Killing Effect of Rat Bone Marrow in Sublethally Irradiated LAF₁ Mice

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Radiation death of lethally irradiated mice can be prevented by postirradiation injection of isologous mouse bone marrow (IBM) (13, 14). Protection in terms of body weight recovery and long-term survival is generally excellent with such treatment. Subsequent studies with foreign bone marrow protection showed transplantation and re-population of the donor's hematopoietic tissues in the irradiated host (7, 11, 15, 24, 25, 28, 30). Thus, similar protection against radiation death is obtained by the postirradiation injection of heterologous rat bone marrow (RBM). In contrast to the recovery of mice given IBM treatment, mice receiving RBM characteristically show a secondary body weight loss and a high percentage of delayed deaths 3–6 weeks after this treatment (2, 3, 15). From these observations and immunologic studies of this problem, Makinodan and Gengozian concluded that the "foreign bone marrow reaction" in lethally irradiated RBM-treated mice was attributable to a delayed immunologic response of the irradiated host to the foreign transplant, which resulted in a chronic in vivo antigen-antibody reaction (8, 15). Additional studies corroborated this (4, 9, 10, 16, 18–23, 27). A subsequent elaboration of this hypothesis stated that the occurrence (treatment-time relation), severity (chronic or acute), and final effects (physical and metabolic) of such an in vivo antigen-antibody reaction would depend on the x-ray dose and the number of RBM cells injected (9). Thus, the radiation-induced injury, the transplantability of the foreign tissues in the host, and the rate and degree of recovery of the host's immune mechanism are functions of the x-ray dose. The amount of foreign bone marrow injected must be considered, however, if the dividing donor cells are to be recognized as proliferating antigens participating in a dynamic in vivo antigen-antibody (host-immune mechanism) reaction. Under appropriate conditions, then, the optimum balance of antigen and antibody in vivo in such a system could conceivably result in an acute reaction, which, compounded with the radiation-induced injury, would lead to death. This was borne out in (C3H × 101)F₁ mice that had received a sublethal x-ray dose of 710 r (LD₁₀) and 140 × 10⁶ RBM cells intravenously after x-radiation. Sixty mice thus treated died within 16 days after treatment (8). The importance of the bone marrow dose was made evident in another study (9) showing that a smaller number of cells (75 × 10⁶) injected into (C3H × 101)F₁ mice irradiated with 710 r did not yield 100 per cent mortality; survivors were negative for the foreign transplant. Injection of a greater number of RBM cells (300 × 10⁶) resulted in a survivor that was positive for the transplant. Further variation of both the x-ray dose and number of bone marrow cells injected provided additional support for the working hypothesis.

This enhanced killing effect has also been reported by Trentin with CBA mice irradiated with 550 r and injected with (CB × CBA)F₁ bone marrow (29). Similar adverse effects among sublethally irradiated (450–550 r) CBA mice treated with homologous C37BL or heterologous RBM have been noted by van Bekkum and Vos (1). Santos, Cole, and Roan, using LAF₁ mice, gave x-ray doses ranging from 600 to 1000 r, followed by intravenous injection of 70 mg. of RBM. Mortality did not increase among the sublethally irradiated mice injected with RBM (26). However, a constant 70-mg. dose of RBM was used, and, as noted in the hypothesis presented earlier, the in vivo antigen-antibody reaction and its manifestations are a function of the x-ray and the bone marrow dose. The present report shows both the “nonkilling” and “killing” effect of RBM in sublethally irradiated LAF₁ mice when a variation of the RBM dose is considered.

MATERIALS AND METHODS

Male and female LAF₁ mice 12 weeks old were used in all experiments. Irradiation conditions were: 250 kv. at 15 ma.; rate in air, 160 r/min.;


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target-skin distance, 60 cm.; inherent filtration, 1.0 mm. of Al and added filtration of 1.0 mm. of Al. One group of mice received a sublethal dose of 640 r (LD0/30), and a second group received a lethal dose of 900 r (LD100/30). Sixteen or more mice of each group then received (a) no treatment, (b) IBM, or (c) RBM. Bone marrow was injected intravenously within 4 hours after irradiation. Isologous marrow was obtained from LAF1 donors, and the contents of one femur shaft (~12 x 10^6 nucleated cells) was injected into the irradiated host. Rat bone marrow was obtained from the femora, tibiae, and humeri of Sprague-Dawley rats. The number of nucleated RBM cells injected per irradiated animal was either 100 x 10^6 or 240 x 10^6. Thirty-day survivors among the RBM-treated mice were tested for the presence of rat red blood cells (RBC) and granulocytes in the peripheral circulation as described by Makinodan (15).

