Three radiation protocols currently used in treatment of leukemia patients before bone marrow transplantation (BMT) were investigated in a murine model (C57BL/6 → C3H/HeJ) for BM allograft rejection. These include (a) a single dose of total body irradiation (8.5 Gy TBI delivered at a dose rate of 0.2 Gy/min), (b) fractionated TBI (12 Gy administered in six fractions, 2 Gy twice a day in 3 days, delivered at a dose rate of 0.1 Gy/min, and (c) hyperfractionated TBI (14.4 Gy administered in 12 fractions, 1.2 Gy three times a day in 3 days, delivered at a dose rate of 0.1 Gy/min). Donor-type chimerism 6 to 8 weeks after BMT and hematologic reconstitution on day 12 after BMT found in these groups were compared with results obtained in mice conditioned with 8 Gy TBI delivered at a dose rate of 0.67 Gy/min, routinely used in this murine model. The results in both parameters showed a marked advantage for the single dose 8.5 Gy TBI over all the other treatments. This advantage was found to be equivalent to a three- to fourfold increment in the BM inoculum when compared with hyperfractionated radiation, which afforded the least favorable conditions for development of donor-type chimerism. The fractionated radiation protocol was equivalent in its efficacy to results obtained in mice irradiated by single-dose 8 Gy TBI, both of which afforded a smaller but not significant advantage over the hyperfractionated protocol. This model was also used to test the effect of radiation dose rate on the development of donor-type chimerism. A significant enhancement was found after an increase in dose rate from 0.1 to 0.7 Gy/min. Further enhancement could be achieved when the dose rate was increased to 1.3 Gy/min, but survival at this high dose rate was reduced. These results demonstrated indirectly that dose rate affects the expression of host-type pluripotent stem cells, the progeny of which appear 3 to 6 weeks after treatment with 8 Gy TBI delivered at a dose rate of 0.1 Gy/min, but which are eradicated if radiation is delivered at a dose rate of 1.3 Gy/min.

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was tested from 6 weeks posttransplant on (T. Lapidot, unpublished observations, 1989).

Several approaches effectively enhanced donor-type chimerism in this model. They included (a) booster irradiation to the spleen 3 to 4 days after TBI11; (b) administration of myeloablative drugs such as dimethyl myleran (DMM),13 busulfan,14 or thiotapec14 in addition to TBI; and (c) an increase in BM cell dose.11-13 The latter approach enabled us to demonstrate that the expression of host-type stem cells and their progeny depends not only on the aggressiveness of the conditioning protocol, but is also a function of BM engraftment rate. Slow donor-type grafts, associated with small BM inocula, will eventually revert to host-type cells, whereas rapid engraftment such as that observed after transplantation of \(10^6\) T-cell–depleted BM cells will not allow recovery of host-type cells, even though the precursors of such cells are not completely destroyed by the conditioning protocol.

In the present study, we used this murine model to compare the efficacy of three major radiation protocols currently used to condition leukemia patients before BMT. These include (a) 8.5 Gy single-dose TBI\(^{15,16}\); (b) fractionated irradiation of 12 Gy TBI, twice a day in 3 days\(^{11}\); and (c) hyperfractionated radiation (originally described by Shank et al\(^{11}\) and later modified by Brochstein et al\(^{19}\)) of 14.4 Gy TBI, administered in 12 fractions (1.2 Gy each) three times daily for 4 days. In particular, we wished to include in this study the most intensive single-dose TBI protocol currently being used in humans.15,16

### MATERIALS AND METHODS

**Mice.** Animals used were 8- to 12-week-old female C3H/HeJ and C57BL/6 mice obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, ME. All mice were kept in small cages (five animals in each cage) and fed sterile food and acid water.

**Irradiation.** Mice were exposed to different radiation protocols, as described in the Results section, from a Gammabeam 150-A \(^{60}\)Co source (produced by the Atomic Energy of Canada, Kanata, Ontario), at a focal skin distance of 25 cm.

**Preparation of T-cell–depleted BM cells.** BM cells were prepared according to Reissner et al\(^{20}\) with minor modifications.9

**Blood testing.** Mice were bled from the retroorbital vein using heparin-coated glass capillaries. Blood samples were counted for hemoglobin, platelets, and WBCs in a Coulter Counter S111 (Coulter Electronics, Luton, England).

