# TRANSPLANTATION OF ALLOGENEIC BONE MARROW WITHOUT GRAFT-VERSUS-HOST DISEASE USING TOTAL LYMPHOID IRRADIATION*

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Graft-versus-host disease (GVHD)[1] is still the most important barrier to successful allogeneic bone marrow (BM) transplantation in man and in laboratory animals (1–3). GVHD is especially severe when donor and recipient are completely allogeneic, and differ at the major histocompatibility genetic region (4, 5). Several investigators have been able to reduce or eliminate GVHD by depleting the BM inoculum of T cells or T-cell precursors (6–8), or by treating the adoptive recipients with potent immunosuppressive drugs for at least several months after marrow transplantation (1, 2).

We recently reported that preparation of allogeneic marrow recipients with high-dose, fractionated, total lymphoid irradiation (TLI) allows for marrow engraftment and stable chimerism in rats and mice without development of clinical GVHD (9).[2] TLI is a relatively safe form of radiotherapy, routinely used to treat lymphoid malignancies in man (10). Two critical features of TLI used in animals and man are the shielding of radiosensitive nonlymphoid organs (e.g., long bones, lungs, kidneys, skull) with lead, and the high dose of irradiation ($3–4 \times 10^3$ rads) achieved by multiple small fractions of $2–2.5 \times 10^2$ rads each.

In the present work, we compared the outcome of allogeneic BM transplantation in BALB/c mice treated with TLI, lethal whole-body irradiation (WBI), or fractionated thymic irradiation. Marrow donors were completely allogeneic (C57BL/Ka), and differed from the hosts at the *H-2* region. The experimental results showed that recipients prepared with WBI developed a virulent GVHD such that the majority of animals were dead within 3 wk. On the other hand,

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[1] *Abbreviations used in this paper:* BM, bone marrow; GVHD, graft-versus-host disease; MEM, minimal essential medium; PBL, peripheral blood lymphocytes; TLI, total lymphoid irradiation; WBI, whole-body irradiation.

[2] S. Slavin, B. Reitz, C. P. Bieber, H. S. Kaplan, and S. Strober. Transplantation tolerance in adult rats using total lymphoid irradiation: permanent survival of skin, heart, and marrow allografts. *J. Exp. Med.* 147:700.

recipients prepared with TLI showed no clinical signs of GVHD, and 85% survived longer than 250 days. The surviving animals were stable chimeras. Recipients given thymic irradiation showed no evidence of marrow engraftment.

## Materials and Methods

*Animals.* Inbred BALB/c (H-$2^{d/d}$) mice, 4–6 mo old, were used as recipients for skin and BM allografts. Inbred C57BL/Ka (H-$2^{b/b}$), 1–4 mo old, were the donors of BM and skin grafts. All mice were bred in pathogen-free conditions in the Division of Radiobiology, Department of Radiology, Stanford University, but they were transferred to conventional housing before use in irradiation experiments. At the onset of irradiation, a broad-spectrum antibiotic (tetracycline hydrochloride; American Cyanamid Co., Princeton, N. J.) was added to the drinking water.

*Radiation Source.* A Philips unit (250 kV, 15 mA; Philips Medical Systems Inc., Shelton, Conn.) delivered x-rays at a rate of 59 rad/min. The source-to-skin distance was 60 cm. 0.25-mm Cu and 1.0-mm Al correction filters were used and the dosimetry was determined using a calibrating ionizing chamber and lithium fluoride thermoluminescence dosimeters.

*Irradiation Procedure.* The irradiation procedure was previously described in detail (9, 11, 12). Anesthetized BALB/c mice were positioned in a lead apparatus, exposing the major lymphoid organs, including the thymus and the spleen with proper shielding of the skull, lungs, ribs, hind legs, and tail. In some experiments, the thymus was shielded by placing a piece of lead (0.5 x 0.5 cm) over the mediastinum. Thymic irradiation alone was accomplished by exposing the mediastinum, and shielding the rest of the body with lead. Irradiation protocols consisted of fractions of 200 rads/day, five times a week, to achieve a total dose of 3,400 rads (unless specified otherwise in the text). WBI was delivered using the same radiation source to administer a single dose of 1,000 rads.

