-old C₅7/BL/6J Oci mice.

RESULTS

Marrow was obtained from six patients for these studies. Table 1 provides the diagnosis made on these individuals and the control values for the colony forming ability of their marrows before irradiation. It may be seen from the table that all the patients had hemoglobins in excess of 9 Gm. per 100 ml.; one patient had lymphoma but the results obtained in this instance did not differ from those of the rest of the group and have not been reported separately. The average value for the colony forming ability of these marrows was 83 colonies/10^5 cells. This value is greater than the average value of 34 colonies/10^5 cells previously reported.¹ CMRL 1066 does not contain l-asparagine; addition of this amino acid in combination with vitamin B₁₂ resulted in improved plating efficiencies in control experiments. In other studies,

<table>
<thead>
<tr>
<th>Subject</th>
<th>Diagnosis</th>
<th>Hb. Gm. %</th>
<th>CFU-C* per 10⁶ Nucleated Cells Plated</th>
<th>95 per cent Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.S.</td>
<td>Iron Deficiency</td>
<td>10.0</td>
<td>64</td>
<td>4.2</td>
</tr>
<tr>
<td>K.M.</td>
<td>Iron Deficiency</td>
<td>9.4</td>
<td>73</td>
<td>4.5</td>
</tr>
<tr>
<td>R.H.</td>
<td>Iron Deficiency</td>
<td>10.9</td>
<td>101</td>
<td>5.3</td>
</tr>
<tr>
<td>W.S.</td>
<td>Herpes Zoster</td>
<td>12.9</td>
<td>74</td>
<td>4.5</td>
</tr>
<tr>
<td>R.L.</td>
<td>Macrocystosis</td>
<td>10.0</td>
<td>91</td>
<td>6.6</td>
</tr>
<tr>
<td>R.M.</td>
<td>Lymphoma</td>
<td>13.4</td>
<td>97</td>
<td>5.2</td>
</tr>
<tr>
<td>Mouse</td>
<td>(4 experiments)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Colony-forming units in culture.
additional B₁₂ was not added to the medium, and no reduction in plating efficiency was found. The improvement in plating efficiency is, therefore, attributed to the addition of l-asparagine to the cultures.

Radiation Survival of Colony Forming Ability

The marrow from each of the patients shown in Table 1 was used in a separate experiment. On each day, a specimen of mouse marrow was also tested. Both human and mouse marrow specimens were diluted as follows: control marrows were plated at cell numbers from $5 \times 10^4$ to $2 \times 10^5$, and aliquots were irradiated and plated at cell concentrations of from $5 \times 10^4$ to $10^6$ cells/ml. For each radiation dose, 12 cultures were made; colonies in these cultures were counted after seven days of incubation using an inverted microscope and $60 \times$ magnification.

The results of all six experiments are in Fig. 1. The figure is a survival curve showing an exponential decrease in colony forming ability with increasing radiation dose. The results obtained for human marrow and mouse marrow were very similar, although the $D_0$ (dose required to reduce survival to 37 per cent of control) for mouse marrow was slightly though significantly ($p = 0.025$) higher than that for human marrow.

DISCUSSION

The results presented in this paper provide direct evidence that the radiation sensitivity of bone marrow progenitor cells is very similar, regardless of whether their source is human or murine. However, the values for $D_0$ given in Fig. 1 of this paper are higher than the $95 \pm 9$ rad obtained when the radiation sensitivity of mouse marrow is measured using the spleen colony technique.8

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Fig. 1.—Survival curves for colony-forming ability, in culture, of cells from human (closed circles) and mouse (open circles) bone-marrow cells. Each point represents pooled values obtained from 12 separate cultures.
Evidence is accumulating that the cell culture technique employed in these experiments measures a class of cells that may be distinct from the pluripotent hemopoietic stem cells detected by the spleen colony method. The class of cells detected in culture may be an early differentiated descendent of the pluripotent spleen colony-forming cell. It is possible, therefore, that the radiation sensitivity value found in our experiments is a genuine reflection of the differences between cells that form colonies in spleen and those that form colonies in culture. However, it is perhaps more likely that the D0 values reflect the difference in the two assay systems used to measure proliferative integrity. Colony formation in the spleen requires at least 20 cell divisions while colony formation in culture is detected after as few as five cell divisions. This difference in requirement for proliferation may account for the results obtained in this paper. The reason for the slight, though statistically significant (p = 0.025) difference between the survival curves for human and mouse marrow colony-forming cells is not obvious; however, colonies derived from human marrow are smaller than those derived from mouse marrow. If this is a reflection of imperfect cultural conditions, nutritional deficiency might be additive with radiation damage.

We may conclude that the sensitivity of human bone marrow to irradiation as measured directly is of the same order as that predicted on the basis of experiments in mice. Indeed, when marrow from both species is assayed using the same method similar sensitivities to radiation are found. These data provide some justification for the extrapolation of results obtained from radiation studies in rodents to the human situation.

SUMMARY

A culture technique for measuring the proliferative capacity of a class of human hemopoietic cells is presented. This method is used to assess the radiation sensitivity of this class of human hemopoietic cells, and to compare them directly to mouse hemopoietic cells tested in the same system. We conclude that the sensitivity of human bone marrow to irradiation as measured directly is of the same order as that predicted on the basis of experiments in mice.

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