**Chimerism analysis.** Chimerism analysis was made by cytofluorimetry using anti-H2K\(^b\) and anti-H2K\(^k\) monoclonal antibodies (MoAbs), as described by Lapidot et al.11 The cells were pressed through stainless steel sieves to make cell suspensions in P\(_2\)/NaCl, and divided equally into three test tubes. The cells were then stained by indirect immunofluorescence; the first MoAb used was either anti-H2K\(^b\) MoAb specific for donor (20-8-45, a gift from D. Sachs) or anti-H2K\(^k\) specific for host-type major histocompatibility complex (MHC) antigens (H-100-5R28, a gift from G. Hammerling), and the second MoAb was FITC-conjugated goat anti-mouse IgG2A (Nordic Immunological Laboratories, Tilburg, The Netherlands). The relative staining of the chimeric cells with each antibody was recorded in a fluorescence-activated cell sorter (FACS). Forward light scatter was used to gate out erythrocytes and dead cells, and the threshold for positive staining among the total leukocyte population was such that the number of cells stained with the second MoAb alone did not exceed 10%. Donor-type chimeras were recorded when more than 30% of the cells were positive with anti-H2K\(^b\) antibody, and mixed chimeras were recorded when the number of positive cells with anti-H2K\(^b\) and anti-H2K\(^k\) exceeded 30% for each antibody. The 30% cutoff was established based on preliminary experiments in which the anti-H2K\(^b\) and anti-H2K\(^k\) antibodies were tested on spleen cells from normal C3H/HeJ and C57BL/6 mice. Such a high cutoff was necessary to avoid false positives.

### RESULTS

Effect of different radiation protocols on durable engraftment of T-cell–depleted BM allografts. The following radiation protocols were compared for their ability to enhance donor-type chimerism in mice receiving allogeneic T-cell–depleted BM: (a) a single dose of 8.5 Gy TBI delivered at a dose rate of 0.2 Gy/min; (b) fractionated 12 Gy TBI administered in six fractions (2 Gy, twice a day in 3 days) at a dose rate of 0.1 Gy/min; (c) hyperfractionated 14.4 Gy TBI administered in 12 fractions (1.2 Gy, three times a day in 4 days) at a dose rate of 0.1 Gy/min; and (d) a single dose of 8 Gy TBI at a dose rate of 0.63 Gy/min, routinely used in our mouse model.11 BMT was performed within 24 hours of completion of irradiation.

As shown in Table 1, optimal engraftment of donor-type cells was achieved by conditioning the recipients with the single-dose 8.5 Gy TBI protocol (group 1), whereas the poorest engraftment was found in mice conditioned with 14.4 Gy hyperfractionated radiation (group 3).

In two independent experiments, transplantation of \(10^6\) T-cell–depleted BM cells in group 1 resulted in excellent survival (15 of 15 and 19 of 20) and in a high incidence of donor-type chimeras (87% and 89%, respectively). In group 3, on the other hand, survival was poor (6 of 15 and 8 of 15), and surviving mice did not engraft (the percentage of donor-type chimeras was 12.5 and 0, respectively). This difference in survival and donor-type chimerism was highly significant (\(P < .01\) by Fisher's exact test).

Increasing the BM inoculum dose to \(8 \times 10^6\) or \(12 \times 10^6\) cells enhanced donor-type chimerism in group 3 to 55.5% and 100%, respectively, suggesting that the difference between the two conditioning protocols can be translated into an increase in cell dosage which is greater than an increment of \(4 \times 10^6\) cells and less than or equal to an increment of \(8 \times 10^6\) cells. Thus, the enhancing effect obtained by using a single dose of 8.5 Gy TBI is equivalent to the effect achieved by doubling or tripling the BM inoculum.

The fractionated radiation protocol (group 2) was similar in its effect to the protocol involving a single dose of 8.0 Gy TBI routinely used in our mouse model (group 4) (\(P = .83\)). The percentage of donor-type chimerism after transplantation of \(4 \times 10^6\) cells was 33.3% in group 2 and 37.5% in group 4. This apparent advantage over group 3 was not significant, however (\(P > .05\) by Fisher's exact test).

