Syngeneic and Allogeneic Bone Marrow Engraftment After Total Body Irradiation: Dependence on Dose, Dose Rate, and Fractionation

By J.D. Down, N.J. Tarbell, H.D. Thames, and P.M. Mauch

Murine bone marrow chimera models were used to assess the efficacy of host total body irradiation (TBI) given at different doses, dose rates, and fractionation schemes in providing for engraftment of syngeneic and allogeneic bone marrow. B6-Hbb\(^+\) congenic and LP mice, respectively, were used as donors (10\(^5\) bone marrow cells) for syngeneic and allogeneic (H-2 compatible) transplantation in standard B6 recipients. Stable marrow chimerism was determined from host and donor stem cell-derived hemoglobin phenotypes (Hbb\(^+\) and Hbb\(^-\)) on gel electrophoresis at 3 months posttransplant. Partial engraftment of syngeneic marrow was seen at single doses as low as 2 Gy, with the donor component increasing steadily with increasing TBI dose to a level of 100% at 7 Gy. Immunologic resistance of the host appeared to prevent allogeneic engraftment until 5.5 Gy. A very steep radiation dose response was then observed so that the level of chimerism with 6 Gy and above became comparable with syngeneic engraftment. Low dose rate (5 cGy minute\(^-1\)) and fractionated TBI required higher total doses for equivalent engraftment (radiation dose-sparing) in both syngeneic and allogeneic bone marrow transplantation. This displacement in the dose-response curve on fractionation was seen with interfraction intervals of 3 and 6 hours. A further dose-sparing effect was observed on extending the interval to 18 and 24 hours, but only for allogeneic transplantation, and may therefore be related to recovery of immune-mediated graft resistance. The involvement of multiple target cell populations in determining allogeneic engraftment rendered the application of the linear-quadratic model for radiation cell survival problematic in this case. The recovery in dose when low dose rate and 6-hour interfraction intervals were applied in either syngeneic or allogeneic BMT is consistent with appreciable sub-lethal damage repair in the primitive self-renewing stem cell population of the host marrow. These results contrast with the poor repair capacity of the 11-day spleen colony-forming units (CFUs) population after fractionated irradiation and support the notion that ablation of early stem cells in the pre-CFUs compartment is essential for long-term marrow engraftment.

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immunity, factors that may be of greater importance to the success of BMT.

In the current study, representative murine models of syngeneic and allogeneic BMT have been established to address these issues. Here the effectiveness of different dose-rate and fractionated TBI schemes to allow for marrow engraftment, with or without the need for immune-suppression, are compared with the ability of late 11-day CFUs to survive modified TBI. The results show an appreciable dose-sparing effect that can be attributed most to SLD repair in early pre-CFUs stem cells with long-term repopulating potential.

MATERIALS AND METHODS

Animals and irradiation. Male C57BL/6j (Hbb°), C57BL/6j congeneric (Hbb°), and LP/J (Hbb°) mice (Jackson Laboratory, Bar Harbor, ME) were barrier maintained in filter-top isolator cages and had negative serum titers for known pathogenic organisms (sendai, PVM, HMV, GD-7, REO-3, LCMV, GDV11, MCMV, and Mycoplasma pulmonis). For allogeneic (MHC compatible) transplantation, LP mice were used as a source of bone marrow for infusion intravenously (IV) in pretreated C57BL/6j (B6). These two strains share the same major histocompatibility haplotype (H-2°) and there has been no evidence for clinical graft-versus-host disease (GVHD) on transplantation of untreated LP marrow cells into irradiated B6 recipients. Nevertheless, there are differences in a number of minor non-H-2 loci (H-1, H-3, H-4, H-7, H-8, H-9, H-12, and H-13), and lethal GVHD can be induced by further addition of donor spleen cells to denote potential immunologic disparities between these strains. For syngeneic transplantsion, C57BL/6/Hbb°/Hbb° (B6-Hbb°) congeneric mice served as donors for marrow transplantation into standard B6 hosts, where immunologic disparities are largely removed as factors in engraftment.

At 3 to 6 months of age, recipient animals (B6) were irradiated in groups of 6 to 10 within a cylindrical lucite chamber (21-cm diameter, 3.5-cm deep) with continuous air flow and positioned logic disparities are largely removed as factors in engraftment.

