Observations on the Settling and Recoverability of Transplanted Hemopoietic Colony-forming Units in the Mouse spleen

By B. I. Lord and J. H. Hendry

A series of experiments was designed to study the effect of spleen size on the seeding and retention of spleen colony-forming units (CFU) after injection into irradiated mice. The cellularity of the spleens of recipient mice was varied using a variety of experimental procedures, and the resulting spleen colony production was analyzed with respect to CFU seeding. It was found that the major factor determining the seeding and ultimate retention of CFU in the spleen of a recipient mouse is the size of the spleen. It is concluded that the most reasonable value of the fraction of injected CFU that produces spleen colonies should be determined from the number of CFU recoverable from the fully shrunken spleen. In these mice, the fraction was 9.5%.

THE FRACTION (f) of spleen colony-forming units (CFU) injected into a lethally irradiated mouse that produces hemopoietic colonies in the spleen has usually been determined by the secondary transplantation method of Siminovitch et al.1 Several more recent reports have shown that the number of CFU that can be recovered from the spleen of an irradiated animal following an injection of hemopoietic cells depends on the interval of time between the injection and the assay.2-4 One of these5 demonstrated that the number of CFU recoverable is directly related to the number of cells in the spleen at the time of assay. The explanation suggested for this relationship was that after injection CFU enter the spleen, but as the spleen collapses due to the radiation, the CFU are expelled or destroyed. It was concluded that the calculation of the f number should be based on the number of CFU recoverable from the fully shrunken spleen, rather than on the maximum numbers recoverable, i.e., about 2 hr after injection. It had earlier been suggested5 that the initial decrease (after about 2 hr) in the number of CFU per spleen was due to an inability to extract and disperse all the CFU from the spleen. This same suggestion has been enforced more recently by Till and McCulloch,6 who have reported experiments in which colony production in W/W' mice receiving different doses of radiation (including no radiation) remained the same.

In an attempt to clarify the fate of CFU entering the spleen after intravenous injection, a variety of experiments have been carried out and are reported in this paper.

From the Paterson Laboratories, Christie Hospital, and the Holt Radium Institute, Manchester, England.

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MATERIALS AND METHODS

Male BDF₁ (C57Bl ♂ × DBA2♂) mice aged 9–12 wk-old or male W/W⁰ mice aged about 14 wk-old, together with their normal (+/+ ) litter mates, were used in the following experiments.

(1) Spleen Colony Production in Normal and Irradiated W/W¹ Mice

Colonies will develop in the spleens of W/W⁰ mice following a bone marrow cell injection, whether or not the animal has been irradiated. Since radiation affects the size of the spleen, these animals, therefore, were used to study the effect of a reducing spleen cellularity on colony production.

Groups of 16 W/W⁰ mice were given 0, 300, or 600 rads x-rays at a dose rate of 30 rads/min and were then injected within 1 hr with 5 X 10⁵ bone marrow cells from normal +/+ mice. At 24 and 48 hr after the injection, three mice from each group were killed, and the nucleated cell content of their spleen was measured. The remaining ten mice from each group were killed at 9 days for spleen colony counting.

(2) Spleen Colony Production in Relation to Time of Recipient Irradiation

Injecting cells immediately after irradiation allows CFU the opportunity of seeding in a spleen of normal size, together with the subsequent effects of the splenic collapse. By irradiating the recipient animal some hours in advance of the cell injection, splenic collapse may be complete before CFU seeding is initiated. Thus, the numbers of CFU seeding in spleens of different sizes can be investigated.

Three groups of 15 BDF₁ mice were given 800 rads x-rays (30 rads/min) at 48 hr, 24 hr, and less than 1 hr, respectively, before an injection of 3 X 10⁴ normal bone marrow cells from the same cell suspension. Spleen colony counts were made 8 days after the cell transfer, since in this experiment some of the animals had been irradiated up to 2 days before the cell transfer.

(3) CFU Settling in Spleens of Artificially Maintained Size

The reverse situation of experiment (2) can be obtained if the splenic collapse can be prevented or delayed during the course of colony development. Foreign protein, e.g., horse serum, can be used for this purpose.

Six BDF₁ mice were irradiated with 800 rads x-rays (30 rads/min) and injected with 3.3 X 10⁵ normal bone marrow cells within 1 hr of the irradiation. Three of the mice were then immediately injected with 1 ml of sterile horse serum and again 7 hr later. After 24 hr, the cellularity and CFU content of the spleens of each group were determined.