**Engraftment rate after different radiation protocols.** In preliminary experiments (data not shown), when 2 to \(10^6\) T-cell–depleted BM cells from C57BL/6 donors were transplanted into 8 Gy TBI-treated C3H/HeJ recipients, the rate of hematopoietic reconstitution 2 weeks posttransplant correlates to the BM cell dose. In later periods (3 to 4 weeks) posttransplant, the differences in hematologic parameters
between mice receiving varying amounts of BM cells do not correlate with BM cell dose and the differences will gradually disappear. Therefore, hematopoietic reconstitution was tested on day 12 posttransplant in mice receiving different radiation regimens.

As shown in Table 2, engraftment rate was correlated to the percentage of donor-type chimerism, as well as with long-term survival data. After a transplant of $4 \times 10^6$ BM cells, most hematologic parameters in group 1 were markedly superior to those of the other groups, whereas the poorest engraftment rate was observed among recipients of the hyperfractionated protocol (group 3). The average leukocyte number, hemoglobin level, and platelet count in group 1 were $2.2 \times 10^7/\mu L$, 13 g/dL, and $437 \times 10^3/\mu L$, respectively, as compared with $1.0 \times 10^7/\mu L$, 6.7 g/dL, and $127 \times 10^3/\mu L$, respectively, in group 3 (Table 2, experiment 2). This difference was highly significant ($P < .01$ by Student’s $t$ test) for the hemoglobin values and the platelet numbers. The hematologic status in group 1 after transplantation of $1 \times 10^6$ BM cells was slightly superior to that of group 3 after transplantation of $4 \times 10^6$ BM cells. The average leukocyte number and platelet count were comparable. Hemoglobin levels were higher in group 1 (8.4 ± 0.7 g/dL, $P < .05$ by Student’s $t$ test). When the BM dose was increased from $4 \times 10^6$ to $12 \times 10^6$ cells, the engraftment rate in mice receiving hyperfractionated radiation (group 3) was slightly lower than that found after $3 \times 10^6$ BM cells were transplanted into mice receiving a single dose of 8.5 Gy TBI (group 1). The differences in leukocyte and platelet numbers were not significant, whereas hemoglobin levels were significantly higher in group 1 ($P < .01$). Thus, the difference between the two radiation protocols, as reflected by engraftment rate, is equivalent to at least a fourfold increment in the BMT inoculum.

**Table 1. Effect of Different Radiation Regimens on Chimerism Status, Six to Eight Weeks After BMT**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Radiation Regimen*</th>
<th>Transplanted Cells ($\times 10^4$)</th>
<th>Survival 45 d Posttransplant</th>
<th>Chimerism Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>4</td>
<td>15/15</td>
<td>Host</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>18/20†</td>
<td>18/15†</td>
<td>Donor</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>8/15†</td>
<td>6/15†</td>
<td>Mixed</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>8/10†</td>
<td>1/1</td>
<td>12.5†</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>13/14</td>
<td>2/1</td>
<td>80.0</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>9/14†</td>
<td>3/5</td>
<td>55.5†</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>5/7†</td>
<td>1/3</td>
<td>60.0†</td>
</tr>
</tbody>
</table>

- Four radiation regimens were tested: (a) single-dose 8.5 Gy TBI (0.2 Gy/min); (b) fractionated 12 Gy TBI, 2 Gy twice daily in 3 days (0.1 Gy/min); (c) hyperfractionated 14.4 Gy TBI, 12 Gy three times daily in 4 days (0.1 Gy/min); and (d) single-dose 8.0 Gy TBI (0.63 Gy/min). T-Cell-depleted BM cells from C57BL/6 donors (H-2K$^b$) were transplanted 24 hours after completion of irradiation into C3H/HeJ (H-2K$^b$) recipients. Statistical significance in reference to radiation regimen 1 was performed according to Fisher’s exact test and is represented as follows: †not significant ($P > .05$), ‡$P < .01$, and §$P < .05$.

