Patterns of Hemopoietic Recovery in Irradiated Mice

By WILLIE W. SMITH, ROBERT Q. MARSTON AND JEROME CORNFIELD

With the assistance of ILO M. ALDERMAN, RUTH E. GILLESPIE and H. JEANETTE RUTH

THE RECENT FINDING that an injection of endotoxin induces hemopoiesis in irradiated mice and hamsters,1 and the observations of Brown, Hirsch and their associates2,3 and of Congdon and Urso4 that homologous and heterologous marrow do not promote thymic regeneration and recovery of lymphatic tissue, have renewed our interest in patterns of hemopoietic recovery in irradiated animals under various conditions which influence their survival. Reports have been made which cover the effect on survival of numerous factors in the administration of bone marrow and spleen homogenate (e.g.5,7) and in several instances, the course of leukocyte restoration.5,8,9 Quantitative studies to be described here demonstrate several points not previously clear.

In this report recovery times of circulating granulocytes and lymphocytes are compared when the following conditions are varied: 1.) radiation dose; 2.) spleen homogenate injection, varying radiation dose to recipient, time of injection relative to irradiation, and number of cells injected; 3.) species of donor for bone marrow injection: isologous vs. heterologous; and 4.) induction of recovery by an injection of endotoxin or colchicine.

In addition, recovery of platelet and of hemoglobin concentrations are compared in experiments with isologous and heterologous bone marrow, endotoxin and colchicine.

PROCEDURES

The mice used were females, 3 to 4 months old, of the LAF1 or (BALB/c x DBA/2)F1 (for brevity hereafter designated CDBA strain). They were caged individually, irradiated in groups of 10 to 20, and distributed among treatment groups so that possible variations due to prior caging and grouping were neutralized. All radiation doses were delivered by an integration ionization chamber circuit which was calibrated just before each experiment against a thimble ionization chamber placed in the position to be occupied by the mice. For the experiments listed under 1) and 2) above, we used a clinical x-ray unit operating at 200 KvP, 20 ma., with 0.51 mm. A1 and 0.25 mm. Cu added filtration, HVL 0.76 mm. Cu. Target distance was 50 cm. and dose rate about 55 r per minute. For the remainder of the experiments the Van de Graaff generator described earlier,9 operating at 2.5 MVCP and 0.6 ma., HVL 1 cm. lead, was used. The animals were exposed on a rotating plastic disc 1 meter from the target at a dose rate of 250 to 300 r per minute.*

From the Radiation Branch, National Cancer Institute and Division of Research Services, National Institutes of Health, Bethesda, Md.

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*We are indebted to Dr. Howard L. Andrews for dosimetry and operation of the Van de Graaff generator.
The endotoxin used was a lipopolysaccharide from *Salmonella typhosa* supplied through the generosity of Difco Laboratories. The colchicine and trimethylcolchicine acid methyl ether d-tartrate were preparations #1v and 1136 of reference1. Spleen homogenate was prepared as previously described. Bone marrow was obtained from the shafts of the long bones and suspended in Tyrode's solution. Spleen homogenate and marrow cells were given intravenously, the endotoxin and colchicine intraperitoneally. Streptomycin was given in some of the experiments in order to extend the dose range over which spontaneous recovery could be studied. Cell counts were made from tail blood. Platelets were counted according to the method described by Brecher and Cronkite, with the exception that the glass was not siliconed. Hemoglobin was determined by the Haden-Hausser method.

*Estimation of end point for lymphocyte and granulocyte recovery.*—Lymphocyte and granulocyte counts of LAF1 mice given 475 r (LD₁) or 725 r (LD₉₀) of 200 KeV x-rays are shown in figure 1 for the portion of each recovery curve which appeared to be exponential. The use of a single end point for each constituent studied would greatly simplify comparisons. In the mice given 475 r we observed that the times at which granulocyte counts had recovered to 500 and lymphocyte counts to 1,260 cells per cu.mm. were approximately the same (14 days). In the mice given 600 r or 725 r the times at which granulocyte counts had recovered to 500 and lymphocyte counts to 1,260 were also approximately the same: 19 days for 600 r and 28 to 29 days for 725 r. The times at which these counts were reached in the course of a progressively increasing series of

*Values for lethality refer to deaths of untreated mice within 28 days of exposure. Mice in the experiments shown in figure 1 were given streptomycin, 2.5 mg. per day, subcutaneously, for 2 to 3 weeks, which decreased mortality after 600 r to 10 per cent and after 725 r to 80 per cent. As nearly as we could determine, streptomycin influenced counts only by increasing the number of animals alive at a given time.
counts were, therefore, adopted as end points for recovery. These particular counts were chosen for convenience and have no implications related to physiologic recovery.

