

# Immunosuppressive Effects of $^{131}\text{I}$ -Anti-CD45 Antibody in Unsensitized and Donor Antigen-presentation H2-matched, Minor Antigen-mismatched Murine Transplant Models<sup>1</sup>

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## ABSTRACT

Iodine-131-labeled anti-CD45 antibody has been added to conventional hematopoietic stem cell transplant preparative regimens to deliver targeted radiation to hematopoietic tissues, with the goal of decreasing relapse rates without increasing toxicity. However, higher radiation doses could be delivered to leukemia cells by antibody if the systemic therapy were decreased or eliminated. To examine the ability of  $^{131}\text{I}$ -anti-CD45 antibody to provide sufficient immunosuppression for transplantation across allogeneic barriers, T-cell-depleted BALB.B marrow was transplanted into H2-compatible B6-*Ly5<sup>a</sup>* mice after  $^{131}\text{I}$ -30F11 (rat antimurine CD45) antibody with or without varying dose levels of total body irradiation (TBI). Groups of five or six recipient mice per  $^{131}\text{I}$  or TBI dose level per experiment were given tail vein injections of 100  $\mu\text{g}$  of  $^{131}\text{I}$ -labeled 30F11 antibody 4 days before marrow infusion, with or without TBI on day 0. Engraftment, defined as  $\geq 50\%$  donor B cells at 3 months post-transplant, was determined by two-color flow cytometric analysis of peripheral blood granulocytes, T cells, and B cells using antibodies specific for donor and host CD45 allotypes and for CD3. Donor engraftment of  $\geq 80\%$  recipient mice was achieved with either 8 Gy of TBI or 0.75 mCi of  $^{131}\text{I}$ -30F11 antibody, which delivers an estimated 26 Gy to bone marrow. Subsequent experiments determined the dose of TBI alone or TBI plus 0.75 mCi of  $^{131}\text{I}$ -30F11 antibody necessary for engraftment in recipient mice that had been presensitized to donor antigens before transplant, a setting requiring more stringent immunosuppression. Engraftment was seen in  $\geq 80\%$  of presensitized recipients surviving after 14–16 Gy of TBI or 12–14 Gy of TBI and 0.75 mCi of  $^{131}\text{I}$ -30F11 antibody. However, only 28 of 69 (41%) presensitized mice receiving 10–16 Gy of TBI alone survived, presumably because of rejection of donor marrow and ablation of host hematopoiesis. In contrast, 29 of 35 (83%) presensitized mice receiving  $^{131}\text{I}$ -30F11 antibody and 10–14 Gy of TBI survived, presumably because the additional immunosuppression provided by estimated radiation doses of 53 Gy to lymph nodes and 81 Gy to spleen from 0.75 mCi of  $^{131}\text{I}$ -30F11 antibody permitted engraftment of donor marrow. These results suggest that targeted radiation delivered by  $^{131}\text{I}$ -anti-CD45 antibody provides sufficient immunosuppression to replace an appreciable portion of the TBI dose used in matched sibling hematopoietic stem cell transplant.

## INTRODUCTION

Although HSCT<sup>3</sup> has been used extensively for the treatment of acute leukemia for almost 30 years, recurrent malignancy remains the most common cause of treatment failure. Furthermore, transplant-

related mortality, due in part to the toxicities of high-dose systemic therapy administered, ranges from 10–50%, depending on disease type and stage, patient age, conditioning regimen, and stem cell source. The delivery of targeted radiotherapy to hematopoietic tissues with the use of a radioimmunoconjugate as part of a transplant preparative regimen has the potential to decrease relapse without increasing transplant-related mortality by delivering relatively higher doses of radiation to malignant cells in those tissues as compared with nontarget organs such as lungs and liver.