**Table 2. Effect of Different Radiation Regimens on Hematopoietic Reconstitution Two Weeks After BMT**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Radiation Regimen*</th>
<th>Transplanted Cells ($\times 10^3$)</th>
<th>No. of Mice</th>
<th>Leukocytes ($\times 10^3/\mu L$)</th>
<th>Hemoglobin (g/dL)</th>
<th>Platelets ($\times 10^3/\mu L$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>4</td>
<td>15</td>
<td>5.1 ± 1.6</td>
<td>14.4 ± 1.3</td>
<td>468 ± 116</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>8</td>
<td>13</td>
<td>6.0 ± 3.7</td>
<td>14.6 ± 1.3</td>
<td>724 ± 338</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>18</td>
<td>8</td>
<td>3.6 ± 1.6†</td>
<td>10.4 ± 1.7†</td>
<td>135 ± 50†</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>10</td>
<td>6</td>
<td>5.0 ± 2.5</td>
<td>12.4 ± 3.9</td>
<td>483 ± 298</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>4.0 ± 1.7‡</td>
<td>13.2 ± 0.8‡</td>
<td>345 ± 54‡</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>4.8 ± 1.7</td>
<td>14.5 ± 1.3</td>
<td>742 ± 111</td>
</tr>
</tbody>
</table>

- Radiation regimen numbers refer to experimental groups, as described in the footnote to Table 1. Statistical significance in reference to the data from recipients of $4 \times 10^6$ cells in group 1, was performed according to Student’s $t$ test, and is represented as follows: †$P < .01$, ‡$P > .05$. Statistical significance of differences between all groups receiving $8 \times 10^6$ BM cells was $P < .05$. 

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experiment 1, and 0.1 Gy/min in experiments 2 and 3, was performed according to Fisher's exact test, and is represented as follows:

conditioned with 8.0 Gy TBI delivered at different dose rates. Statistical significance in reference to the data from mice treated with 0.8 Gy/min in experiment 1, and 0.1 Gy/min in experiments 2 and 3, was performed according to Fisher's exact test, and is represented as follows: *P < .05, †P < .01, ‡Not significant (P > .05).

effect of radiation dose rate on BM engraftment in the particular mouse model described above and to compare the results to the effect achieved by substituting one radiation protocol for another.

As shown in Tables 3 and 4, a marked effect was found when a fixed total dose of 8 Gy TBI was administered but the radiation dose rate was changed. On a change in dose rate from 0.1 to 1.3 Gy/min in recipients of 4 x 10³ T-cell-depleted BM cells, donor-type chimerism was enhanced from 0% to 88.6%. Increasing the BM cell dose to 8 x 10³ T-cell-depleted BM cells after conditioning with 0.1 Gy/min only slightly enhanced the percentage of donor-type chimerism, from 0% to 13.3%, whereas a small BM inoculum of 2 x 10³ T-cell-depleted BM cells afforded 66.6% donor-type chimerism in mice conditioned with 1.3 Gy/min. Thus, the effect on BM engraftment observed when the radiation dose rate was increased from 0.1 to 1.3 Gy/min is more pronounced than the effect obtained by a fourfold increase in BM cell dose.

A similar pattern was also reflected in the hematologic parameters of mice tested 12 days posttransplant; eg, the average hemoglobin level noted in mice irradiated at a dose rate of 1.3 Gy/min and transplanted with 2 x 10³ cells was 10.2 g/dL, as compared with 6.5 g/dL in mice irradiated at a dose rate of 0.1 Gy/min and transplanted with 8 x 10³ T-cell-depleted BM cells. Despite enhanced engraftment of donor cells in the group treated with a dose rate of 1.3 Gy/min, the survival of these mice was lower (owing to infection with gram-negative pathogens) than that of mice in the other groups. Thus, among mice treated with dose rates less than 0.7 Gy/min, 0.7 to 0.8 Gy/min, or 1.3 Gy/min, survival was 47 of 60 (78.3%), 44 of 62 (70.9%), and 39 of 64 (60.9%), respectively. These differences were highly significant (P < .001 by Fisher's exact test).

DISCUSSION

In recent years, many researchers have investigated the relationship between radiation dose rate and the fractional survival of colony-forming units (CFU), 8 to 10 days posttransplant. In these studies, most of which were reviewed in 1983 by Glasgow et al and more recently confirmed and extended by Evans et al and Tarbell et al, no significant effect on CFU could be demonstrated, although a marked effect was shown on survival 30 days postirradiation. The short-term CFU assay may not be sensitive enough, however, to detect the low incidence of residual host-type stem cells remaining after the heavy TBI used in the conditioning of leukemia patients.