The end points were estimated separately for each treatment and cell type by fitting, by least squares, straight lines of the form

\[ \log \text{count} = a + bt, \]

where \( t \) is time in days, to that portion of the recovery curve which appeared to be exponential. The end point for lymphocytes is then

\[ (\log 1260 - a) \div b \]

and for granulocytes \((\log 500 - a) \div b\). Confidence limits for these estimates are then obtained by a straightforward application of Fieller's theorem.

Inspection of results (tables 1 to 4) shows that although the recovery times, so defined, ranged from 6 to 29 days, the difference between granulocyte time and lymphocyte time of recovery was usually small in mice recovering spontaneously or after the injection of isologous cells. The span covered by the 95 per cent confidence limits for granulocyte and lymphocyte end points averaged 2.0 days or 16 per cent of the recovery time in 22 groups of mice. A single group \((900 \text{ r}, 2.5 \text{ Mev}, \text{table 4})\), in which the number of counts was small, is not included in this average.

The times at which platelet and hemoglobin concentrations departed from a downward trend were taken as the end points for their recovery. Lines for the decreasing and increasing portions of the curve were separately fitted by the method of least squares and the time of intersection of the two lines computed. Confidence limits are again given by Fieller's theorem.

**RESULTS**

Radiation dose strongly affects the spontaneous leukocyte recovery time but not the time at which the increase in leukocytes is brought about by injected spleen homogenate. Table 1 shows recovery times in two experiments with LAF1 mice in which the recipients were given isologous homogenate following exposure to 475 to 725 r. The delays observed at the higher radiation doses were relatively small. Similar results have been reported in hamsters.\textsuperscript{15}

These results suggest a fixed time for leukocyte repopulation by the transplanted cells as far as the condition of the host is concerned (within reasonable limits, of course), a view which was tested further by irradiating the mice 6 days or 3 days before giving the injection of homogenate (table 2). Results again indicate that these recovery times are relatively insensitive to effects of irradiation upon the host.

The last study in the series of experiments with injected spleen homogenate was made to determine the effect of the number of cells injected upon leu-

<table>
<thead>
<tr>
<th>r, 200 Kev</th>
<th>Streptomycin Only</th>
<th>36 x 10^6</th>
<th>90 x 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gran. Lymph.</td>
<td>Splen Cells</td>
<td>Splen Cells</td>
</tr>
<tr>
<td>475 r</td>
<td>14</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>600 r</td>
<td>20</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>725 r</td>
<td>29</td>
<td>13</td>
<td>10</td>
</tr>
</tbody>
</table>

Mean span of 95 per cent confidence limits, 1.9 days.
TABLE 2.—Time in Days of Recovery to 500 Granulocytes or 1,260 Lymphocytes per cu.mm. (95% Confidence Limits) with Increasing Delays in the Time of Injection

<table>
<thead>
<tr>
<th>Time of Injection</th>
<th>Immediately after Irrad.</th>
<th>3 Days after Irrad.</th>
<th>6 Days after Irrad.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocyte</td>
<td>10 (9–12)</td>
<td>12 (11–12)</td>
<td>15 (14–16)</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>9 (8–10)</td>
<td>12 (11–13)</td>
<td>15 (14–16)</td>
</tr>
</tbody>
</table>

LAF1 mice, 600 r, 200 Kev exposure on different days, 60 × 10⁶ donor cells on the same day.

kocyte recovery time. Results are shown in figure 2. Under the conditions of our experiments the maximum effect was reached with the injection of approximately 22 million nucleated cells, the number obtained from about one-third of a donor spleen, and amounted to a reduction of 9 days or 46 per cent of the time required for spontaneous recovery. Half that number of donor cells was almost equally effective.

