Humoral Factor in Irradiation Protection: Modification of Lethal Irradiation Injury in Mice by Injection of Rat Bone Marrow

CHARLES C. CONGDON AND EGIS LORENZ

From the Laboratory of Biophysics, National Cancer Institute, Bethesda, Maryland, and the Argonne National Laboratory, Division of Biological and Medical Research, Lemont, Illinois

In earlier reports it was demonstrated that the injection of suspensions of normal bone marrow from the same species would prevent the death of animals exposed to total body irradiation. Data indicating protection are available on mice (1), rats (2), hamsters (3), and guinea pigs (4). The results obtained with bone marrow suspensions in irradiated rabbits and dogs were equivocal (4, 5).

Although the hypothesis that seeding of the damaged hematopoietic tissue with non-irradiated bone marrow is responsible for the recovery of the irradiated host is an attractive one, there is mounting evidence that other noncellular factors may be equally, if not more, important. Jacobson (6) postulated such factors following his observations that splenectomy of the shielded spleen 1 hour after irradiation still gave survival of his irradiated mice. Furthermore, he found evidence indicating that hematopoiesis in irradiated rabbits could be stimulated by introducing the spleens of immature mice intraperitoneally. Lorenz et al. (1, 7) also suspected that noncellular factors might be operating following their observation that intravenous injections of guinea pig bone marrow into irradiated mice gave protection, although sporadic. No effect, however, was observed from using dog and rabbit bone marrow in irradiated mice or from using rabbit bone marrow in irradiated guinea pigs. Nevertheless, since guinea pig bone marrow could not repopulate the damaged bone marrow of the mouse (7), a humoral factor, stimulating the damaged mouse hematopoietic tissue to regrowth was postulated. Experiments in which the guinea pig bone marrow failed were explained by the observation that transplanted heterologous bone marrow injected intraperitoneally degenerated within a few days after transplantation. In the two successful experiments it was presumed that the transplanted bone marrow stayed alive long enough to produce sufficient amounts of the hypothetical humoral factor. A search was undertaken for a heterologous bone marrow which regularly would protect irradiated mice; and rat bone marrow was found to fulfill this requirement. This paper is a report of the results obtained in these experiments.

MATERIALS AND METHODS

The irradiated mice were from the genetically homogeneous hybrid LAF1 and Strain A. Both male and female animals were used at approximately three months of age. The 30-day LD50 for LAF1 mice is approximately 650 r, and for Strain A mice approximately 560 r. In the present experiments listed in table I the mice received a tissue dose of 800 r or 900 r. From previous experience it was known that with very rare exceptions either of these exposures are 10-day LD100 for either type of mouse. The irradiation conditions were 186 kvp, 20 ma, beam filtration 0.25 mm Cu + 1.06 mm Al. Two tubes opposite each other were used to obtain a uniform tissue dose; their foci were 54 cm from the center of the mice. The dosage rate was 100 r/s. The bone marrow from the rat was prepared always by the same technique. Either male or female rats from 150-200 gm in weight were killed by decapitation and the humeri and femurs excised. The shafts were split open, and the bone marrow was lifted out and transferred to a watch glass containing buffered saline. A suspension which would pass through a 26-gauge needle was obtained by repeatedly forcing the mass of bone marrow through needles of different calibers.

The amount of bone marrow administered intravenously or intraperitoneally was approximately 20-30 mg by wet weight/mouse. It was noted that Osborne-Mendel rats had redder bone marrow than the other strains of rats used. In one experiment not listed in table I mice which had received 800 r followed by intraperitoneal injection of rat bone marrow were killed and autopsied daily for 10 days to observe the fate of the rat bone marrow in the peritoneal cavity of the mouse.

Received for publication September 14, 1953.

Autopsies were performed on representative animals in many of the experimental groups, and microscopic sections were prepared from most organs.

One group of nine animals from experiment 4, Table I had erythrocyte, leukocyte and differential counts three times a week following irradiation and intravenous injection of rat bone marrow.

RESULTS

Table 1 gives the 21-day survival data in seven experiments following injection of rat bone marrow into mice exposed to a lethal dose of total body irradiation. Excellent results generally were obtained with bone marrow from Osborne-Mendel rats injected intravenously into LAF1 mice. Bone marrow from other strains of rats injected into irradiated mice was less satisfactory in terms of 21-day survival. The intraperitoneal route of bone marrow injection was much less effective than the intravenous route.

