A Difference Between Spleen-derived and Bone Marrow-derived Colony-forming Units in Ability to Protect Lethally Irradiated Mice

By A. L. Kretschmar and W. R. Conover

Suspensions of cells in the bone marrow and in the spleen of normal mice were prepared. Two groups of lethally irradiated mice were given cells from bone marrow and two groups of lethally irradiated mice were given cells from the spleen of the same donor mouse. One of the groups injected with bone marrow and one injected with spleen cells were killed at 9 days for determination of CFU. The remaining animals were followed for 100 days to determine the mortality statistics. The mortality among mice not given cells was 100 per cent; among mice injected with cells, the mortality was reduced. The data permit a comparison of the reduction in mortality (protection) following injection of bone marrow CFU with protection following injection of spleen CFU. Protection with CFU of bone marrow was different from protection with CFU of spleen. It seems possible that both hemopoietic pluripotent “stem cells” and “early differentiated progenitors” can form spleen colonies and that the ratio of these CFU may be different for bone marrow and spleen.

Mice exposed to total-body X-irradiation in the 700–1000 R dose-range die because of destruction of the hemopoietic cell-renewal system.1 This outcome (death in the second or third week after irradiation) can be largely prevented by intravenous infusion of a suspension of cells from hemopoietic organs of unirradiated animals of the same or different strain and even by infusion of cells from a different species.2 The protective effect of cells is generally ascribed1,2 to repair of the hemopoietic system of the irradiated mice brought about by transplantation of progenitors of the hemopoietic cell-renewal system. A possible contribution from mature functional elements (white blood cells and platelets, for example) in the suspensions of the donor cells can probably be neglected since the studies reported below are concerned with infusion of minimal doses of cells. Thus, the protection (in terms of per cent survival among treated animals) afforded by various suspensions of cells ought to be a function of the number of progenitors in the cell suspensions used. Many experiments1,2 support this expectation.

When small numbers of hemopoietic cells are injected into lethally irradiated mice and these animals are sacrificed 7–9 days later, gross nodules may be found in their spleens.3 The nodules are colonies of hemopoietic cells of mixed
or relatively pure lines of differentiation. These colonies appear to be clonal, i.e., all cells are progeny of a single progenitor. An assay of hemopoietic stem cells which has been widely employed in experimental hematology is based on this kind of experiment.

The considerations outlined above suggest that the spleen colony assay should predict the efficacy of cell suspensions with regard to protection against the lethal effect of total-body irradiation. This expectation was not realized in the experiments reported below. Three times as many “colony-forming units” in suspensions of the cells from normal spleen (CFU-Sp) had to be infused to reduce mortality to the same level in lethally irradiated mice as when we used the colony-forming units in suspensions of the cells from normal bone marrow (CFU-B) of the same donor. Thus, two kinds of colony-forming units, CFU-Sp and CFU-B, are distinguishable with respect to efficiency in protection of lethally irradiated mice for 100 days.

**Materials and Methods**

Male and female C3H mice were used. They were born and raised in the vivarium of the Memorial Research Center and transferred when they were about 5 weeks of age to the Biology Division, Oak Ridge National Laboratory, where they were held in quarantine for 19 days before they were used. Mice serving as recipients were 14–16 weeks old when they were irradiated. Cells from unirradiated male donors were infused into male recipients and female cells into female recipients.

Cell suspensions were prepared in cold Tyrode’s solution. The number of cells in a given volume of suspension was determined by counting with a Coulter electronic particle counter with occasional cross-checks on these counts using a hemocytometer. The bone marrow suspensions were made by flushing the femurs with Tyrode’s solution. The cells were then dispersed and suspended by drawing them back and forth through hypodermic needles of decreasing bore size to No. 27. Appropriate dilutions were made in Tyrode’s solution so that the number of cells (indicated in tables and figures) could be injected intravenously in a volume of 1 ml.

Suspensions of spleen cells were counted, diluted and injected as described for bone marrow. The cleanly dissected spleen was fragmented in cold Tyrode’s solution with fine scissors and then the cells were suspended by gently forcing the pulp through stainless steel wire gauze or by manual manipulation of the Teflon pestle of a homogenizer.