BMT. Bone marrow cell suspensions from donor mice were prepared by flushing the medullary cavity of the humerus, tibia, and femur with cold Hanks balanced salt solution (HBSS). Nucleated cell counts were made in crystal violet using a hemocytometer. Cell suspensions were kept on ice until use and injected IV into the lateral tail vein 2 to 4 hours after the end of recipient irradiation. In each BMT experiment the CFUs content of the donor marrow was measured in syngeneic (donor) and allogeneic (recipient) hosts administrated 9.5 Gy TBI (100 cGy/minute), 10° cells, and killed 8 days later for spleen colony counts (8-day CFUs).

Determination of erythroid engraftment. Transplant models were designed to use the Hbb erythroid marker as a measurement of stable donor-marrow engraftment at 3 months after BMT. Here the host and donor allelic forms of the hemoglobin β-chain were distinguished using polyacrylamide gel electrophoresis modified from Kudisch et al. Animals were tail bled to 20-μL heparinized micropipettes and the blood washed in 10 mL cold saline. The samples were centrifuged at 1,200 rpm for 10 minutes, the saline aspirated off to the red blood cell pellet, and stored at −20°C until analysis. A 12% acrylamide gel was prepared containing Triton-X 100 and a final concentration of 6 mol/L urea. A pre-electrophoresing period of 1 hour was performed with 0.1 mol/L cysteamine. Blood samples were hemolysed with 1.0 mL distilled water. A tracking buffer was made containing acetic acid, mercaptoethanol, urea, and pyronine Y. Four microliters of sample was added to 36 μL of tracking buffer. Buffered samples were added to the wells and run at 6-W constant power until the pyronine marker reached the base of the verticle gel (approximately 2 hours). Gels were stained for 1 hour in Coomasie Brilliant Blue dye and destained in methanol/acetic acid solution for approximately 2 days. The mouse strains used here in experimental BMT either have the hemoglobin phenotype Hbb° characterized by a single band (standard B6 mice) or Hbb° characterized by a diffuse pattern of β-major and β-minor bands (LP and B6-Hbb° congeneric mice). Quantitative measurements of the two Hbb allelic forms in stable mixed chimera was made with a scanning densitometer (Hoeffer GS300 with a GS350 data system). Sensitivity of the assay was within 5% in controlled mixing experiments.

Determination of CFUs radiation dose-survival. To assess the radiation sensitivity of bone marrow CFUs with fractionated irradiation, donor B6 mice were administered doses of TBI (100 cGy/min) as a single fraction or four equal fractions (0.4 to 1.5 Gy/fraction) twice per day with 6- and 18-hour intervals. Bone marrow from the tibia and femur was removed 18 hours after treatment to allow for any potential lethal damage that could occur in situ. The number of donor mice in each dose group varied from 4 (at low total doses) to 20 (at high total doses). Secondary irradiated recipients were prepared with 12.5 Gy (7.0 and 5.5 Gy 3 hours apart). Prophylactic treatment with neomycin (3.5 g/L drinking water) starting 1 week before recipient irradiation was administered to ensure good survival up to 11 days. Animals were injected with an appropriate number of donor marrow cells and killed 11 days later for spleen colony determination by the method of Till and McCulloch. Total spleen colonies and colonies of above 1.2-mm diameter were counted under a calibrated dissecting microscope. Eight mice were used for each average CFUs determination. Because total marrow cellularity decreases after TBI, the surviving fractions were calculated on the basis of CFUs yield per hind limb and not as a proportion of cells injected.

Analysis of radiation dose-effect parameters. Radiation dose-response curves for each treatment schedule were obtained either from engraftment data or as a quanatual response, ie, no or partial engraftment as failure and complete (>95%) engraftment as success. The latter then became amenable to probit analysis using the statistical package GLIM® and, in applying Fieller’s theorem, the EDα value (radiation dose to produce 50% complete engraftment) with 95% confidence limits (CL) was determined. Moreover, direct analysis® was applied to pooled sets of data to estimate dose-response parameters without the requirement for determination of EDα values.