*BM Transplantation.* The BM cells were flushed out of isolated long bones (femur, tibia, and humerus) using a 25-gauge needle, and minimal essential medium (MEM). The cells were washed once, filtered through a nylon mesh, and resuspended in MEM. 0.25-ml aliquots of medium containing $10 \times 10^6$ or $30 \times 10^6$ nucleated cells were injected into the lateral tail vein of recipient mice.

*Skin Transplantation.* Full-thickness C57BL/Ka skin obtained from the abdomen of 4- to 6-wk-old female mice were transplanted to the flank of BALB/c recipients (13). Grafts were considered rejected at the time of complete sloughing, or when a dry scab was formed.

*Purification of Peripheral Blood Lymphocytes (PBL).* Blood obtained from the retro-orbital veins was placed in preservative-free heparin coated glass tubes. Samples were diluted (1:5) in phosphate-buffered saline (PBS), and the PBL were purified using a Ficoll-Hypaque gradient (14). Contaminating erythrocytes were lysed with ammonium chloride (15).

*Assay for Chimerism.* Chimerism of the PBL of BM recipients was assayed using a complement-dependent microcytotoxicity assay with a specific anti-H-$2^b$ alloantiserum (B10.A(5R) × A)$F_1$ anti-B10.A(2R) (kindly supplied by Dr. D. B. Murphy, Department of Medicine, Stanford University) as described previously (13). Purified PBL were suspended in medium 199 (Microbiological Associates, Inc., Bethesda, Md.) containing 5% heat-inactivated fetal calf serum ($2 \times 10^6$ cells/ml). 10 $\mu$l of the lymphocyte suspension was incubated with 10 $\mu$l of anti-H-$2^b$ antiserum (1:10) for 30 min at room temperature in 6 x 50-mm glass tubes (Kimble Products, Div. Owens-Illinois, Inc., Toledo, Ohio). 5 $\mu$l of guinea pig complement was added for an additional 45 min at room temperature. The cells were spun at 250 *g* for 5 min, and resuspended in 15 $\mu$l of medium 199. 15 $\mu$l of a 0.1% solution of trypan blue was added before examining the viability of cells in a standard hemocytometer. The cytotoxicity index was calculated by comparing the number of viable cells present after treatment with anti-H-$2^b$ antiserum to that after treatment with normal mouse serum. Negative (BALB/c; PBL ≤5% cytotoxicity) and positive (C57BL/Ka; PBL, 95–100% cytotoxicity) controls were performed in each experiment. Cytotoxicity assays were done in triplicate, and read by a blind observer. Data shown in tables (percent of donor [H-$2^b$] type cells) is the mean ± SD of triplicate values of the cytotoxicity index.

## Results

*Effect of TLI on Allogeneic Skin and Marrow Survival.* Adult BALB/c mice were given marrow and/or skin allografts 1 day after the completion of TLI. TLI

TABLE I

*Survival of C57BL/Ka BM and Skin Allografts in BALB/c Mice Treated with Different Radiation Protocols*

| Recipient treatment | Number of C57BL/Ka BM cells injected | Number of mice | C57BL/Ka skin graft survival | | Fraction of mice with long-term skin graft survival (>100 days) | Percent of donor-type cells in PBL of recipients* | |
|---|---|---|---|---|---|---|---|
| | | | Mean | Range | | Skin graft >100 days survival | Skin graft <100 days survival |
| *rads* | | | *days* | | | | |
| TLI, 3,400 | 0 | 16 | 49.1 | 35–67 | 0/16 | – | – |
| TLI, 3,400 | $10 \times 10^6$ | 15 | >250‡ | | 8/15 | 44 | 0 |
| TLI, 3,400 | $30 \times 10^6$ | 27 | >100§ | | 24/27 | 91 | 37 |
| TLI, 1,400 | 0 | 7 | 18.4 | 13–27 | 0/7 | | |
| TLI, 1,400 | $30 \times 10^6$ | 11 | 17.6‖ | 13–24‖ | 1/11 | ND | 0 |
| TLI, 3,400 with thymus shield | 0 | 10 | 18.0 | 16–25 | 0/10 | – | – |
| Thymus only 3,400 | 0 | 4 | 15.7 | 15–16 | 0/4 | – | – |
| Thymus only 3,400 | $30 \times 10^6$ | 6 | 16.3 | 14–18 | 0/6 | – | 0 |
| None | 0 | 12 | 10.7 | 10–13 | 0/12 | – | – |

* Chimerism was determined in pooled blood samples obtained 7,100 days after BM transplantation. Representative results of one of four experiments are shown.