Autopsies on representative experimental and control animals dying within the 21-day period showed the findings (8) associated with death due to total body irradiation, including destruction of hematopoietic and lymphatic tissues. Bacterial infection was common.

The animals in the experimental groups found dead after the 21-day period showed at autopsy regeneration of hematopoietic and lymphatic tissue, but in some instances bacterial infection developed in spite of the injection of bone marrow. In a few animals the cause of death was not apparent. The experimental animals that were killed and autopsied at 30-90 days in order to terminate the experiments showed regeneration of hematopoietic and lymphatic tissues.

In experiments 10 and 13 in which the rat bone marrow was given intraperitoneally hematopoietic cells were often found on the omental serosa. These cells showed mitotic activity and appeared to be producing blood forming cells. In a separate experiment not listed in Table I, 20 LAF1 mice were exposed to 800 r and given Osborne-Mendel rat bone marrow intraperitoneally. Two mice were killed and autopsied each day after irradiation for 10 days. These animals showed the same feature as noted in experiments 10 and 13 of Table I. The rat bone marrow was readily demonstrated throughout the 10-day period on the omental serosa or was sometimes attached to the pancreas. It appeared to be

<table>
<thead>
<tr>
<th>Exper.</th>
<th>Donor Rat Strain</th>
<th>Recipient Mouse Strain</th>
<th>Rate of Infection</th>
<th>No. of Animals</th>
<th>No. Dying on Indicated Number of Days</th>
<th>Mortality, %</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>800 Osborne-Mendel</td>
<td>LAF1</td>
<td>IV*</td>
<td>31</td>
<td>21</td>
<td>0</td>
<td>4 late deaths 48-56 days</td>
</tr>
<tr>
<td>2</td>
<td>800 Osborne-Mendel</td>
<td>LAF1</td>
<td>IV</td>
<td>15</td>
<td>1</td>
<td>0</td>
<td>3 late deaths 24-40 days</td>
</tr>
<tr>
<td>3</td>
<td>800 Osborne-Mendel</td>
<td>LAF1</td>
<td>IV</td>
<td>32</td>
<td>1</td>
<td>1</td>
<td>28 late deaths 24 days</td>
</tr>
<tr>
<td>4</td>
<td>900 Osborne-Mendel</td>
<td>LAF1</td>
<td>IV</td>
<td>31</td>
<td>2</td>
<td>1</td>
<td>2 dead 29 days, 1 dead 31 days</td>
</tr>
<tr>
<td>5</td>
<td>800 Osborne-Mendel</td>
<td>A</td>
<td>IV</td>
<td>31</td>
<td>2</td>
<td>1</td>
<td>2 dead 40 days, 1 dead 48 days</td>
</tr>
<tr>
<td>6</td>
<td>800 Osborne-Mendel</td>
<td>A</td>
<td>IV</td>
<td>31</td>
<td>2</td>
<td>1</td>
<td>2 dead 40 days, 1 dead 48 days</td>
</tr>
<tr>
<td>7</td>
<td>800 Sprague-Dawley</td>
<td>LAF1</td>
<td>IV</td>
<td>31</td>
<td>2</td>
<td>1</td>
<td>2 late deaths 24, 36 days</td>
</tr>
<tr>
<td>8</td>
<td>800 Sprague-Dawley</td>
<td>LAF1</td>
<td>IV</td>
<td>31</td>
<td>2</td>
<td>1</td>
<td>2 late deaths 24, 36 days</td>
</tr>
<tr>
<td>9</td>
<td>800 Sprague-Dawley</td>
<td>LAF1</td>
<td>IP</td>
<td>31</td>
<td>2</td>
<td>1</td>
<td>2 late deaths 24, 36 days</td>
</tr>
<tr>
<td>10</td>
<td>800 Sprague-Dawley</td>
<td>LAF1</td>
<td>IP</td>
<td>31</td>
<td>2</td>
<td>1</td>
<td>2 late deaths 24, 36 days</td>
</tr>
<tr>
<td>11</td>
<td>800 Sprague-Dawley</td>
<td>A</td>
<td>IV</td>
<td>31</td>
<td>2</td>
<td>1</td>
<td>2 late deaths 24, 36 days</td>
</tr>
<tr>
<td>12</td>
<td>800 Fischer</td>
<td>A</td>
<td>IV</td>
<td>31</td>
<td>2</td>
<td>1</td>
<td>2 late deaths 24, 36 days</td>
</tr>
<tr>
<td>13</td>
<td>800 Fischer</td>
<td>LAF1</td>
<td>IP</td>
<td>31</td>
<td>2</td>
<td>1</td>
<td>2 late deaths 24, 36 days</td>
</tr>
</tbody>
</table>

* Intravenous  † Intraperitoneal
viable and definitely recognizable as hematopoietic tissue.