The dose-sparing effect of fractionated and low dose-rate irradiation were interpreted in terms of 2 the linear-quadratic model of dose-survival of target cells. This is given by: ln S = −(αD + βD²), where the existence of the reparable component of radiation damage is in the β term only. This model can be related to dose-response data on the basis of the following assumptions. It is supposed that a particular response in a tissue (eg, successful bone marrow engraftment) corresponds to precisely one level of depletion of a critical target cell population capable of preventing engraftment. The survival of these target cells after a particular fractionated or low dose-rate exposure is assumed to follow the “incomplete-repair” (IR) model,® an extension of the linear-quadratic model, and furthermore cellular proliferation is assumed to have a negligible effect. The parameters of the IR model are: (1)
the natural logarithm of the number of target cells at risk (ln k); (2) the coefficient of total dose (α); (3) the coefficient of total dose x dose per fraction (β); and (4) the repair rate (μ).

Under these assumptions, if D is the total fractionated dose administered in individual fractions of size d separated by time intervals Δt, and if the exposure time per fraction is t, then the probability (p) of engraftment is given by:

\[ p = e^{-\ln k - \alpha D - \beta D/d + \gamma} \]

where g, c, and h are functions of μ, Δt, t, and the number of fractions. Quantities of particular interest that may be estimated by direct analysis include: (1) k, the target clonogen number; (2) α/β, an inverse measure of repair capacity of the tissue; and (3) T1/2 = (ln 2)/μ, the repair halftime in the tissue.

The α/β ratio has the dimensions of dose and indicates the dose at which cell kill from the linear-low-dose term is equalled by cell kill from the dose-squared term. Tissues of low α/β ratios have a large capacity for dose-sparing from fractionated and low dose-rate irradiations. For comparison between different tissues or endpoints, the α/β ratio has advantages over many other parameters of fractionation dose-sparing because it is theoretically independent of the level of iso-effect studied. However, this parameter is applied empirically in the present study to acknowledge that other processes (eg, proliferative repopulation, multiple target cell determinants) may influence the final estimation of α/β.

RESULTS

Animal survival. All recipient mice survived up to 6 months posttransplant with no physical signs of GVHD. Animals that failed to engraft exhibited host marrow repopulation providing adequate hematopoietic support after TBI.

Engraftment after single acute doses of TBI. Figure 1 compares the dose-response curve for syngeneic (B6-Hbbd in standard B6-Hbbd recipients) with data obtained from the major histocompatibility complex (MHC) compatible allogeneic system (LP in B6) after single-dose, high dose-rate TBI. This clearly shows that TBI was capable of inducing partial engraftment of syngeneic marrow at doses as low as 2 Gy. In contrast, no allogeneic engraftment at all was seen at doses below 5.5 Gy, and this denotes an immunologic resistance of B6 hosts against LP donor marrow. However, the dose-response curve for allogeneic engraftment was dramatically steep so that data at 6 Gy and above were all comparable, suggesting very effective immune suppression at this dose.

Each transplantation was accompanied by an estimation of the 8-day CFUs content in the donor marrow and the average between experiments was found to be 12.3 ± 1.6 (SD) and 7.5 ± 3.6 CFUs per 10^6 nucleated cells in B6-Hbbd and LP donors, respectively, as assessed in syngeneic hosts prepared with 9.5 Gy. The colony numbers did not change significantly when LP CFUs were assayed in allogeneic B6 recipients (9.7 ± 2.8). An amount of 9.5 Gy was found to be sufficient in preventing the endogenous formation of host-derived CFUs in all nontransplanted controls (26 mice).

Syngeneic engraftment after protracted or fractionated TBI. The radiation dose-dependent transplantation of B6-Hbbd marrow in B6-Hbbd mice was extended to investigate the effect of modifying TBI with: (1) single doses at continuous low dose rate (5 cGy minute^-1); (2) two doses separated by a 6-hour interval; (3) two doses separated by an 18-hour interval; (4) multiple 2-Gy doses administered continuously (2 Gy/F cont.) every 6 hours, and (5) multiple 2-Gy doses administered twice daily (2 Gy/F twice per day) at alternate 6- and 18-hour intervals. These were compared with high dose rate (100 cGy minute^-1) single dose controls. Figure 2 displays the dose-response relationships for these different TBI schemes to show that low dose rate and two fraction treatments had the effect of shifting the dose-response curve to higher doses by approximately 2 Gy with no difference between 6- and 18-hour interfraction intervals. Even greater dose sparing was seen on multiple dose fractionation, requiring more than a doubling in dose to achieve near complete chimerism. Here there appeared to be no difference between the 2 Gy/F cont. and 2 Gy/F twice per day treatments.