‡ Data shown is mean of 8/15 mice with long-term skin grafts. In 7/15 mice, C57BL/Ka skin survival was 34–90 days.

§ Data shown is mean of 24/27 mice with long-term skin grafts. In 3/27 mice, C57BL/Ka skin survival was ≤ 20 days.

‖ Data shown is mean and range of 10/11 mice with skin survival ≤ 24 days.

ND, not done.

(3,400 rads) prolonged the survival of C57BL/Ka skin allografts (mean, 49.1 days) about five times as compared to that (mean , 10.7 days) on untreated, control BALB/c recipients (Table I). 8 of 15 recipients treated with TLI (3,400 rads) and given $10 \times 10^6$ C57BL/Ka BM cells intravenously, maintained skin allografts for longer than 100 days (Table I). Increasing the dose of BM cells to $30 \times 10^6$ resulted in long-term (>100 days) skin allograft survival in 24 of 27 recipients.

Typing of the histocompatibility antigens (H-2) of the peripheral blood lymphocytes of BALB/c mice bearing long-term C57BL/Ka skin grafts showed that these animals were stable chimeras (Table I). The level of chimerism was related to the dose of BM cells injected (Table I).

*Effect of Dose of Irradiation on Allogeneic Skin and BM Graft Survival.* To determine the effect of the dose of irradiation on skin and marrow allograft survival, several BALB/c mice were given TLI with a total dose of 1,400 rads. Skin graft survival was slightly prolonged (mean, 18.4 days) as compared to controls (Table I). 1 of 11 mice given TLI (1,400 rads) and $30 \times 10^6$ C57BL/Ka BM cells maintained a C57BL/Ka skin graft for longer than 100 days. The remaining 10 mice rejected their grafts by day 24, and showed no evidence of chimerism 100 days after BM transplantation (Table I). This indicates that

uniform long-term BM graft survival is critically dependent upon the dose of irradiation exceeding 1400 rads.

*Effect of Thymic Irradiation on Allogeneic Skin and BM Graft Survival.* Table I shows that C57BL/Ka skin grafts placed on BALB/c mice given TLI (3,400 rads) with thymic shielding survived for a much shorter period of time (mean, 18.0 days) than did grafts placed on recipients given TLI (3,400 rads) without thymic shielding (mean, 49.1 days). This suggests that the thymus plays an important role in the chronic immunosuppression induced by TLI. To examine the immunosuppression induced by high dose, fractionated irradiation of the thymus alone, several BALB/c mice received 3,400 rads (200-rad fractions) to the thymus with shielding of all other tissues. The survival of skin allografts placed on the latter recipients was only slightly prolonged (mean, 15.7 days) as compared to unirradiated controls (Table I). Mice given thymic irradiation and $30 \times 10^6$ allogeneic BM cells showed no evidence of chimerism after 100 days. Skin grafts transplanted on the day after the injection of the allogeneic BM survived no more than 18 days (Table I). This shows that both the thymus and the peripheral lymphoid tissues must be included in the irradiation fields in order to achieve the full immunosuppressive effects of TLI.

*GVHD after BM Transplantation in Mice Given WBI.* Fig. 1 A shows that a single dose of 1,000 rads of WBI killed 100% of BALB/c mice within 11 days. All mice given WBI could be rescued by a single intravenous injection of $30 \times 10^6$ syngeneic BM cells on the day after irradiation (Fig. 1 A). Although $30 \times 10^6$ allogeneic BM cells prolonged the lives of BALB/c mice given WBI, the majority of recipients died by day 12, and 95% died by day 61 (Fig. 1 A). The latter recipients showed typical changes of GVHD, including weight loss, hunched back, diarrhea, and alopecia, and skin erythema.

*Lack of GVHD after BM Transplantation in Mice Given TLI.* Fig. 1 B shows that TLI (3,400 rads) alone is not a lethal form of radiotherapy, since 90% of irradiated mice survive longer than 100 days, and over 80% survive longer than 250 days. However, we have observed considerably higher mortality figures during periods of known intercurrent infection in our mouse holding rooms. We therefore chose to present mortality data of mice given either WBI or TLI during a period of time when there was no obvious infection of animals in our holding facility.