Using the same criteria for granulocyte and lymphocyte recovery we compared the effect of isologous and heterologous bone marrow cells in LAF1 mice (fig. 3). Three groups of 30 mice were irradiated with 850 r, 2.5 Mev X-rays, after which group I was injected with 70 × 10⁶ mouse cells and group II with 100 × 10⁶ Osborne-Mendel rat cells. Rat granulocytes were distinguished by the presence of granules containing alkaline phosphatase.¹⁶ Fifteen “takes” and 10 “non-takes” survived long enough to be used in the study.

The time of granulocyte recovery in mice with isologous marrow was advanced from 19 days to 7 days after irradiation, as shown in table 3. Circulating rat granulocytes in “takes” reached 500 cells per cu.mm. in 8 days. Recovery time in “non-takes” did not differ significantly from that in controls. The time at which hemoglobin concentration began an upward trend was also the same in “takes” as in mice given isologous marrow: 10 days as compared to 16 and 15 days for controls and “non-takes.”

The lymphocytes in “takes” failed to show an advance in recovery time. A cell concentration of 1260 per cu.mm. was reached in 6 days in mice given isologous marrow as compared to 25 days for “takes” and 21 days for controls. This observation is of particular interest in view of the findings of Brown and Hirsch and their associates and of Congdon and Urso regarding the poor recovery of lymphatic tissue in mice given marrow from donors of a foreign strain.²⁴

An unexpected finding in this experiment was the statistically significant failure of platelets in “takes” to show an advance in recovery time comparable to that seen for granulocytes and hemoglobin.

The pattern of hemopoietic recovery in mice given an injection of endotoxin or colchicine prior to irradiation was studied in the last group of experiments to be described here. The advance in bone marrow cellularity, granulocyte and platelet count and hemoglobin concentration induced by an endotoxin injection has been previously reported,¹ as well as the increase in survival following the use of either endotoxin¹⁰ or colchicine.¹⁷,¹⁸

*We wish to thank Miss Marie Grenan for making these preparations.*
The chief points of interest in this group of experiments, shown in figure 4 and table 4 are:

1. No distinct difference in recovery pattern was detected between treatment with endotoxin and colchicine.

2. The recovery times for granulocytes and hemoglobin were as early in mice given endotoxin as in mice given isologous bone marrow (see LAF1 mice, 850 r, tables 3 and 4). Lymphocytes did not respond favorably in the LAF1 mice given endotoxin but showed varying degrees of improvement over controls in other experiments with endotoxin or colchicine. Platelet recovery was significantly advanced, but we do not have data for comparison at the higher radiation doses.

3. Radiation dose, within the limits of our experiments, was not an important determinant of granulocyte recovery time in the mice treated with endotoxin or colchicine. Recovery to 500 cells occurred 10 days later in CDBA controls given 900 r than in those given 700 r, while in corresponding groups treated with the colchicine derivative, colchicine or endotoxin it occurred in 4 to 5 days regardless of the radiation dose. Lymphocyte recovery time was also relatively unaffected by the 200 r difference in radiation dose.

**DISCUSSION**

The time of hemopoietic recovery is the most important determinant of survival in mice receiving LD$_1$ to LD$_{90}$ irradiation. The fact that different treatments may alter the recovery times of the various components independently is of considerable interest not only in radiation biology but in immunology and hematology as well.
Fig. 3.—Granulocyte, lymphocyte, platelet and hemoglobin concentrations in LAF1 mice given bone marrow from mice of the same strain or from Osborne-Mendel rats. Only the cells showing alkaline phosphatase granules (about 85 per cent of the total) were counted in the granulocytes of “takes.” Controls and mice given rat marrow were treated with streptomycin.

Table 3.—Time (in Days) of Recovery to 500 Granulocytes of 1,260 Lymphocytes per cu.mm. (95% Confidence Limits) and Time at which the Slope for Recovery Intersects the Downward Slope for Log Platelet Count or Hemoglobin Concentration in LAF, Mice Injected with Bone Marrow from the Same Strain or from Osborne Mendel Rats following Exposure to 850 r 2.5 Mev X-Rays