Allogeneic engraftment after protracted or fractionated TBI. Dose-response curves for treatment of B6 mice with continuous low dose rate (5 cGy minute^-1 and 2-Gy fractions administered every 6 hours (cont.) or twice daily (b.i.d.) are shown for allogeneic LP engraftment in Fig 3 as (1) ordinal data and (2) quantal data. This study showed that while 7 Gy as a single acute exposure was capable of inducing complete donor engraftment, fractionated TBI at 2 Gy per fraction every 6 hours only allowed complete engraftment at a total dose of 14 Gy. A further dose-sparing effect was apparent when an overnight period of 18 hours was inserted between the twice-per-day irradiations so that full engraftment was not seen until 22 Gy. TBI at 5 cGy minute^-1 also required higher doses, but to a smaller extent.

The influence of extending the interfraction interval and overall treatment time was studied directly by separating two equal TBI doses with different time periods ranging from 3 hours to 6 days. As shown in Fig 4, the calculated ED50 dose (to achieve 50% complete engraftment) rose by

\[ \text{ED}_{50} \text{ dose} = \frac{1}{4} \text{sum of doses} \]

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Fig 2. Radiation dose-sparing effect of modified TBI as compared with single-dose high dose-rate controls (●) on the level of syngeneic marrow engraftment (B6-Hbb\(^{a}\) in B6-Hbb\(^{b}\) recipients). (Top) Two equal fractions with 6- (▼) and 18-hour intervals (▲) and multiple 2-Gy fractions administered every 6 hours (▼) or at alternate 6- and 18-hour intervals (▲). (Bottom) Continuous low dose rate (5 cGy/min) (■). Error bars represent ±1 SEM for 5 to 12 mice from one or two experiments.

about 1.7 Gy when the two doses were interrupted by a 3- or 6-hour break, but a further significant increase was seen when the rest period was extended to 18 or 24 hours (giving an iso-dose increment of about 3 Gy). There was no significant change in the ED\(_{50}\) dose when the interval was extended further to 6 days. Table 1 lists the ED\(_{50}\) values corresponding to complete engraftment of LP marrow (10\(^7\) cells) in B6 hosts prepared with fractionation schemes of varying doses per fraction and interfraction intervals.

Dose-response parameters were estimated for the pooled data (treatment times up to 6 days), and for various shorter interfraction or overall times using direct analysis with the IR model. Several trends were noted: with increasing time estimates of \(\alpha\), \(\alpha/\beta\), and ln k decreased while T\(\frac{1}{2}\) increased. These changes were more dependent on average time between fractions than on the overall treatment time. Inclusion of 18- and 24-hour intervals in the fractionation schemes all gave \(\alpha/\beta\) ratios of negative radiation dose. This does not appear to be an influence of repopulation because, on theoretical grounds,\(^{26}\) repopulation among surviving cells would be expected to result in increased estimates of ln k. Positive and biologically meaningful \(\alpha\) values resulted when the analysis was restricted to short (≤6 hours) interfraction intervals. A relatively low value of \(\alpha/\beta\) resulted with repair halftime of about 1 hour (Table 2).

Fig 3. Effect of fractionated and low dose-rate TBI on erythroid LP engraftment in B6 recipients. Single doses were delivered at 100 cGy/min (high dose rate, HDR) and 5 cGy/min (low dose rate, LDR). Fractionated TBI was administered at high dose rate in 2-Gy doses either twice daily (b.i.d.) at 6- and 18-hour intervals or continuously (cont.) every 6 hours. (Top) Radiation dose-response curves for the level of engraftment as ordinal data. (Bottom) Radiation dose-response curves for proportion of recipients with complete engraftment as quantal data. The curves and their 95% confidence limits at the ED\(_{50}\) level (horizontal error bars) were computed by probit analysis.