Transplantation of allogeneic BM cells ($30 \times 10^6$) slightly increased the mortality of BALB/c mice given TLI. However, 80% of marrow recipients survived longer than 110 days. None of the marrow recipients showed clinical evidence of GVHD (diarrhea, hunched back, ruffled fur). Although recipients sometimes lost 20–35% of their body weight during the irradiation treatment, animals gained weight after BM transplantation. The majority of recipients regained their pretreatment weight within 4–6 wk. None of the long-term survivors showed clinical evidence of GVHD. The cause of death of the 15% of animals is unclear, since none of these BM recipients showed obvious signs of GVHD. Autopsy specimens frequently showed evidence of pneumonitis and enteritis, without splenomegaly. We have observed similar autopsy findings in untreated control mice that have succumbed during residence in the same animal rooms (9).

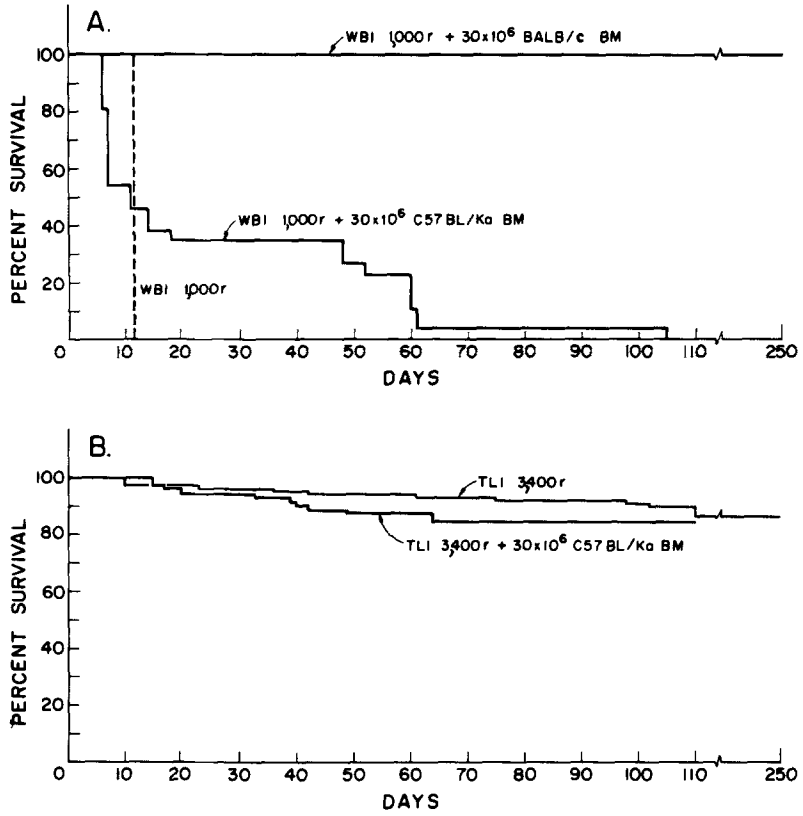


FIG. 1. Survival of BALB/c mice given either TLI, 3,400 rads, or WBI, 1,000 rads, and 30 $\times$ $10^6$ C57BL/Ka BM cells. (A) Survival of 8 mice given WBI and no BM cells, 26 mice given WBI and 30 $\times$ $10^6$ C57BL/Ka BM cells, and 8 mice given WBI and 30 $\times$ $10^6$ BALB/c BM cells. BM cells were injected intravenously 1 day after WBI. (B) Survival of 146 mice given TLI and no BM cells, and 71 mice given TLI and 30 $\times$ $10^6$ C57BL/Ka BM cells. The latter group of mice were set up later than the former, and survival is shown up to the present time. r, rads.

*GVHD after the Transfer of Allogeneic Spleen Cells to Mice Given WBI or TLI.* Fig. 2 shows that BALB/c recipients given WBI (1,000 rads) and 30 $\times$ $10^6$ C57BL/Ka spleen cells intravenously, all died within 10 days after irradiation and cell transfer. In addition, all mice given TLI (3,400 rads) and 30 $\times$ $10^6$ allogeneic spleen cells succumbed by day 46 (Fig. 2). Both groups of mice showed signs of vigorous GVHD. These findings show that spleen cells induce a more rapid GVHD than do BM cells in mice given WBI, and that mice given TLI are protected against clinical GVHD by allogeneic BM, but not by spleen cells.