<table>
<thead>
<tr>
<th></th>
<th>Irad. Control</th>
<th>Mouse Marrow</th>
<th>“Takes”</th>
<th>Rat Marrow “Non-Takes”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocyte</td>
<td>19 (18-20)</td>
<td>7 (6-7)</td>
<td>8 (8-10)*</td>
<td>17 (16-19)</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>21 (16-28)</td>
<td>6 (5-8)</td>
<td>25 (22-40)</td>
<td>20 (18-21)</td>
</tr>
<tr>
<td>Platelet</td>
<td>11 (10-12)</td>
<td>6 (5-7)</td>
<td>11 (10-12)</td>
<td>11 (10-12)</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>16 (15-17)</td>
<td>10 (2-11)</td>
<td>10 (5-11)*</td>
<td>15 (9-17)</td>
</tr>
</tbody>
</table>

*Based on rat granulocytes only, which averaged 85 per cent of the total.
†68 per cent confidence limits. Other limits are for 95 per cent confidence.

It was possible to choose, empirically, end points for recovery time which were essentially the same for granulocytes and lymphocytes of mice recovering spontaneously after various radiation exposures, namely, the time at which granulocytes reached 500 and lymphocytes 1,260 per cu.mm. in the course of recovery. Granulocyte and lymphocyte recovery, evaluated in this way, also occurred simultaneously in mice treated with isologous spleen homo-
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Fig. 4.—Granulocyte, lymphocyte, platelet and hemoglobin concentrations in CDBA mice given 10 μg. S. typhosa endotoxin 24 hours before or 40 μg. colchicine 48 hours before irradiation.

TABLE 4.—Recovery Times for Granulocytes, Lymphocytes, Hemoglobin and Platelets in Mice Treated with Endotoxin, Colchicine or a Colchicine Derivative prior to Irradiation

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Treatment</th>
<th>Recovery Time, Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Granulocyte</td>
</tr>
<tr>
<td>LAF1 850 r</td>
<td>Control</td>
<td>20 (19-22)</td>
</tr>
<tr>
<td></td>
<td>Endotoxin*</td>
<td>7 (6-7)</td>
</tr>
<tr>
<td>CDBA 700 r</td>
<td>Control</td>
<td>11 (10-12)</td>
</tr>
<tr>
<td></td>
<td>Endotoxin*</td>
<td>4 (4-5)</td>
</tr>
<tr>
<td></td>
<td>Colchicine†</td>
<td>5 (3-6)</td>
</tr>
<tr>
<td>CDBA 900 r</td>
<td>Control</td>
<td>21 (18-27)§</td>
</tr>
<tr>
<td></td>
<td>Colchicine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Derivative†</td>
<td>5 (4-6)</td>
</tr>
</tbody>
</table>

*10 μg. S. typhosa i.p. 24 hrs. before irradiation.
| 40 μg. i.p. 48 hrs. before irradiation.
| 1 mg. trimethylcolchicinic acid methyl ether d-tartrate i.p. 24 hrs. before irradiation.
| §66 per cent confidence limits. Other limits are for 95 per cent confidence.

genenate or bone marrow regardless of radiation dose to the host, number of cells injected or time of injection.

These findings are in marked contrast to the asynchronous condition seen after the successful administration of heterologous marrow, in which neither lymphocytes nor platelets showed an accelerated recovery. It seems obvious
that the isologous cells repopulate lymphatic as well as myelocytic tissue, since these elements show comparable recovery in the circulating blood. Under the conditions of our experiment with heterologous marrow, however, the host must either have rejected the lymphocyte precursors or failed to support their growth. Differences between the response to graded radiation doses in lymph nodes, which is dose-dependent regardless of species, and bone marrow, which is correlated with mortality regardless of species, have been reported by De Bruyn\textsuperscript{19} and Brecher and Cronkite.\textsuperscript{20} Differences in their recovery following the administration of heterologous as compared to isologous bone marrow have been mentioned earlier.\textsuperscript{21} Gengozian and Makinodan\textsuperscript{22} and LaVia et al.\textsuperscript{23} have reported a delayed ability to produce antibodies in animals treated with foreign as compared to isologous cells.