RESULTS

The cumulative 30-day mortality data are given in Table 1. Of the 20 mice receiving 640 r only, none died within the 30-day period. Injection of IBM into a group of 20 mice that had received 640 r also showed 0 per cent mortality. Of nineteen mice receiving 640 r and 100 x 10^6 RBM cells, 1 died on the 13th day after treatment, a 30-day mortality of 5 per cent. All survivors of this group at 30 days were negative for rat RBC and granulocytes in the peripheral blood. A fourth group of sixteen mice receiving 640 r and 240 x 10^6 RBM cells all died within 19 days.

Among the lethally irradiated (900 r) mice, IBM provided excellent protection. Only one of twenty died in the 30-day interval. Injection of 100 x 10^6 RBM cells into 22 lethally irradiated mice gave a 30-day mortality of 75 per cent, whereas injection of 240 x 10^6 RBM cells into sixteen lethally irradiated mice yielded a 12 per cent mortality in this time interval. Survivors of the latter two groups, when tested for rat RBC and granulocytes in the peripheral circulation at this time interval, showed values of 50-75 per cent for rat RBC, and all showed positive tests for alkaline phosphatase in the granulocytes, characteristic of rat-type cells.

Increased mortality beyond the 30-day interval occurred only among the lethally irradiated RBM-treated mice. Since the numbers involved were small, no attempt was made to compare the delayed mortality occurring in the low-dose group (100 x 10^6) with that of the high-dose group (240 x 10^6).

DISCUSSION

The experiments reported here demonstrate the enhanced killing effect in sublethally irradiated LAF1 mice, when a certain dose of RBM is used. The results thus corroborate our previous findings in the (C3H X 101)F1 strain and substantiate the in vivo antigen-antibody reaction hypothesis presented at that time predicting the enhanced killing phenomenon. In this study, the nonkilling effect observed with the low dose of 100 x 10^6 RBM cells in the 640 r animals can be explained by the relative efficiency of the irradiated host's

**TABLE 1**

<table>
<thead>
<tr>
<th>X-RAY DOSE (r)</th>
<th>TREATMENT</th>
<th>NO. MICE</th>
<th>CUMULATIVE MORTALITY (PER CENT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Days after treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>640</td>
<td>None</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>640</td>
<td>IBM*</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>640</td>
<td>RBM+100X10^6</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>640</td>
<td>240X10^6</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>900</td>
<td>None</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>900</td>
<td>IBM</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>900</td>
<td>RBM</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>900</td>
<td>100X10^6</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>900</td>
<td>240X10^6</td>
<td>16</td>
<td>0</td>
</tr>
</tbody>
</table>

* IBM = isologous bone marrow.
RBM = rat bone marrow.
† Nucleated cells injected intravenously.

The table shows the effects of various treatments on the mortality of LAF1 mice irradiated with different doses of X-rays. The data are divided into treatment groups based on the type of treatment (IBM or RBM) and the dose of bone marrow cells injected. The cumulative mortality is given for each group at various time points after treatment.
immune mechanism in eliminating the foreign cells (antigen) before complete transplantation had occurred. This results in a mild reaction with little or no damaging effect on the host. Conversely, the killing effect of the high dose of $240 \times 10^4$ RBM cells in the $640 \text{r}$ animals may be explained by a greater degree of transplantation and proliferation of the injected foreign cells, which, in conjunction with the host's immune mechanism, results in a sustained dynamic in vivo antigen-antibody reaction. The effects of this reaction compounded with the radiation-induced injury leads to death.