Iodine-131-labeled anti-CD45 antibody has been demonstrated to deliver higher radiation doses to hematopoietic tissues including marrow, spleen, and lymph nodes as compared with nonhematopoietic tissues in mice, macaques, and humans (1–5) and has been used to deliver supplemental hematopoietic tissue radiation in combination with standard myeloablative regimens in several clinical protocols (4, 5). BC8 antibody, a murine antihuman CD45 antibody, has been administered as an  $^{131}\text{I}$ -labeled radioimmunoconjugate to more than 100 patients with acute myeloid or lymphoid leukemia. Radiation doses of 5–10 Gy delivered by antibody to liver, the normal organ receiving the highest dose, have been well tolerated when combined with either cyclophosphamide and TBI (4) or busulfan and cyclophosphamide (5) regimens. On average, radiolabeled antibody delivered estimated radiation doses to marrow that were 2.3-fold higher than those delivered to liver and delivered doses to spleen that were 4.8-fold higher than those delivered to liver.

Higher total doses of radiation could be delivered to target tissues by radiolabeled antibody if the systemic therapy, particularly TBI or busulfan, could be decreased or eliminated. Such an approach might not only further decrease the risk of posttransplant relapse but also decrease the toxicity of the transplant procedure by decreasing the total doses delivered to nonhematopoietic tissues such as lung, liver, kidney, and mucous membranes. However, because one purpose of the preparative regimen administered before transplantation is to provide enough suppression of the host immune system to prevent rejection of donor HSCs, the ability of radiation delivered by  $^{131}\text{I}$ -anti-CD45 antibody to mediate such immunosuppression must be determined before substituting radiolabeled antibody for systemic therapy. Of particular concern is the very low dose rate at which radiation is delivered by  $^{131}\text{I}$ -labeled antibody, given the ability of immune-competent cells to repair sublethal radiation damage and the demonstrated requirement for higher radiation doses to achieve an equivalent degree of immunosuppression when delivered at a lower exposure rate (6–10).

Matthews *et al.* (3) have demonstrated previously in murine transplant models that radiation from 0.5 mCi of  $^{131}\text{I}$  delivered by anti-CD45 antibody alone provided sufficient myeloablation to allow engraftment of T-cell-depleted congenic marrow (C57BL/6, *Ly5<sup>b</sup>*) that differed from the host by CD45 allotype (B6-*Ly5<sup>a</sup>*), a difference that does not provoke immune recognition. In contrast, in the most demanding test of immunosuppression, the delivery of 1.5 mCi of  $^{131}\text{I}$  by anti-CD45 antibody to the same B6-*Ly5<sup>a</sup>* recipients did not reliably

Received 1/30/01; accepted 4/30/01.

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<sup>1</sup> Supported by NIH Grant CA71077. D. C. M. is a Clinical Scholar of the Leukemia and Lymphoma Society, and I. D. B. is a Clinical Research Professor of the American Cancer Society.

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<sup>3</sup> The abbreviations used are: HSCT, hematopoietic stem cell transplant; TBI, total body irradiation; HSC, hematopoietic stem cell; MoAb, monoclonal antibody; GVHD, graft-versus-host disease.