(4) Effect of Radiation Dose Rate on Spleen Colony Production

To investigate the effect of the radiation dose rate on the reduction of spleen cellularity and CFU content, groups of 15 BDF₁ mice were given 150 rads or 450 rads x-rays at dose rates of 30, 148, or 645 rads/min. Twenty-four hours after 150 rads, spleen cellularities were measured, and the splenic CFU content was assayed in further groups of irradiated BDF₁ mice (800 rads x-rays, 30 rad/min). Endogenous colony production at 8 days was measured in the groups given 450 rads.

RESULTS

As shown in Table 1, the CFU concentration in +/+ bone marrow, as assayed in unirradiated W/W⁰ mice, was 9.0 CFU/10⁵ cells in the first experiment. When the recipients had been irradiated with 300 or 600 rads, the CFU concentrations appeared to have been 4.4 and 2.4/10⁵ cells, respectively. These reduced colony yields correspond to spleens that had shrunk in the first 48 hr after injection by a factor of 3.5 from about 215 × 10⁶ cells to about 60 × 10⁶ cells. The apparent CFU concentrations for the two irradiated groups were reduced by factors of 2.0 and 3.7, as compared with the concentration...
Table 1. Spleen Colony Formation in Normal and Irradiated W/W<sup>v</sup> Mice

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>300 Rads</th>
<th>600 Rads</th>
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<tbody>
<tr>
<td>Spleen cellularity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 24 hr (x 10&lt;sup&gt;-6&lt;/sup&gt;)</td>
<td>224</td>
<td>70</td>
<td>60</td>
</tr>
<tr>
<td>At 48 hr (x 10&lt;sup&gt;-6&lt;/sup&gt;)</td>
<td>288</td>
<td>77</td>
<td>69</td>
</tr>
<tr>
<td>Spleen cellularity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colonies/10&lt;sup&gt;5&lt;/sup&gt; bone marrow cells injected</td>
<td>9.0 ± 1.6&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4.4 ± 0.6&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.4 ± 0.8&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean spleen weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 9 days (mg)</td>
<td>102</td>
<td>67</td>
<td>48</td>
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</table>

*Standard error.

given by the unirradiated W/W<sup>v</sup> mice. A repeat of this experiment gave essentially the same results. Following radiation, the spleen cellularities fell by a factor of about 3.9 times in 24 hr, while the apparent CFU concentration fell by 2.4 times compared with unirradiated W/W<sup>v</sup> mice.

Irradiation of recipient BDF<sub>1</sub> mice 24 and 48 hr before injecting normal bone marrow cells (Table 2) produced no significant change in colony yield by comparison with the recipients that had been irradiated immediately prior to the injection. CFU concentrations of 17.3, 18.6, and 14.0 CFU/10<sup>5</sup> cells were obtained from the 0-, 24-, and 48-hr preirradiated recipients, respectively.

Twenty-four hours after the injection of normal bone marrow cells, plus two injections of horse serum, the spleens of BDF<sub>1</sub> recipient mice still contained 42 x 10<sup>6</sup> cells as compared with 26.5 x 10<sup>6</sup> cells in those with no serum (Table 3). The fractions of the injected CFU recoverable from these spleens were 14.1% and 9.5%, respectively, and the ratios of the two cellularities to f numbers were identical.

Varying the radiation dose rate to BDF<sub>1</sub> mice produced significant differences both in cellularity and CFU content of the spleen. The results of replicate experiments are shown in Table 4. Twenty-four hours after 150 rads x-rays, the spleen contained 23 x 10<sup>6</sup> cells when the radiation had been given at 30 rads/min, 40 x 10<sup>6</sup> cells (148 rads/min), or 98 x 10<sup>6</sup> cells at the highest rise rate, 645 rads/min. These differences were reflected by the number of transplantable CFU that could be extracted from the spleen; approximately five times more were available following the high dose rate than the low dose rate. The same pattern was also evident when considering endogenous colony development following 450 rads x-rays. The colony yield was again up to five times higher following irradiation at the high dose rate.