Mice were exposed to 900 R total-body X-irradiation in a revolving lucite chamber 60 cm. from the target of the X-ray tube. A constant potential Phillips machine was used with factors: 250 KV., 15 mA., HVL, 0.5 mm. Cu, and dose rate in air of 160 R/min. Mice were caged in groups of 10 after irradiation and infusion of cell suspensions.

Separate cell suspensions were prepared from the spleen and bone marrow of the same donor mouse. Cells were then counted, diluted and injected into irradiated recipients for assay of colony-forming units and determination of 100-day mortality. Thus, spleen and bone marrow cells from the same mouse were tested for both their capacity to give rise to gross splenic nodules at 9 days and their capacity to protect lethally irradiated mice for 100 days.

**Results**

Mortality data are plotted on the abscissa of Fig. 1 against total CFU injected. The data suggest that mortality varies inversely with respect to number of CFU injected. Thus, the more CFU injected, the lower the mortality and, hence, the greater the therapeutic efficacy of the cell suspensions that contain the CFU. However, the data relating CFU-Sp and mortality is completely separated from that relating CFU-B and mortality. The ratio between the
two is roughly three to one, that is one CFU-B is equal to about three CFU-Sp.

Results since 1966 are summarized in Table 1. The mortality, although variable, averaged 56 per cent in mice given 1·10⁶ bone marrow cells and 66 per cent in mice given 2·10⁶ spleen cells. Thus, it takes more than 20 times as many spleen cells as bone marrow cells to achieve 50 per cent protection. The relative CFU assays are shown in Fig. 2. The bone marrow suspensions contained 7.8 times as many CFU/10⁹ cells as the spleen. Thus, the bone marrow was >20/7.8 or approximately three times as effective as would be expected on the basis of the spleen colony assay.

The last two columns of Table 1 show results of three experiments with mixtures of bone marrow and spleen cells. The data show that 0.5·10⁵ bone marrow cells were approximately equivalent to 10⁶ spleen cells (assuming linear mortality versus cells over the range 73–41 per cent mortality with 2·4·10⁶ spleen cells). This ratio of 20 is what was observed for bone marrow and for spleen cells alone, thus, no evidence for an “enhancing” factor in bone marrow or an “inhibiting” factor in spleen was obtained.

Table 1.—Cumulative Mortality in Per Cent at 100 Days*

<table>
<thead>
<tr>
<th>Date</th>
<th>X-Ray Dose (R)</th>
<th>None</th>
<th>Bone Marrow Cells</th>
<th>Spleen Cells</th>
<th>Spleen Cells Plus Bone Marrow Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose (10⁵)</td>
<td>Mortality</td>
<td>Dose (10⁶) Mortality</td>
</tr>
<tr>
<td>Dec. 1966</td>
<td>850</td>
<td>100</td>
<td>1·10⁵</td>
<td>30</td>
<td>1·5·10⁶</td>
</tr>
<tr>
<td>Jan. 1967</td>
<td>900</td>
<td>100</td>
<td>1·10⁵</td>
<td>32</td>
<td>1·5·10⁶</td>
</tr>
<tr>
<td>May 1967</td>
<td>900</td>
<td>100</td>
<td>0.75·10⁵</td>
<td>74</td>
<td>2·10⁶</td>
</tr>
<tr>
<td>Nov. 1967</td>
<td>900</td>
<td>100</td>
<td>0.65·10⁵</td>
<td>58</td>
<td>1·5·10⁶</td>
</tr>
<tr>
<td>Sept. 1968</td>
<td>900</td>
<td>100</td>
<td>0.8·10⁵</td>
<td>78</td>
<td>2·10⁶</td>
</tr>
<tr>
<td>1969</td>
<td>900</td>
<td>100</td>
<td>0.9·10⁵</td>
<td>100</td>
<td>2·5·10⁶</td>
</tr>
<tr>
<td>Feb. 1969</td>
<td>900</td>
<td>100</td>
<td>0.8·10⁵</td>
<td>93</td>
<td>2·10⁶</td>
</tr>
<tr>
<td>June 1969</td>
<td>900</td>
<td>100</td>
<td>1·10⁵</td>
<td>76</td>
<td>4·10⁶</td>
</tr>
<tr>
<td>Nov. 1969</td>
<td>900</td>
<td>—</td>
<td>2·10⁵</td>
<td>57</td>
<td>4·10⁶</td>
</tr>
</tbody>
</table>