To determine whether or not allogeneic spleen cells can induce GVHD in stable chimeras, several BALB/c mice given TLI (3,400 rads) and 30 $\times$ $10^6$ C57BL/Ka BM cells were injected with C57BL/Ka spleen cells after chimerism had been documented. Fig. 2 shows the survival of C57BL/Ka $\rightarrow$ BALB/c chimeras given intravenous injections of 30 $\times$ $10^6$ spleen cells on days 78, 92, 106, 118, 150, and 160 after BM transplantation. All animals in the group survived at least 90 days after the last spleen cell injection, and showed no

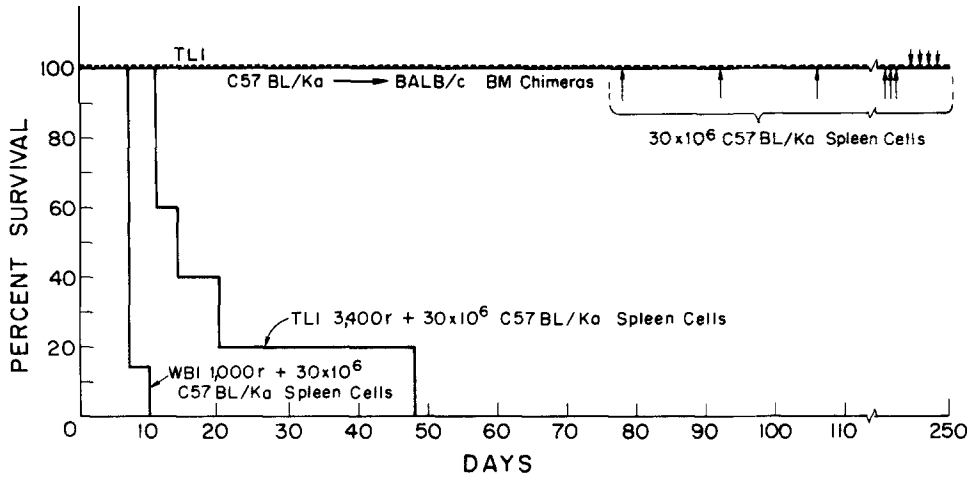


FIG. 2. Survival of BALB/c mice given either TLI (3,400 rads) or WBI (1,000 rads) and 30 $\times$ $10^6$ C57BL/Ka spleen cells. Results from four groups of mice are shown. The first group (14 mice) was given WBI, and a single intravenous injection of spleen cells 1 day later. The second group (6 mice) was given TLI and a single intravenous injection of spleen cells 1 day after the completion of TLI. The third group (4 mice) was given TLI, and six intravenous injections of spleen cells on days 180, 194, 220, 240, 268, and 280 after the completion of TLI (indicated by arrows). The fourth group (6 mice) was given TLI, and 30 $\times$ $10^6$ C57BL/Ka BM cells 1 day after the completion of TLI. In addition, these animals received six intravenous injections of spleen cells on days 78, 92, 106, 118, 150, and 160 after the completion of TLI. The latter recipients were all shown to be stable chimeras before spleen cell injections were begun. r, rads.

evidence of GVHD. This shows that C57BL/Ka spleen cells cannot induce GVHD in C57BL/Ka → BALB/c chimeras despite the fact that the chimeras are specifically tolerant to C57BL/Ka transplantation antigens (9). In control experiments, BALB/c mice given TLI without BM cells received C57BL/Ka spleen cell injections more than 100 days after irradiation. None of the latter animals developed GVHD. However, our previous experiments indicate that the latter recipients recover their ability to respond to allogeneic lymphocytes in vitro by 100 days (9).[3] Therefore, it is likely that these mice rejected the allogeneic spleen cells.

*Effect of Presensitization on BM and Skin Allograft Survival in Mice Given TLI.* A significant problem in clinical organ and BM transplantation is the presensitization of prospective recipients with blood products which share transplantation antigens with donor tissues (1). We therefore sensitized normal BALB/c mice with two 0.25-ml intravenous injections of C57BL/Ka blood, 18 and 11 days before initiating TLI. The latter recipients received 30 $\times$ $10^6$ C57BL/Ka BM cells and a skin allograft at the end of TLI. Table II shows that all skin grafts were rejected after 24 days. None of these mice showed evidence of chimerism of the peripheral blood lymphocytes 100 days after BM and skin transplantation. Thus, presensitization with peripheral blood prevented the

[3] S. Slavin, S. Nimelstein, and S. Strober. Specific humoral transplantation tolerance to H-2 antigens in mice treated with total lymphoid irradiation. Manuscript in preparation.