Fig 4. Split-dose recovery (seen as an increase in ED\(_{50}\) dose) at different time intervals separating two equal doses of TBI for allogeneic (LP in B6) engraftment. ED\(_{50}\) values (dose for 50% complete engraftment) and 95% confidence limits were obtained from dose-response curves using probit analysis.
Table 1. Iso-Effect TBI Doses for Allogeneic Engraftment (LP → B6) at Different Doses per Fraction and Interfraction Interval

<table>
<thead>
<tr>
<th>No. Experiments</th>
<th>Dose/Fraction (Gy)</th>
<th>No. Fractions</th>
<th>Fraction Interval (GY)</th>
<th>ED_{50} (95% CL) (Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6.1</td>
<td>1</td>
<td>0</td>
<td>6.1 (6.0-6.4)</td>
</tr>
<tr>
<td>2</td>
<td>3.9*</td>
<td>2</td>
<td>3 h</td>
<td>7.8 (7.3-8.3)</td>
</tr>
<tr>
<td>2</td>
<td>3.9*</td>
<td>2</td>
<td>6 h</td>
<td>7.9 (7.6-8.2)</td>
</tr>
<tr>
<td>1</td>
<td>4.6*</td>
<td>2</td>
<td>18 h</td>
<td>9.1 (8.7-9.5)</td>
</tr>
<tr>
<td>2</td>
<td>4.6*</td>
<td>2</td>
<td>24 h</td>
<td>9.2 (8.8-9.7)</td>
</tr>
<tr>
<td>3</td>
<td>4.4*</td>
<td>2</td>
<td>6 d</td>
<td>8.7 (8.0-9.2)</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>5-8</td>
<td>6 h</td>
<td>12.6 (11.9-13.3)</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>8-11</td>
<td>6 + 18 h</td>
<td>19.7 (19.0-21.0)</td>
</tr>
<tr>
<td>1</td>
<td>2.8*</td>
<td>5</td>
<td>6 + 18 h</td>
<td>14.0 (12.0-16.0)*</td>
</tr>
<tr>
<td>1</td>
<td>3.8*</td>
<td>4</td>
<td>24 h</td>
<td>15.3 (15.0-15.5)*</td>
</tr>
</tbody>
</table>

*Interpolated values for ED_{50}.
†The five fraction twice per day data would not converge on probit analysis and so the ED_{50} is given as the partial response with 0 and 100% engraftment.
‡The four daily fraction data were taken from the published report of Mauch et al. 19

CFUs dose survival after single and fractionated TBI. Radiation dose-survival curves for the 11-day CFUs population are presented in Fig 5. All curves showed a straight exponential decrease in CFUs survival on increasing total radiation dose with no indication of a shoulder. The position and slope (D_{0}) of the cell survival curve appeared unaffected by fractionated irradiation and the results obtained from total or large spleen colony counts remained similar.

DISCUSSION

These experiments involving both syngeneic and allogeneic BMT conditioning have clearly demonstrated that the success of donor hematopoietic engraftment is critically dependent on TBI dose and its modification by dose rate and fractionation. Allogeneic chimerism was particularly sensitive to changes in TBI dose. Here single acute exposures of TBI gave a very steep dose-response curve with complete autologous marrow recovery at 5 Gy and complete donor marrow chimerism at 7 Gy. This dose range is close to the threshold of 7.5 Gy observed clinically for HLA-matched allograft rejection. 1 However, in many other murine BMT models the radiation doses are well beyond the narrow margin for MHC-compatible marrow engraftment. 3 Furthermore, this resistance to fully allogeneic marrow at high radiation doses has been correlated with residual host clonable T cells. 30 Such a correlation would appear not to operate for H-2-compatible engraftment as seen in LP into B6 at lower doses of radiation.

Apart from the immune suppressive effects of radiation in allowing donor marrow stem cell growth is the prevention of competitive autologous relapse from surviving host colonies. Our use of a syngeneic model with a hemoglobin marker for quantifying donor erythroid engraftment provides an opportunity to investigate this process without the added problems of immunologic rejection. The position and shape of the dose-response curve in comparison with allogeneic engraftment shows that the relative importance of immune suppression and host stem cell ablation is exquisitely dependent on the radiation dose level. Hence, at doses of 5 Gy and below the absence of H-2-compatible engraftment appears to reflect inadequate immune suppression, while at 6 Gy and above the dose responses for syngeneic and allogeneic chimerism converge to indicate an abrupt and complete suppression of immune-mediated rejection. The radiation doses required for removing the

Table 2. α/β and Repair Halftime (T\textsubscript{H}) Estimates for Allogeneic Engraftment (LP → B6) With Interfraction Intervals of ≤6 Hours Using Direct Analysis

<table>
<thead>
<tr>
<th>TBI Schedules</th>
<th>α/β (Gy)</th>
<th>T\textsubscript{H} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD (100 and 5 Gy min\textsuperscript{-1})</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>2 Fx (3 and 6 h intervals)</td>
<td>(1.3-2.9)*</td>
<td>(0.9-1.9)*</td>
</tr>
<tr>
<td>2 Gy/Fx (every 6 h)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*95% CL.
remaining host component and establishing a complete allogeneic chimeric state therefore appear to be limited to the final eradication of repopulating host stem cells.