Using the same murine model as that used in the present study, we previously showed that transplantation of a small dose of 1 x 10³ T-cell-depleted BM cells enhanced survival to 82% as compared with a survival rate of 29% in irradiated mice that did not receive a transplant. This enhancement was associated with a transient engraftment of donor-type cells during the initial posttransplant period. In these transient chimeras, host-type cells remaining after conditioning gradually replaced the initial donor-type graft. Similar "reversals" were reported in other murine models using different mouse strains, or different conditioning protocols. Thus, the presence of a marked signal of host type cells, 30 to 60 days

**Table 3. Effect of Radiation Dose Rate on Chimerism Status of Mice Conditioned Six to Eight Weeks After BMT**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dose Rate (Gy/min)</th>
<th>Transplanted Cells (x 10⁶)</th>
<th>Survival 45 d Posttransplant</th>
<th>Chimerism Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Host</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Donor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Donor (%)</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>2</td>
<td>19/22</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>2</td>
<td>12/22*</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>4</td>
<td>15/20</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>4</td>
<td>17/20†</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>4</td>
<td>9/20†</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>4</td>
<td>15/22†</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>8</td>
<td>15/20</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>8</td>
<td>12/20†</td>
<td>1</td>
</tr>
</tbody>
</table>

T-cell-depleted BM cells from C57BL/6 (H-2Kb) were transplanted 24 hours after completion of irradiation into C3H/HeJ (H-2K') recipients and conditioned with 8.0 Gy TBI delivered at different dose rates. Statistical significance in reference to the data from mice treated with 0.8 Gy/min in experiment 1, and 0.1 Gy/min in experiments 2 and 3, was performed according to Fisher's exact test, and is represented as follows: *P < .05, †P < .01, ‡Not significant (P > .05).

**Table 4. Effect of Radiation Dose Rate on Hematopoietic Reconstitution Two Weeks After BMT**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dose Rate (Gy/min)</th>
<th>Transplanted Cells (x 10⁶)</th>
<th>No. of Mice</th>
<th>Leukocytes (x 10³/µL)</th>
<th>Hemoglobin (g/dL)</th>
<th>Platelets (x 10³/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>2</td>
<td>20</td>
<td>1.4 ± 0.5</td>
<td>7.4 ± 1.6</td>
<td>75 ± 48</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>2</td>
<td>19</td>
<td>1.4 ± 0.3†</td>
<td>8.2 ± 1.6†</td>
<td>73 ± 2†</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>2</td>
<td>12</td>
<td>1.2 ± 0.4†</td>
<td>10.2 ± 2.5*</td>
<td>123 ± 120†</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>8</td>
<td>15</td>
<td>1.1 ± 0.5</td>
<td>6.5 ± 3.0</td>
<td>68 ± 51</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>8</td>
<td>12</td>
<td>1.9 ± 1.1*</td>
<td>9.5 ± 2.7‡</td>
<td>163 ± 129*</td>
</tr>
</tbody>
</table>

Experimental groups were as described in the footnote to Table 3. Statistical significance in reference to the data from mice treated with 0.1 Gy/min was performed according to Student's t test and is represented as follows: *P < .05, †P < .05, ‡P < .01.
posttransplant, has been shown to serve as a sensitive indicator for the effect of different radiation regimens or drugs on the expression of host pluripotent stem cells.

Our results contradict those based on the short-term CFU assay, in that they clearly show a pronounced effect of radiation dose rate on the late expression of host-type cells (progeny of residual host-type pluripotent stem cells). Indeed, this effect was found to be similar in its magnitude to that achieved by adding a myeloablative drug such as busulphan or DMM to the conditioning protocol involving 8 Gy TBI delivered at a dose rate of 0.67 Gy/min.

These conflicting results can be resolved if we assume that changes in radiation dose rate may selectively affect the very early stem cells, which play a critical role in BM engraftment and in establishment of durable donor-type chimerism but do not affect the short-term CFU. A similar selective effect on CFU that possess a greater self-renewal capacity has been attributed in several studies to busulphan.

The late appearance of host-type blood cells may be a reflection not only of host stem cell survival, however, but also of host-type lymphocytes, which may reject donor cells and indirectly alter the balance in favor of host-type cells.

