Experimental Treatment of Total-body Irradiation Injury: A Brief Review

By C. C. Congdon

WIDE INTEREST in the experimental treatment of total-body irradiation injury by postirradiation injection of living blood-forming cells is evident from the many reports in this field. Discussions among biologists and medical workers indicate an active interest in the potential applications of this particular radiobiologic research to human disease.

The results of an animal experiment in this laboratory illustrate how dramatic the experimental treatment can be in preventing death from the acute radiation syndrome (table 1).

Treatment of total-body injury from x-rays has been most extensively studied. However, injection of bone marrow cells was also effective in lethal external gamma and fast neutron exposures.1, 2 Acute mortality from the mixed radiations given off by intravenous radon in equilibrium with its decay products was reduced by administration of bone marrow at the proper interval after the radon injection.1

The amount of bone marrow or other blood-forming tissue injected after a lethal exposure determines the percentage survival at 30 days. The data in mice vary somewhat from one strain to another or from one F1 hybrid to another, probably depending on variation in individual resistance to irradiation. In general, 1 to $10^6$ nucleated bone marrow cells gives optimum 30-day survival in mice after exposure to 900 r of x-rays. However, as few as 50,000 nucleated blood-forming cells will allow a low percentage of mice to survive 30 days in some experiments.3-4 If, instead of 30-day survival, the bone marrow of the irradiated host is quantitatively studied to determine the effect of different doses of bone marrow administered, response is proportional to dose over a much greater range than when survival is used as the endpoint.3

The effective cell type, or types, in the administered blood-forming tissue has not been clearly determined. Since dividing cells are necessary, the stem cells and reticulum cells of the hematopoietic tissues are implicated.5

The intravenous route of injection gives the best 30-day survival in mice and guinea pigs. Intraperitoneal injection is effective but not so effective as intravenous. Intrapleural and intrasplenic injections were also effective, but intramuscular and subcutaneous injections did not cause survival of lethally irradiated mice.6

The time of injection of bone marrow may be delayed in lethally irradiated mice for at least 3–4 days after exposure (see reference 1 and unpublished data by Congdon). With massive doses of bone marrow, this period could probably be extended a few more days and still get recovery of some animals. In chronic

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TABLE 1.—Effect of Bone Marrow Treatment on Survival after Lethal Irradiation

<table>
<thead>
<tr>
<th>No. of mice*</th>
<th>X-ray dose (c)</th>
<th>Treatment</th>
<th>30-day survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>900</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>10</td>
<td>1100</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>1300</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>900</td>
<td>Bone marrow†</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>1000</td>
<td>Bone marrow</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>1100</td>
<td>Bone marrow</td>
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<tr>
<td>10</td>
<td>1400</td>
<td>Bone marrow</td>
<td>50</td>
</tr>
</tbody>
</table>

* Female C3H × 101F, hybrid mice about 3 months of age.
† Approximately 12 × 10⁶ normal isologous bone marrow cells given intravenously to each mouse after x-irradiation.

gamma irradiation of guinea pigs, the bone marrow was administered several months after the start of the irradiation.¹

Treatment with bone marrow permits about a doubling of the LD₅₀ 30-day exposure. In (L × A)F₁ mice 3 months of age, the control LD₅₀ 30-day value was 641 r (617 r, 668 r), and with bone marrow treatment, the value was 1155 r (1100 r, 1230 r) (Congdon, unpublished data).

When preirradiation treatment with a dose-reducing chemical agent was given and then bone marrow was injected after irradiation, the LD₅₀ 30-day value was nearly tripled.⁷

Preservation of living blood-forming cells and tissue culture of these cells was studied by several groups. The preserved or cultured cells were tested by their ability to cause recovery of lethally irradiated animals.

Short-term preservation of bone marrow cell suspensions at 2–5 C. was good for about 4 days.⁸ Long-term preservation of blood-forming tissues in frozen glycerol was good for at least 83 days.⁹ Tissue-cultured bone marrow cells were effective after 21 days in culture at 25 C.¹⁰

It has been known for many years that exposure of the total body to ionizing radiation may result in death within a few weeks from the destruction of blood-forming tissues. The inability of these tissues to produce peripheral blood elements is frequently the immediate cause of death, since the low granulocyte levels result in ulceration, bacteremia, and often a fatal septicemia. Similarly, massive fatal hemorrhage may ensue from the rapidly dropping blood platelet levels. If the exposed animal lives long enough, anemia appears through loss of elements in the bone marrow that produce red blood cells. The lethally irradiated animal that has had a small portion of its blood-forming tissue shielded during the irradiation or that has received a postirradiation injection of living blood-forming cells does not die, because the destroyed bone marrow is replaced in a few days by active blood-forming cells, which replenish the peripheral blood elements and thus prevent the infection, hemorrhage, and anemia (fig. 1).¹¹, ¹²