The effect of administering TBI in different fractionation and dose-rate schemes on the position of the dose-response curve provides further insights into the radiobiologic properties of the target cell(s) determining hematopoietic engraftment. Multiple 2-Gy fractions gave dramatic dose sparing for both syngeneic and allogeneic engraftment, as shown by a shift of the respective dose responses to higher total doses of TBI. The extra dose sparing on inclusion of 18-hour intervals in the twice-per-day treatments was a surprising finding that distinguished allogeneic from syngeneic engraftment. This difference was also evident in the two fraction data where extra split-dose recovery was seen when the interval was extended from 6 to 18 and 24 hours, and signifies an additional recovery process confined to allogeneic marrow rejection. The lack of further dose sparing beyond 24 hours to 6 days indicates minimal continuous repopulation over this period and tends to exclude proliferative recovery as being responsible for the large dose-sparing effect of fractionation.

The initial recovery in dose using 3- and 6-hour fraction intervals is consistent with the faster process commonly ascribed to sub-lethal damage repair. The kinetics of radiation repair also provide a basis for explaining the dose-sparing effect of protracting the radiation exposure with 5 cGy/min.23,23 In the latter situation, intracellular repair can operate within the shortest possible treatment time and under the minimal influence of cellular proliferation. Therefore, we suspect that radiation repair in a critical cell population, as witnessed within 6 hours between doses, can account for most of the marked dose sparing from accelerated multiple 2-Gy fractions.

An alteration in TBI conditioning with either low dose rate or fractionation was a subject of investigation in early studies on mice transplanted with xenogeneic rat bone marrow.34,35 However, the possible contribution of lethal GVHD renders these data difficult to interpret. In a later study of fully allogeneic BMT in mice, GVHD was avoided by donor marrow T depletion, and here fractionated TBI became worse than single doses in achieving engraftment and survival.26 A similar conclusion has recently been obtained from a canine model of DLA-identical bone marrow engraftment7 and supports the murine H-2-compatible allogeneic data gained from the present study.

Our use of a range of total radiation doses to construct full dose-response curves for each TBI scheme offers the first opportunity to measure the actual extent of dose sparing and apply informative fractionation parameters for comparison with other tissue systems. The LQ model is especially useful in this respect because it can also be used to define the dose-survival curve of a particular target cell population where the α and β components may be considered to reflect respective radiation cell kill from irreparable and reparable lesions. The relative contribution of these lesions will ultimately dictate the amount of dose recovery for certain tissues with changes in dose rate and dose per fraction. The ratio α/β can be readily obtained from iso-effect doses for various tissue types and is generally regarded to be higher (above 6 Gy) for early radiation reactions.36,39 Under ideal circumstances this parameter should theoretically provide an inverse index of overall repair during fractionated irradiation. For allogeneic marrow engraftment as seen in the present study, the apparent extra dose-sparing effect of immune recovery produced unrealistically low values of α/β. However, when 6-hour interfraction intervals were used, this effect appeared minimal and the data at 100% allogeneic engraftment became comparable with syngeneic transplantation. Here the α/β value was still remarkably low and falls well within the range of 1 to 6 Gy normally ascribed for later responding tissues.39 Indeed, the α/β ratio for bone marrow engraftment lies close to that obtained from fractionated and low dose-rate experiments on the lung.40,42 Hence, in situations where full bone marrow chimerism is the desired effect and pneumonitis becomes the radiation dose-limiting complication, no therapeutic advantage is envisaged by increasing the tolerated total dose with fractionated TBI. This consideration does, of course, neglect other important treatment and disease variables commonly experienced by BMT patients such as combined chemotherapy, donor marrow T depletion, opportunistic infections, GVHD, and relapse of malignancy. While it still remains necessary for all these factors to be evaluated with respect to TBI conditioning, current clinical data do suggest poorer engraftment when TBI is fractionated5,53,64 and have favored the continued use of single-dose TBI in some BMT centers.65 Similar problems may arise from a decrease in radiation dose rate where clinical evidence for higher leukemic relapse has already emerged.66