In a recent study, we demonstrated the relative advantage of myeloablative drugs over the lymphotoxic drug cyclophosphamide in our murine model. We showed that although administration of 120 mg/kg cyclophosphamide abrogated clonable T cells more effectively than DMM, thiotepa, or busulphan, it did not enhance donor-type chimerism when added to 8 Gy TBI. Considering the marked enhancement of BM allografts achieved by addition of myeloablative drugs to 8 Gy TBI, we tend to favor the possibility that direct elimination of stem cells is critical for donor-type engraftment in this particular setting. Further studies using transplantation between congenic strains of mice may be needed to resolve this issue, however.

The sensitivity of our mouse model in detecting the expression of residual host stem cells also permitted us to investigate different radiation protocols currently used in the conditioning of leukemia patients before BMT. Two conclusions can be drawn from our results:

First, the single dose 8.5 Gy TBI protocol similar to that used by Kersey et al. is by far the most effective in attaining donor-type chimerism after transplantation of T-cell-depleted BM. This simple procedure may be particularly suitable for conditioning of human leukocyte antigen (HLA)-mismatched recipients, in whom engraftment after conditioning with fractionated or hyperfractionated radiation is extremely problematic.

Second, until the toxicity of the single-dose protocol is evaluated, fractionation of radiation is desired. Our results suggest that of the two protocols that involve fractionation of radiation, fractionated radiation (six times 2 Gy, group 2) may be slightly superior to hyperfractionated radiation (group 3) in its effect on donor-type chimerism or on host-type stem cell "relapse." This minor advantage was of low statistical significance, however, and may be negligible ($P > .05$ by Fisher's exact test).

Clearly, in leukemia patients, usefulness of the single-dose TBI regimen depends not only on its toxicity or its myeloid and lymphoid ablative potential, but also on its ability to abrogate pathologic cells. A major prospective randomized clinical study, comparing a single-dose 10 Gy TBI protocol to a fractionated 12 Gy TBI protocol, showed that in both groups the leukemia relapse rate was identical, suggesting that the two regimens were roughly equivalent in terms of leukemic cell kill. Survival was better in patients receiving the fractionated protocol, owing entirely to a decreased rate in deaths caused by complications. If leukemic cells from acute leukemias or chronic myelogenous leukemia have cell survival characteristics similar to those of normal BM stem cells, our present study suggests that the leukemic cells may be abrogated more efficiently by the single-dose 8.5 Gy TBI protocol.

Shank et al. suggested that hyperfractionated radiation may ablate normal bone BM cells as well as leukemic cells more effectively because it was believed that cell cycle effects may exist that alter radiosensitivity in succeeding fractions. Our results do not support this hypothesis, in that "relapse" of normal host-type blood cells is significantly higher in mice conditioned with hyperfractionated TBI. Conflicting results reported in the literature appear to show greater variation in the response of leukemic cells to radiation as compared with normal cells, however.

The radiation protocols we tested are generally supplemented in the clinic with exactly the same chemotherapy (2 x 60 mg/kg cyclophosphamide). Therefore, variabilities in the outcome of different conditioning protocols probably should be linked to the radiation component of the protocol.

Thus, in our experiments we studied the effect of the radiation component to elucidate its ablative role in the absence of chemotherapy. The outcome of the combined protocol (TBI plus cyclophosphamide) may not be predicated on the effect of TBI alone, however, as some synergism between these modalities may occur. Further studies in our mouse model using both TBI and cyclophosphamide are required to establish the relevance of the present results to clinical BMT.

Finally, several studies in different animal models indicate that, in principle, single-dose TBI will afford more efficient engraftment of BM allografts than fractionated radiation. The novelty of the present study lies in its direct comparison of detailed conditioning protocols currently used in different clinical centers, using a well-calibrated mouse model for transplantation of T-cell-depleted BM. In this model, the relative advantage of a given protocol can be translated into an equivalent increase in BM inoculum. Thus, we are able to quantify engraftment efficiency, as well as the repression of host-type stem cells, the progeny of which can emerge only if mice are kept alive long enough after BMT.

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Induction of donor-type chimerism in murine recipients of bone marrow allografts by different radiation regimens currently used in treatment of leukemia patients

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