* C3H mice given single-dose total-body X-irradiation and then isologous bone marrow or spleen cells or a mixture of bone marrow and spleen cells.
**PROTECTION OF LETHALLY IRRADIATED MICE**

0 BM CFU

Spleen CFU .8

a U,

[Image]

**Fig. 2.**—Spleen colony assay data for the cell suspensions used for the experiments summarized in Table 1. The vertical lines at the symbols indicate SE of the mean.

**DISCUSSION**

It is clear from these results that the spleen colony assay measures a population of cells that is not homogeneous with respect to the functional properties required to protect mice from death after 900 R total-body X-irradiation. Thus, CFU-B are more efficient than CFU-Sp in reducing mortality.

Normal mouse blood contains about 2·10⁶ mature myeloid cells per ml.; therefore, the number of mature cells injected in 10⁵ bone marrow cells would be equivalent to transfusion of less than 0.05 ml. whole blood into the irradiated mouse. It seems unlikely that this small number of mature cells could contribute very much if anything to the protective effect of the bone marrow suspensions. Dividing differentiated cells, e.g. myelocytes, with several amplifying divisions might be equivalent to substantially more than 0.05 ml. whole blood but any sustained proliferation would make such cells countable in the CFU assay. Barnes and colleagues¹⁰ have reported that mixing lymphocytes with small doses of bone marrow cells improved survival statistics. We might, therefore, expect that spleen-cell suspensions, with their large numbers of mature lymphocytes, would be relatively more effective than marrow-cell suspensions at minimal cell-dose levels instead of less. Thus, our results should be considered primarily from the point of view of relative numbers of hemopoietic progenitors contained in the spleen and bone marrow suspensions.

The relative protection with spleen cells and bone marrow cells and the relative numbers of CFU found in these studies is comparable to results published by other investigators.¹² Thus, what we have observed for C3H may be valid for other strains of mice. The distribution of colonies of various morphologic differentiation categories was not very different in our material. For example, of 28 spleen cell-derived colonies, 61 per cent were erythroid, 18 per cent granuloid, the rest megakaryocytic, undifferentiated, or mixed; and, of 20 bone marrow cell-derived colonies, 65 per cent were erythroid, 20 per cent granuloid, and the remainder were various other categories, as for the spleen cell-derived colonies.

An interpretation that would be consistent with the work of Bennett and Cudkowicz,¹¹ Silini et al.,⁵ Schofield and Cole,¹² and Lahiri and van Putten¹³ is that cells counted as CFU on the basis of the number of nodules found on
spleens at 7–9 days are not homogeneous with respect to potential for differentiation and proliferation. It seems likely that some are pluripotent6 with great proliferative potential, while others may be "early differentiated precursor cells."11 These latter cells may have restricted proliferative potential and yet be measured as CFU in the usual spleen colony assay. Lahiri and van Putten13 show that there is a difference in proliferative potential in bone marrow-derived and spleen-derived CFU. Moreover, Worton et al.14 have shown directly that there are subclasses of CFU which can be physically separated in bone marrow suspensions that differ in capacity for self-renewal.

Thus, perhaps it is only the pluripotent cells with relatively unrestricted proliferative potential that contribute to long-term survival after irradiation and the ratio of these cells among CFU in bone marrow is higher than among the CFU in spleen. If this is correct, it is not possible to equate the results of spleen colony assay for CFU with the kinetics of a pluripotent hemopoietic stem cell until methods are worked out to reliably separate such a cell from the other CFU.

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REFERENCES


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