In the experiments reported by Santos, Cole, and Roan (26), treatment of LAF$_1$ mice with $640 \text{r}$ only and $640 \text{r}$ plus $70 \text{mg.}$ of RBM yielded 30-day mortalities comparable to those obtained in this study with mice receiving $640 \text{r}$ only and $640 \text{r}$ plus $100 \times 10^4$ RBM cells. In the discussion of their inability to corroborate our data concerning the enhanced killing effect, Santos et al. refer to our publication (8) that predicted and showed the effect of $710 \text{r}$ and RBM treatment on (C3H $\times$ 101)$_1$F$_1$ mice. Although that study demonstrated the phenomenon clearly, we subsequently elaborated our working hypothesis in another report stating "injection of foreign bone marrow into x-radiated mice may result in an in vivo antigen ... antibody ... reaction, the degree (chronic or acute) and effects of which are dependent on the two variables (a) x-ray dose applied to the host and (b) number of bone marrow cells injected" (9). The dependency of the killing effect on these two variables was shown in that study. Strains of mice may also differ in their immunologic capacity toward soluble or particulate antigens (5, 6, 12), and Makinodan et al. (20–22) placed emphasis on this point, particularly in regard to radiation-bone marrow treatment studies. Thus, given the same experimental conditions, one cannot expect identical results in two different strains of mice. The increased mortality among sublethally irradiated RBM-treated mice, basically caused by an in vivo antigen-antibody reaction involving incompatibility of host-versus-graft tissue, is quite dependent on the rate and degree of recovery of the host's immune mechanism after irradiation and also the systemic injury sustained by radiation, factors that may vary among the strains of mice. The extreme sensitivity of this strain variation was made apparent in this laboratory where (101 $\times$ C3H)$_1$F$_1$ mice, given $710 \text{r}$ (LD$_0$) and $140 \times 10^4$ RBM cells, showed a 30-day mortality of 57 per cent, whereas similar treatment of the reciprocal hybrid, (C3H $\times$ 101)$_1$F$_1$ has always yielded 100 per cent mortality (Makinodan and Shekarchi, unpublished data). As is evident from this report, it would be theoretically possible to obtain 100 per cent mortality in the $(101 \times \text{C3H})_1$F$_1$ mice by slightly increasing the RBM dose.

The results obtained with the lethally irradiated LAF$_1$ mice receiving the two different RBM doses also substantiate our previous findings with the (C3H $\times$ 101)$_1$F$_1$ mice; i.e., a higher RBM dose yields a greater 30-day survival among lethally irradiated mice. Similar data showing greater percentage survival with increasing doses of foreign bone marrow into lethally irradiated mice have been presented by van Bekkum and Vos (1). What limit, if any, this treatment with a high dose of bone marrow has on increasing survival is not known; thus far, however, no deleterious effect on lethally irradiated mice has been observed with this experimental procedure. Again, strain differences may necessitate a qualification of the effectiveness of higher bone marrow doses in promoting survival. In the report by Santos et al., injection of penicillin (3840 units) on the day of bone marrow treatment lowered the 30-day mortality. As shown here, increasing the RBM dose to $240 \times 10^4$ cells also resulted in a significant decrease in the 30-day mortality. Although the therapeutic value of penicillin is to be expected in bone marrow protection studies, it would be difficult to explain on the basis of bacterial contamination the enhanced killing effect with the high dose of bone marrow in sublethally irradiated mice, on the one hand, and, on the other, the increased survival of lethally irradiated mice receiving the same bone marrow treatment. This phenomenon is plausible, however, if one accepts our basic hypothesis of a dynamic in vivo antigen-antibody reaction occurring in x-irradiated mice that have received foreign bone marrow.

**SUMMARY**

Injection of a high rat bone marrow (RBM) dose ($240 \times 10^4$) into LAF$_1$ mice that had received $640 \text{r}$ (LD$_0$) resulted in 100 per cent mortality 19 days after treatment. Injection of a smaller amount ($100 \times 10^6$) of RBM cells into similarly irradiated mice gave a mortality comparable to that obtained with x-rays only. Treatment of lethally irradiated ($900 \text{r}$) mice with the two different bone marrow doses showed a greater 30-day survival among those receiving the higher dose treatment. The data are discussed in terms of our hypothesis, which states that an in vivo antigen-antibody reaction may occur in irradiated...
mice treated with foreign bone marrow, depending on the amount and type of bone marrow and the x-ray dose.

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