In the chimeras of LAF1 mice given Osborne-Mendel rat marrow following exposure to mid-lethal radiation there was, in addition, a failure of new platelet production. Since total rejection, even for granulocytes, occurred in about half of the mice injected with rat marrow, it seems possible that the specific failure of lymphocyte and platelet generation may represent selective rejection of certain hemopoietic components. The observations have a bearing not only upon the question of differential antigenicity of precursor cells and cells in the course of differentiation, but also upon the origin of granulocytes, lymphocytes, erythrocytes and platelets. If, for example, the same precursor gives rise to granulocytes and lymphocytes but only granulocytes appear in the circulation, then possibly the cells of a later stage of lymphocytic differentiation are intolerably antigenic. The same might apply also to foreign megakaryoblasts. Congdon\textsuperscript{23} describes an acute tissue reaction in the white pulp of the spleen in mice given foreign bone marrow which, he suggests, may be related to the prompt destruction of injected foreign cells. Rat plasma and serum did not produce the reaction.

Turning to recovery induced by endotoxin or colchicine in the irradiated animal’s own damaged hemopoietic tissues, one sees an asynchronous condition again. Here recovery times of granulocyte, hemoglobin and platelet concentrations were comparable to those observed in mice given isologous bone marrow, while lymphocytes showed a recovery time generally earlier than that of the controls but not as early as that of mice given isologous marrow.

When cells of the hemopoietic system are derived from donor cells, the degree of radiation damage to the host has relatively little influence on the time required for repopulation. When the irradiated animal’s own cells are the parents of the new generations, however, it seems reasonable to expect that the increasing damage assumed to accompany increasing radiation would exert a major influence on recovery time. We can only state that this was not the case in the experiments with endotoxin and colchicine. Survival data in mice pretreated with a colchicine derivative\textsuperscript{18} show an abrupt termination of the beneficial effect at essentially the same radiation level at which the effectiveness of isologous bone marrow comes to an end. Clearly, endotoxin and colchicine are not “dose reducing” in the sense suggested for sulfhydryl
compounds.\textsuperscript{24} They tend, rather, to have an effect more nearly like the all-or-none effect of injected isologous hemopoietic cells.

Tissue reactions to foreign cells cannot, of course, be responsible for the asynchrony in recovery observed in the mice treated with endotoxin or colchicine. These treatments apparently represent cases of selective hemopoietic activation and may exert their effect through the same, as yet unknown, mechanism. Other chemically dissimilar substances may be capable of producing the same or still other patterns of hemopoietic recovery. Such differing patterns can be useful in clarifying both qualitative and quantitative aspects of the functions of the various components involved, and may also contribute pertinent information to some of the perplexing problems of hematologic cell lineage.

**SUMMARY**

We have demonstrated three distinct patterns of altered hemopoietic recovery in irradiated mice:

1. That seen in mice given isologous hemopoietic cells, in which granulocytes, lymphocytes, hemoglobin and platelets all showed an early recovery. The time required for granulocyte and lymphocyte recovery was relatively unaffected by the amount of radiation to which the host was exposed (LD\textsubscript{1.99}).

2. That seen in mice given rat bone marrow, in which only granulocytes and hemoglobin showed an early increase in concentration, with lymphocytes and platelets recovering near the control times.

3. That seen in mice given endotoxin or colchicine prior to irradiation, in which the lymphocyte recovery was variable while the granulocytes, hemoglobin and platelets compared favorably with mice given isologous bone marrow.

**SUMMARIO IN INTERLINGUA**

Nos ha demonstrate tres distincte configurationes de alterate restauration hematópoietic in muses irradiate:

1. Le configuration monstrate in muses que recipeva isologe cellulas hematopoietic. Le granulocytos, le lymphocytos, le hemoglobina, e le plachettas monstrava omnes un restauration precoce. Le tempore requirite pro le restauration de granulocytos e lymphocytos esseva afficite relativamente paucite per le quantitate de radiation al qual le recipiente esseva exponite (DL\textsubscript{1.99}). Le tempore inter injection e restauration esseva etiam relativemente constante.

2. Le configuration monstrate in muses que recipeva medulla ossea de ratto. Solmente le granulocytos e le hemoglobina monstrava un precoce augmento in lor concentration, durante que le restauration del lymphocytos e del plachettas occurreva a approximativemente le mesme tempore como in le animales de controlo.

3. Le configuration monstrate in muses que recipeva endotoxina o colchicina ante le irradiation. Le restauration del lymphocytos esseva variabile durante que le restauration del granulocytos, del hemoglobina, e del plachettas esseva comparabile con illo del muses que recipeva isologe medulla ossee.
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