Table 2. Dependence of CFU Assay on Time of Recipient Irradiation in BDF<sub>1</sub> Mice

<table>
<thead>
<tr>
<th>Interval Between Recipient Irradiation and Cell Injection</th>
<th>Less Than 1 Hr</th>
<th>24 Hr</th>
<th>48 Hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies/spleen (3 x 10&lt;sup&gt;4&lt;/sup&gt; bone marrow cells injected)</td>
<td>5.2 ± 0.5&lt;sup&gt;*&lt;/sup&gt;</td>
<td>5.6 ± 0.2&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4.2 ± 0.7&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
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</table>

*Standard error.
Table 3. \( \frac{f}{\text{Number}} \) Determination in Normal Irradiated Recipients and in Irradiated Recipients Injected With Horse Serum in BDF\(_1\) Mice

<table>
<thead>
<tr>
<th></th>
<th>Spleen Cellularity 24 hr After Cell Injection</th>
<th>( f ) Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>( 26.5 \times 10^6 )</td>
<td>( 9.5 \pm 1.2 )</td>
</tr>
<tr>
<td>Horse serum injected</td>
<td>( 42.0 \times 10^6 )</td>
<td>( 14.1 \pm 2.2 )</td>
</tr>
<tr>
<td>Ratio of serum injected:control</td>
<td>( 1.58 )</td>
<td>( 1.49 \pm 0.21 )</td>
</tr>
</tbody>
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**DISCUSSION**

A series of experiments was designed to investigate one aspect of the behavior of CFU injected into irradiated mice. This was related to whether the number of colonies produced in the spleen is derived from the large numbers of CFU that can be recovered from the spleen 2 hr after injection or from the much smaller number that can be recovered 24 hr after injection.

In the first experiment, irradiated and nonirradiated W/W* mice were used to allow colony development in spleens that, subsequent to the injection, may or may not shrink. If the collapse of the spleen presents a mechanical problem only to the operator in trying to recover the cells from the spleen, then one would expect no difference in colony yield between the nonirradiated and the irradiated groups. This was not the case; the colony yield was reduced in the irradiated groups, and this appears contrary to the findings of Till and McCulloch. However, it is also interesting to note that the spleen cellularity was reduced in the irradiated groups by a factor of less than four, whereas in normal strains of mice one would expect to lose about 90% of the spleen cells after 600 rads x-rays. Consequently, if expulsion from, or destruction of, the CFU due to spleen shrinkage occurs, one would expect the relatively small reduction in colony yield in irradiated W/W* mice. Consideration of the radiation dose rate (Table 4) shows that 30 rads/min (as used for the W/W* mice) gave a considerably greater reduction in spleen cellularity than

Table 4. Effect of Radiation Dose Rate on Spleen Cellularity and CFU Content in BDF\(_1\) Mice

<table>
<thead>
<tr>
<th>X-Ray Dose Rate (rads/min)</th>
<th>30</th>
<th>148</th>
<th>645</th>
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<tbody>
<tr>
<td><strong>24 hr after 150 rads</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen cellularity (( \times 10^{-6} ))</td>
<td>23</td>
<td>40</td>
<td>98</td>
</tr>
<tr>
<td>Transplantable CFU/spleen, i.e., CFU obtained from spleen 24 hr after 150 rads x-rays</td>
<td>4.5 ± 0.4</td>
<td>15.4 ± 1.1</td>
<td>25.9 ± 2.2</td>
</tr>
<tr>
<td>150 rads x-rays</td>
<td>15.5 ± 2.6</td>
<td>35.0 ± 3.5</td>
<td>—</td>
</tr>
<tr>
<td><strong>9 days after 450 rads</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endogenous colonies per spleen, i.e., endogenous hemopoietic colony formation resulting from sublethal radiation</td>
<td>4.3 ± 1.4</td>
<td>4.9 ± 0.9</td>
<td>19.8 ± 1.8</td>
</tr>
<tr>
<td>8.5 ± 1.3</td>
<td>—</td>
<td>19.5 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>5.3 ± 0.6</td>
<td>9.1 ± 1.6</td>
<td>—</td>
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</tbody>
</table>
the high dose rate. It may well be that at dose rates higher than 30 rads/min little change would occur in the spleen cellularity of W/W' mice and, thus cause, little reduction in the colony yield. Till and McCulloch used a dose rate of 100 rads/min.

The greater effectiveness of x-rays delivered at a rate of 30 rads/min, as compared with 148 and 645 rads/min, in reducing the spleen cellularity and CFU content is somewhat surprising. One might expect some degree of recovery “Elkind type” occurring with the lower dose rates. However, the total radiation times for the high and low dose rates are only 0.7 min and 15 min for 450 rads. In these short intervals, and with an extrapolation number for spleen cells close to unity, (0.8 quoted by Guzman and Lajtha7 for splenic CFU) no recovery of this kind could be reliably detected.