TABLE II

*Effect of Presensitization of BALB/c Mice with Donor-Type Blood on the Long-Term Survival of C57BL/Ka BM and Skin Allografts after TLI**

| Presensitization protocol | Donor-type cells in PBL | Mean C57BL/Ka skin allograft survival | Fraction of recipients with long-term (>100 days) skin graft survival |
|---|---|---|---|
| | % | *days* | |
| 0 | 91‡ | >100§ | 24/27 |
| C57BL/Ka Blood 0.25 ml days −18, −11 | 0‖ | 18.7 (range 17–24) | 0/8 |

* Experiments involved $30 \times 10^6$ C57BL/Ka BM cells, and TLI with 3,400 rads.

‡ Chimerism was determined in pooled blood sample in mice with long-term skin grafts.

§ The data shown is the mean of 24/27 mice with long-term skin grafts.

‖ Chimerism was determined on pooled blood sample obtained >100 days after skin and marrow transplantation.

TABLE III

*Effect of Presensitization of BALB/c Mice with Donor-Type Skin on C57BL/Ka Skin Allograft Survival after TLI**

| Presensitization | Irradiation | Number of mice | C57BL/Ka Skin | |
|---|---|---|---|---|
| | | | Mean | Range |
| | | | *days* | |
| None | None | 12 | 10.7 | 10–13 |
| None | TLI | 12 | 49.1 | 35–67 |
| C57BL/Ka skin graft‡ | TLI | 6 | 23.0 | 20–27 |
| C57BL/Ka skin graft§ | None | 4 | 8.2 | 7–12 |

* TLI carried out with 3,400 rads.

‡ Presensitizing allograft placed 24 days before TLI. Final graft placed 1 day after TLI.

§ Presensitizing allograft placed 6 mo before final C57BL/Ka skin graft.

establishment of stable chimerism observed after TLI and BM transplantation in unsensitized hosts (Table II).

The effect of presensitization of recipients was further studied by immunizing several BALB/c mice with C57BL/Ka skin grafts 24 days before the initiation of TLI. At the end of TLI, all mice received a second skin allograft. Table III shows that presensitization markedly reduced the survival of the skin allografts (mean survival, 23 days) as compared to that observed with unsensitized hosts (mean survival, 49.1 days). However, graft survival was still twice as long as that of grafts placed on unirradiated, unsensitized BALB/c mice (Table I), and three times as long as that on unirradiated, presensitized recipients (Table III).

## Discussion

The object of the present work was to further investigate the use of TLI in allogeneic BM transplantation. Our previous studies showed that BALB/c mice given TLI (3,400 rads) accepted C57BL/Ka BM without clinical GVHD. How-

ever, long-term BM engraftment occurred in only 53% of recipients given $10 \times 10^6$ cells, and the vigor of GVHD in this strain combination was not tested in recipients prepared with lethal WBI.

The present investigation shows that increasing the dose of injected BM cells from $10 \times 10^6$ to $30 \times 10^6$ increased the percentage of recipients with long-term (>100 days) BM grafts from 53 to at least 90%. The percentage of donor-type lymphocytes in the blood also increased from 44 to 91%. All of the chimeric animals maintained skin allografts for more than 100 days, and showed no clinical signs of GVHD (hunched back, weight loss, diarrhea, etc.).

Reduction of the cumulative dose of TLI to 1,400 rads substantially reduced the nonspecific immunosuppressive effects observed with 3,400 rads, since skin allograft survival with the low dose (mean, 18.4 days) was considerably less than that with the high dose (mean, 49.1 days). Reduction of the dose of TLI also decreased the frequency of recipients with long-term BM engraftment to <10% after the injection of $30 \times 10^6$ BM cells.