Early in the development of this field of research by Jacobson,¹³ Lorenz,¹⁴ and others the mechanism of action of the shielded or injected blood-forming cells
was thought to be either humoral stimulation or cellular transplantation. In the first hypothesis, the shielded or injected cells stimulated the damaged bone marrow to quick recovery from injured primitive precursors. In the second hypothesis, the injected cells transplanted to the destroyed blood-forming tissues and by subsequent growth completely replaced the destroyed tissue. These hypotheses were generally thought to be mutually exclusive and focused attention from the start on the most basic aspect of the experiments. A number of reviews treating the then existing data in terms of these hypotheses have appeared.\(^6,^{12,15,16}\) Many attempts were made to show that chemicals and components from blood-forming or non–blood-forming tissues would cause recovery from total-body irradiation injury in a way that would exclude the transplantation-repopulation hypothesis.\(^{13–18}\) Some of these experiments have still not been completely evaluated, and important work continues along these lines. Numerous indirect attempts were also made to establish the humoral hypothesis. These include the interspecies experiments with bone marrow,\(^{20}\) bone,\(^{21}\) and spleen,\(^{22}\) the use of tumor tissue to cause recovery from radiation injury;\(^4\) and the use of mature peripheral blood elements.\(^5\)

A series of experiments were carried out that established that injection of living blood-forming cells into the blood stream of an irradiated animal is followed by localization of the injected cells in the destroyed blood-forming tissues. The donor cells multiply in these tissues and produce peripheral blood elements of the donor type.
Three experiments were performed: (1) isologous—in which the recipient and donor mice were of the same inbred strain, (2) homologous—in which the recipient and donor animals were of the same species but of different strain or genetic constitution, and (3) heterologous—in which bone marrow cells from a normal rat were given to an irradiated mouse. The last two experiments are feasible because irradiation of the total body temporarily injures the immune response of the recipient.

Evidence for prompt localization after intravenous injection comes from several laboratories. Nowell and co-workers, using the heterologous “rat-to-mouse” experiment, could identify selectively the donor cells by alkaline phosphatase staining. They observed within a few hours after injection of rat bone marrow that the irradiated mouse had rat-type cells in his bone marrow and that the recovered bone marrow contained nearly 100% rat-type cells by 15 days after irradiation. In a homologous “mouse-to-mouse” experiment, Merwin and Congdon found that the recipient irradiated mouse had donor-type cells in his blood-forming tissues within a few hours after injection and were still present at 180 days. In these experiments, an immunologic tissue transplantation test was used to demonstrate the presence of the donor cells. At Harwell, Ford and associates found transplantation of blood-forming cells in a homologous “mouse-to-mouse” experiment. They used a cytologic marker, a chromosome translocation for identifying the blood-forming cells of the donor in the recipient mouse. These workers also reported in the heterologous “rat-to-mouse” experiment that the chromosomes of the bone marrow of the recovered mouse were of the rat type both in shape and number.

Graevsky has reported that intravenous injection of blood-forming cells would raise sharply the low spleen cell and bone marrow cell counts of animals irradiated 2 days earlier. This has been confirmed by others.

Equally important are the demonstrations of the character of the peripheral blood elements; i.e., whether they are of the donor or recipient type in the recovered irradiated animal. Lindsley et al. have found in the homologous “rat-to-rat” experiment, where the donor rat had red blood cells of one antigen type and the irradiated recipient rat had red blood cells of another type that the peripheral blood of the irradiated recipient developed red blood cells of the donor type after intravenous injection of bone marrow from the donor animal. In the “rat-to-mouse” experiment, Makinodan observed that the red blood cells in the irradiated mouse were rat type in their response to the appropriate anti-red blood cell serum and that the red blood cells of the mouse type disappeared. He further demonstrated that the serum proteins in this experiment remained those of the recipient. Vos et al. also found rat red blood cells in irradiated mice given rat bone marrow. Nowell et al. showed that the peripheral blood leukocytes in the “rat-to-mouse” experiment were of the rat type, as determined by their alkaline phosphatase staining, and remained so for as long as 16 months after injection.

Smith, Makinodan, and Congdon demonstrated that the blood platelets were of the donor type in the “rat-to-mouse” experiment. It should be mentioned that histologic evidence of local transplantation of bone marrow in irradiated animals, even between species, has been in existence for some time.
ments reported here go considerably beyond this in demonstrating orthotopic transplantation after intravenous injection and the subsequent production of peripheral blood elements of the donor type.

New and interesting problems have been raised by the establishment of the transplantation-repopulation theory. The cell type, or types, in the bone marrow responsible for this remarkable transplantation has not been determined. The rapidity and uniformity of the repopulation suggest a basic cell that can go to any blood-forming site and develop into the blast cell types of the bone marrow, or that interchange of blast cells between blood-forming tissue sites is rapidly and easily accomplished.

Application of the transplantation-repopulation theory to spleen and thigh shielding experiments and pre- and postirradiation parabiosis would indicate that the latter situation is possible.