The difference in the spleen cellularities as a result of radiation at different dose rates is, however, reflected by the colony-forming capacities of these spleens. The transplantable CFU content of the spleens 24 hr after 150 rads is still subject to the criticism that CFU may be simply inaccessible. Endogenous colony formation, however, is not subject to this criticism because only the animal handles the cells. The fact that endogenous colony formation, therefore, is in line with the spleen cellularity changes seems good support for the hypothesis that postirradiation collapse results in the exclusion or destruction of CFU from the spleen.

An alternative explanation of the radiation dose rate effects is that the different rates might produce different degrees of CFU killing. However, it has been shown8 that dose rate has no significant effect on CFU survival over a range of 9–600 rads/min. In endogenous colony production, there is no problem of seeding of colony-forming cells to spleens of different sizes, and thus, the only factor relevant to the reduction of colony yield is the reduction in spleen size due to irradiation.

If the number of spleen colonies produced is dependent on the number of CR1 that can be detected in the spleen 2 hr after injection, and the lower 2-hr f values obtained by preirradiation of the primary recipients2'4'9 are due to fewer CFU being admitted into the shrunken spleen, then preirradiation of recipient mice for CFU assays should give a lower colony yield. In fact, this is not the case. There is no loss of colony production when the spleens of the recipient mice have shrunk before the cell transfer. This observation confirms that of Shadduck et al.,4 who also reported that preirradiation of the recipient mice does not alter the final colony yield. However, they also reported significantly lower f values in the preirradiated recipients and suggested that a higher migration or replication rate of CFU in those animals made up the difference. Measurements of f values reported recently from these laboratories9 show little difference when measured 24 hr after injection of bone marrow cells into either pre- or freshly irradiated mice. Consequently, in our mice, subsequent migration of CFU appears to be relatively insignificant. An explanation of these differences may lie in the fact that Shadduck et al. used female mice in their study. Female spleens are significantly larger than male spleens10 and so are subject to more dramatic postirradiation cellularity
changes. Since Shadduck et al. reported that the minimum spleen size occurred 4 days after radiation in their mice, it is clear that CFU proliferation in the freshly irradiated group will be occurring while the spleen is still shrinking. This factor may well modify the CFU growth curve. In our experiments using male mice, these effects are minimized because of the initially smaller spleen. As a result, both the f values and the final colony yields are very similar in pre- and freshly irradiated mice. It appears, therefore, that the smaller spleen of the preirradiated recipient mouse admits the same number of CFU as that which is recoverable from the spleen when the mouse is irradiated immediately prior to the cell injection.

Schooley demonstrated that by injecting recipient mice with rabbit serum before irradiating and injecting the bone marrow, the recipient's spleen was very much enlarged. This permitted a larger intake of CFU by the spleen, and consequently he recorded large f values 2 hr after the injection of cells. Retarding the collapse of the spleen by injecting horse serum immediately after the cell injection, a slower subsequent loss of CFU from the spleen has now been demonstrated (Table 3), with the f value in serum-treated mice 14% as compared to 9.5% in control animals. Smith et al. showed an increase of CFU in spleens of endotoxin-treated mice, especially after multiple injections. These treatments also had the effect of producing splenic hyperplasia and thus allowing more CFU to reside there. Boggs et al. showed that splenic hyperplasia produced by injections of foreign plasma or endotoxin, or by bleeding before irradiation, resulted in increased endogenous colony formation. Similarly, Lord and Murphy demonstrated that in the preestrus stage of the estrous cycle, spleen cellularities were much increased; the splenic CFU content under these conditions was correspondingly higher. On the other hand, Morley et al. showed that splenic hyperplasia due to injections of colloidal carbon did not allow increased colony formation, nor did it demonstrate an increased 2-hr f number. The difference in these observations, however, may reflect the fact that foreign serum or endotoxin stimulates an immune reaction while carbon stimulates the reticuloendothelial system.

A further point is illustrated by Barnes et al. who showed that one transplanted CFU gives rise on the average to 1.3 colonies in the spleen. This indicates that during early proliferation of CFU (in the first 48 hr) migration within the spleen is not only possible but does occur. It is unlikely, therefore, that these cells would become irretrievably lodged in the spleen stroma during this time.

It is concluded that loss of CFU from the spleen as a result of radiation-induced spleen shrinkage does, in fact, take place. Consequently, it is suggested that the f number should be determined from the CFU content of the fully shrunken spleen. For normal bone marrow CFU in these animals, therefore, the f number is approximately 10%.

ACKNOWLEDGMENT

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REFERENCES

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