One other variable that deserves attention is the influence of changing the quality and quantity of the donor marrow inoculum. In the present study the number of transplanted marrow cells was held constant at 107, and at this cell dose level the effective radiation doses were similar to those used in clinical BMT.7 Lowering the donor stem cell content may necessitate an increase in TBI dose to achieve equivalent engraftment, but unacceptable radiation toxicities may then become the limiting factor. Further experimental studies are currently under way to investigate the inter-relationship between host TBI and donor marrow doses in producing bone marrow chimeras.

A proper understanding of the radiobiologic factors involved in the therapeutic application of BMT requires the identification of the host target cell population(s), whose radiation kill determines donor marrow engraftment. For immunologic rejection of allogeneic marrow stem cells, it is conceivable that low TBI doses allow survival of particular lymphocyte subpopulations capable of initiating a host-versus-graft reaction. The radiation sensitivity of this process may vary according to the genetic disparity between donor and host, with fully allogeneic BMT needing higher TBI doses than either semiallogeneic67 or H-2-compatible68 transplants. Such comparisons can be complicated by additional differences in host-versus-graft radiosensitivity noted
among different inbred mouse strains. Cytotoxic T cells appear to be responsible for rejection of H-2 disparate donor marrow, while natural killer cells are mainly implicated in hybrid (semiallogeneic) resistance. Little is currently known regarding the critical immunocompetent cell involved in H-2-compatible allogeneic marrow rejection in relation to TBI dose and dose schedule.

In both syngeneic and allogeneic BMT, the formation of a stable bone marrow chimera presumably requires engraftment of primitive stem cells with high self-renewal at the expense of the analogous radiation sterilized cells of the host. These cells are expected to have an appreciable SLD repair capacity to account for the dose-sparing effect of fractionated and low dose-rate TBI in syngeneic BMT. The recovery of CFUs after TBI can, however, be enhanced by protracting the irradiation, and this may result from SLD repair in an earlier repopulating stem cell population. The CFUs population is now commonly regarded to be heterogeneous with respect to proliferative potential, cycling activity, cell surface antigens, rhodamine retention, and sensitivity to various cytotoxic agents, and it is generally accepted that later-appearing spleen colonies arise from earlier stem cells. The absence of any dose sparing with fractionated TBI on 11-day CFUs survival, whether it be for all colonies or for those above 1.2 mm (see Fig 5), still contrasts with results obtained from syngeneic marrow engraftment (see Fig 2) and is consistent with the concept that even late-forming CFUs do not reflect survival of the most primitive stem cell. Clearly, alternative methods for directly measuring the clonogenicity of cells residing in the pre-CFUs compartment are needed for extrapolation to marrow engraftment data. Ploemacher et al have developed an in vitro limiting dilution assay capable of assessing marrow cells of very high repopulating potential as well as the committed hematopoietic sub-populations corresponding to early and late CFUs. This technique has recently provided evidence for appreciable SLD repair between two radiation doses in the more primitive stem cells (Ploemacher: unpublished observations, December 1989), and this compares well with the split-dose recovery obtained from corresponding syngeneic marrow engraftment as presented in Fig 2. The resting proliferative status of primitive pre-CFUs stem cells may provide conditions that favor repair during extended irradiation and hence confer a low α/β ratio akin to the classical late radiation responses of slow cell-renewing tissues. While the CFUs population now appears to be a poor candidate as the target cell responsible for persistent marrow engraftment, these cells may still be important in avoiding acute marrow failure, especially those that give rise to late spleen colonies. When transplanted in irradiated recipients, this population could offer transient engraftment and hematopoietic support during the critical period of host CFUs depletion. Depending on the irradiation dose and schedule, this process may allow enough time for autologous marrow recovery and subsequent disappearance of donor chimerism.

In conclusion, these investigations have shown that radiation dose and its pattern of delivery have a major impact in determining the success of long-term marrow engraftment and need to be seriously considered in the design of experimental and clinical BMT protocols. Continuing radiobiologic studies on this topic should provide further opportunities for identifying the critical cell populations that govern bone marrow engraftment.

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