The nature of the recovered immune mechanism in the irradiated animal needs to be determined. Preliminary work indicates that the immune response remains that of the irradiated recipient rather than becoming that of the donor animal. The lymphatic tissues are one of the main problems that remain to be settled.

Merwin and Congdon reported donor-type antigen, presumably representing donor-type cells in the lymph nodes and thymus of irradiated mice receiving homologous bone marrow. Ford et al. observed chromosomes of the donor type in the lymph nodes and thymus of irradiated mice receiving homologous spleens.

The presence of pathologic changes in lymph nodes and splenic white pulp and thymus of most irradiated mice receiving foreign bone marrow makes interpretation of the work of Merwin and Congdon and Ford et al. difficult. Extramedullary granulopoiesis in lymph nodes of mice recovering from irradiation injury is often seen and might account for the finding of donor-type cells in the lymph nodes. It becomes particularly important to settle this matter since bone marrow transplantation and spleen shielding will prevent radiation-induced thymic leukemia in the mouse.

The violent secondary reactions that appear in many irradiated mice receiving foreign bone marrow are another major problem in this general field of research. It was observed in lethally irradiated mice given rat bone marrow that after apparent recovery from the radiation injury, the mice often died at intervals of 3 weeks to 100 days after exposure. A similar situation was seen in irradiated mice that received bone marrow from a foreign strain.

The secondary reactions referred to as delayed foreign bone marrow reactions are characterized by temporary recovery of the irradiated mice generally paralleling that seen with nonforeign (isologous) bone marrow transplantation. During the period of temporary recovery bone marrow and peripheral blood elements return to normal or nearly normal status. The reaction begins about 3 weeks after exposure with a steady loss of body weight in the presence of normal food intake. Graying of hair that occurs in isologous bone marrow-treated mice does not take place in a uniform manner. The animals are ruffled, and dermatitis is often present. Autopsy revealed extreme atrophy of lymphatic tissues as an end stage of severe reactive changes in lymph nodes and white pulp of the spleen. These changes were interpreted as representing a reaction of the host animal's
immune mechanism to the foreign bone marrow and blood elements. The radiation injury to the immune mechanism is only temporarily damaged at these exposure levels. The severe metabolic disorder of weight loss in the presence of a normal food intake has not been satisfactorily explained, and it is not known whether this represents a part of the immunologic reaction.

The current status of the humoral hypothesis for bone marrow recovery in irradiation injury deserves comment. It is no longer useful, operationally, to think of the problem in terms of the original mutually exclusive hypotheses. For many years, hematologists have considered the possibility that one or more phases of blood formation were under humoral or endocrine-like control, with feedback mechanisms for maintaining appropriate levels of peripheral blood elements. It was natural to look for evidence of these concepts in bone marrow recovery after lethal irradiation. Developments suggest that erythropoietic stimulating factors exist in the plasma of appropriately stressed animals to which irradiated marrow can respond.40

A number of unrelated, nonliving materials ranging from ground glass41 to inactivated virus42 seem to influence survival from radiation injury at some exposure levels.18-41 Possibly in the same category is the demonstration that mouse spleen cells will enhance survival in irradiated rabbits.42 Mechanism studies in all these experiments and others like them not cited in this review have been very limited. No adequate explanation for the results observed exists at the present time. None of these experiments, however, have been so successful as the treatment of lethal irradiation injury with living blood-forming cells. The search for nonliving materials that will duplicate the recovery from lethal irradiation caused by injection of living blood-forming cells remains a vital area for advancement of the treatment of irradiation injury.

The human application of the findings developed by this area in experimental research has received consideration from the outset by most people familiar with the field. No doubt we could more easily contemplate human clinical research had we achieved by this time a humoral substance that would reproduce the effect of injecting living bone marrow cells as shown in figure 1. Lacking this, we are confronted with the most remarkable finding that an entire organ system destroyed by irradiation can be replaced by intravenous injection of a few normal cells of the same type. It is of additional interest that Maxwell and Weston43 showed that chemical injury to bone marrow in the rat, like irradiation damage, can be reversed by homologous bone marrow injections.

SUMMARIO IN INTERLINGUA

Es presentate un revista del labores complite in le campo del tractamento experimental de lesiones de irradiation del corpore integre—irradiation roentgen e altere—per varie methodos de injection postirradiational de vive cellulas hematopoietico.

Es citate le resultatos dramatic obtene al Laboratorios National Oak Ridge e alterubi in prevenir morte post irradiation per injicar medulla ossee e altere tessutos hematopoietico in le animales studiate. Es presentate le conclusiones publicate per varie autores in re le aspectos quantitativo e chronologic del injectiones. Studios relative al preservabilitate del material a injicar es reportate.
Le autor discute experimentos in re le localisation del cellululas injicite e in re le relation inter animal donator e animal recipiente. (Le irradiation del corpore integre paralysa temporarimente le responsa immunologic del recipiente contra injectiones de tessuto non compatibile.) Es alludite in conclusion a effortios a trovar substantias mineral de efficacia simile a illo del injectiones de cellulas hematopoietic.

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