The critical importance of irradiation of the thymus during TLI was demonstrated by the marked decrease in the survival of C57BL/Ka skin grafts transplanted to recipients given TLI with thymic shielding as compared to that in recipients given TLI with the thymus exposed. However, fractionated, high-dose irradiation (3,400 rads total, 200 rads/fraction) of the thymus alone resulted in only marginal prolongation of skin graft survival (mean survival, 18 days). None of the recipients given thymic irradiation alone accepted long-term BM grafts. These findings suggest that irradiation of both the thymus and peripheral lymphoid tissues is necessary to achieve the full immunosuppressive effects of TLI.

To study the vigor of the GVHD in the C57BL/Ka → BALB/c strain combination, BALB/c mice were given a single lethal dose (1,000 rads) of WBI and $30 \times 10^6$ C57BL/Ka BM cells. The majority of recipients died within 12 days, and 95% died within 61 days. All irradiated recipients given $30 \times 10^6$ syngeneic BM cells survived longer than 250 days. Allogeneic marrow recipients showed typical signs of GVHD with alopecia, weight loss, hunched back, and diarrhea. In contrast, >80% of mice given TLI alone, or TLI and $30 \times 10^6$ allogeneic BM cells survived at least 110 days after BM transplantation. Although the marrow recipients were chimeric, none showed clinical signs of GVHD. The cause of death of those animals which died in these latter groups is not clear. Autopsy results were similar to those observed in untreated control mice which succumbed in our holding rooms during the same period of time. These findings show that preparation of BM recipients with TLI allows for engraftment without GVHD in a strain combination which shows severe GVHD when WBI is used for preparation.

Although the mechanism by which TLI protects against GVHD remains to be elucidated, the severe GVHD induced by $30 \times 10^6$ allogeneic spleen cells in recipients given TLI suggests that the usual target organs are susceptible to immunological attack by mature immunocompetent T cells. Protection against GVHD by allogeneic BM cells may be due in part to the lack of development of mature T cells and/or to the development of suppressor cells from immature cells in the BM. Even susceptibility to GVHD induced by spleen cells was transient in mice given TLI, since no GVHD was observed after multiple

injections of spleen cells into stable chimeras or into mice allowed to spontaneously recover from TLI for a period of >100 days. It is likely that the allogeneic spleen cells can only proliferate rapidly in the lymphoid tissues before repopulation by either progeny of the allogeneic BM cells, or that of the host's own BM cells.

One important problem in clinical BM transplantation is presensitization by blood transfusions (1). To investigate this point in our experimental model, several BALB/c mice were given 0.25-ml blood transfusions from C57BL/Ka donors on two separate occasions before the initiation of radiotherapy and BM transplantation. The experimental results show that the blood transfusions prevented long-term engraftment of BM in recipients given TLI. This suggests that TLI cannot abrogate immunity to allogeneic BM cells induced by the blood elements. Further studies on the effects of TLI on the rejection of skin allografts in presensitized mice showed that TLI substantially prolongs allograft survival, even in mice that had previously rejected a skin allograft. However, the survival time (mean, 23 days) was less than half of that in unsensitized mice given TLI (mean, 49.1 days).

In conclusion, this work suggests that TLI may be a useful procedure in clinical BM and organ transplantation. TLI has already been used extensively in humans for treatment of lymphoid malignancies, and is a relatively safe form of radiotherapy (10).

## Summary

Bone marrow (BM) and skin allografts from C57BL/Ka (H-$2^{b/b}$) mice were transplanted to BALB/c (H-$2^{d/d}$) recipients treated with total lymphoid irradiation (TLI), whole-body irradiation (WBI), or fractionated thymic irradiation. TLI prolonged skin allograft survival about five times as long as that in untreated controls, and allowed for permanent engraftment of BM cells in ≅90% of recipients. None of the BM recipients showed clinical signs of graft-versus-host disease (GVHD) (diarrhea, weight loss, hunched back, etc.). On the other hand, recipients given WBI and allogeneic BM cells developed severe clinical GVHD. The majority of the latter recipients died within 12 days after BM transplantation, and 95% died within 61 days. Although TLI protected BALB/c mice against GVHD induced by BM cells, all recipients given TLI and allogeneic spleen cells developed lethal GVHD. Thymic irradiation alone marginally prolonged skin allograft survival, and did not allow for allogeneic BM engraftment. These results suggest that TLI may be a useful regimen in clinical BM transplantation, since this form of radiotherapy is used extensively in humans and has few severe side effects.

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