Figure 1 gives the average erythrocyte and leukocyte counts on nine mice from experiment 4, Table 1. The 900-r control peripheral blood counts on LAF1 mice as well as the counts of those receiving homologous bone marrow were taken from an earlier experiment, and represent the average blood counts on 10 mice for each group. These data show essentially the same disappearance and return of peripheral blood cells for injection of heterologous bone marrow as was observed when homologous bone marrow was used.

DISCUSSION

The failure to obtain reproducible results in the protection of irradiated mice with guinea pig bone marrow (1, 7) left unsettled the question whether or not a humoral factor can operate across species barriers in protection against total body irradiation. The unequivocal demonstration in the present work that bone marrow from the rat will prevent the death of irradiated mice shows that the mechanism of protection is not species specific. However, the work on heterologous bone marrow suspensions also seems to indicate that the closer the relationship of the donor and the recipient species (mouse), the more likely the success of the postirradiation protection. This, however, may be entirely a function of the survival time of the heterologous bone marrow.

In the present experiments it seems highly improbable that the rat bone marrow cells repopulate the bone marrow of the mouse or transform into cells that repopulate the lymph nodes, splenic nodules or thymus of the mouse. From the observations on rat bone marrow injection intraperitoneally it seems clear that rat bone marrow survived in the peritoneal cavity of the mouse. In limited observations (1) on the fate of guinea pig bone marrow in the peritoneal cavities of irradiated mice, it was noted that necrosis of most of the injected bone marrow was the usual reaction, and only a few hematopoietic cells survived as long as seven days. If these data are considered in reference to intravenous injection in mice, a situation in which the injected cells cannot be identified histologically, it appears that the rat bone marrow cells have a greater chance of survival and hence of production of a humoral factor than guinea pig bone marrow cells. Should such an explanation be the correct one, then the humoral factor produced by the injected cells, if it can be isolated, may not have the species limitations that so far have been observed for the transplantation of cells of the bone marrow.

The effectiveness of rat bone marrow in irradiated LAF1 mice compares favorably with homologous bone marrow in irradiated LAF1 mice. However, the amounts of rat bone marrow injected in these experiments were approximately 20 times as great as was used in the earlier experiments with homologous LAF1 bone marrow. No data are available concerning the minimum amount of rat bone marrow necessary to give protection. The deaths occurring after 21 days were exceedingly rare with homologous bone marrow in LAF1 mice, but were occasionally observed in irradiated Strain A mice receiving homologous bone marrow (6). In the present work a few late deaths were observed in nearly every experiment including those in which irradiated LAF1 mice were used. Most of these were attributed to bacterial infection.

It is interesting to note that bone marrow
of Osborne-Mendel rats afforded the best protection. It was observed that the suspensions prepared with bone marrow of this strain were darker red than those of the two other strains. It may be that there are strain differences among rats as to the amount of blood forming tissue present in the femurs and humeri at a given body weight. The gross observation that Osborne-Mendel rats had a redder bone marrow than the other strains supports this idea. However, to evaluate strain differences in rat bone marrow more detailed study than that presented here would be required.

CONCLUSIONS

The data presented give further evidence for a humoral factor in postirradiation protection by injection of bone marrow suspensions. Total body exposures of 800 r and 900 r are 100% lethal to LAFl and Strain A mice. Using rat bone marrow the following mortalities were observed in irradiated mice. Bone marrow from Osborne-Mendel rats given intravenously to irradiated LAFl mice resulted in 0-30% mortality at 21 days. In irradiated Strain A mice, bone marrow from Osborne-Mendel rats resulted in a 45% mortality at 21 days. Bone marrow from Sprague-Dawley rats given intravenously to irradiated LAFl mice gave 30-80% mortality at 21 days. When given intraperitoneally a 100% mortality occurred. Sprague-Dawley rat bone marrow given intravenously to irradiated Strain A mice gave an 85% mortality at 21 days. Bone marrow from Fischer rats did not alter the 100% mortality of irradiated Strain A mice when given intravenously. The same type of bone marrow given intraperitoneally to irradiated LAFl mice resulted in 86% mortality at 21 days.

REFERENCES

9. LORENZ, E. AND C. C. CONGDON. Unpublished data