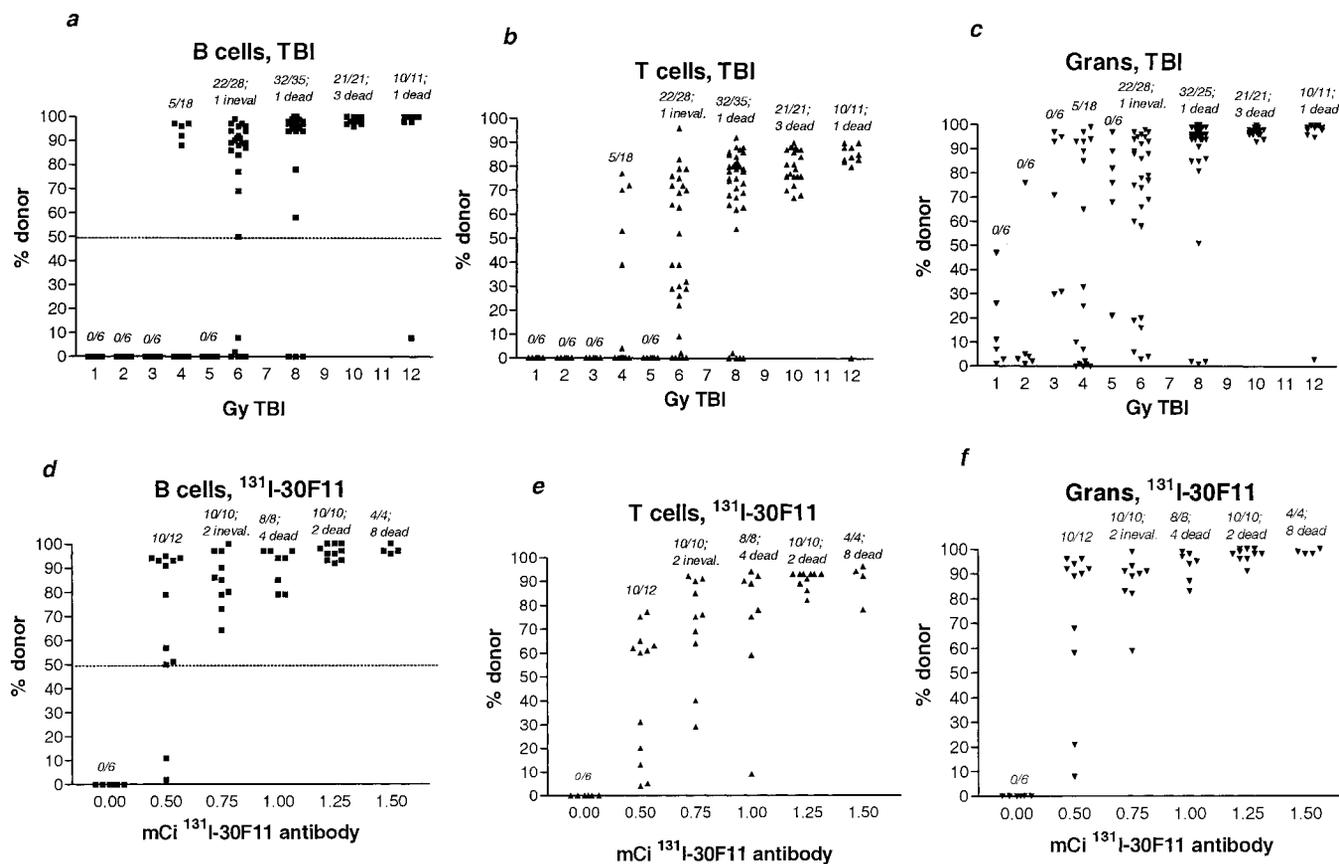


Fig. 1. Engraftment of B cells (a and d), T cells (b and e), and granulocytes (c and f) after 1–12 Gy of TBI (a–c) or 0–1.5 mCi of <sup>131</sup>I-30F11 antibody (d–f). Data points on or above the 50% line in a and d represent animals meeting the criteria for donor engraftment. The number of mice meeting the criteria for engraftment relative to the number of mice surviving to 3 months posttransplant and the number of deaths are indicated for each treatment group. Data in a–c are the combined results of five experiments, and data in d–f are the combined results of two experiments.

allow engraftment of T-cell-depleted, H2-mismatched marrow from BALB/c donors. However, in the latter model, the radiation delivered by 0.75 mCi of <sup>131</sup>I-antibody combined with 6–8 Gy TBI resulted in engraftment, thus demonstrating that the radiation delivered by antibody could substitute for approximately half of the 14 Gy of TBI required for engraftment in H2-mismatched recipients.

Until the recent availability of HLA-matched unrelated HSC donors, the most commonly used source of stem cells in human transplantation has been a sibling donor who is matched for major histocompatibility antigens but mismatched for multiple minor histocompatibility antigens. Therefore, we examined the immunosuppression and myeloablation provided by <sup>131</sup>I-anti-CD45 antibody in a similar H2-compatible, minor antigen-mismatched murine transplant model, using BALB.B donors and the same B6-*Ly5<sup>d</sup>* recipient mice. We also studied the immunosuppression provided by radiolabeled antibody when recipient mice had been presensitized to donor alloantigens by injecting donor splenocytes 4 weeks before <sup>131</sup>I-antibody/TBI conditioning and T-cell-depleted transplant, a setting in which maximum immunosuppression is needed to allow engraftment.

## MATERIALS AND METHODS

**Mice.** Male B6-*Ly5<sup>d</sup>* mice were bred at the Fred Hutchinson Cancer Research Center (Seattle, WA) and housed in a pathogen-free environment with acidified water and autoclaved chow. Male BALB.B mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were between 6 and 12 weeks old at the start of each experiment. Both B6-*Ly5<sup>d</sup>* and BALB.B mouse strains express the H2<sup>b</sup> haplotype but differ for CD45 allotype and at multiple minor histocompatibility loci.

**MoAbs.** MoAbs GK1.5 (rat IgG<sub>2b</sub>, anti-CD4), 30-H12 (rat IgG<sub>2b</sub>, anti-*thy* 1.2), and 2.34 (rat IgG<sub>2b</sub>, anti-CD8) were isolated from culture supernatants of cell lines obtained from either American Type Culture Collection (GK1.5 and 2.34) or Dr. Jeffrey Ledbetter (30-H12) of Bristol Myers Squibb (Seattle, WA). Hybridoma secreting antibody 30F11, a rat IgG<sub>2b</sub>, recognizing all isoforms of murine CD45, was the gift of Dr. Jeffrey Ledbetter. Hybridoma cell lines secreting murine IgG<sub>2a</sub> antibodies 104, which recognizes the Ly5.2 epitope encoded by the *Ly5b* allotype of murine CD45, and A20, which recognizes the Ly5.1 epitope encoded by the *Ly5a* allotype of murine CD45, were the gifts of Dr. Shoji Kimura of Sloan Kettering Institute (New York, NY). Ascites containing each MoAb were prepared in BALB/c mice under pathogen-free conditions, and batch extraction of each MoAb was performed with the use of Abx exchange resin (J. T. Baker, Phillipsburg, NJ) and high-pressure liquid chromatography (Biosys 510; Beckman, Fullerton, CA).

**Iodination and Characterization.** Antibodies were labeled with Na<sup>131</sup>I (ICN, Irvine, CA) by using the chloramine-T method for high specific activity labeling (11). The immunoreactivity, or the percentage of counts able to bind at antigen excess, was determined by incubating antibody at 5 ng/ml (low concentration) with  $1.0 \times 10^7$  to  $3.0 \times 10^8$  B6-*Ly5<sup>d</sup>* splenocytes for 1 h at room temperature and measuring unbound antibody as described previously (12).

**Bone Marrow Transplantation.** In the presensitization experiments, groups of five or six B6-*Ly5<sup>d</sup>* recipient mice were given tail vein injections of  $1.0 \times 10^6$  BALB.B splenocytes 4 weeks before marrow infusion. In all experiments, groups of five or six recipient B6-*Ly5<sup>d</sup>* mice received tail vein injections of 100 μg of 30F11 antibody labeled with 0.5–1.5 mCi of <sup>131</sup>I in 200–300 μl administered 4 days before marrow infusion. The estimated radiation doses delivered to various organs by these doses of <sup>131</sup>I-30F11 antibody are summarized in Table 1, as calculated from previously published biodistribution studies of trace <sup>131</sup>I-labeled 30F11 antibody (3). Control mice received 200-μl injections of RPMI 1640 alone in initial experiments. Whole-

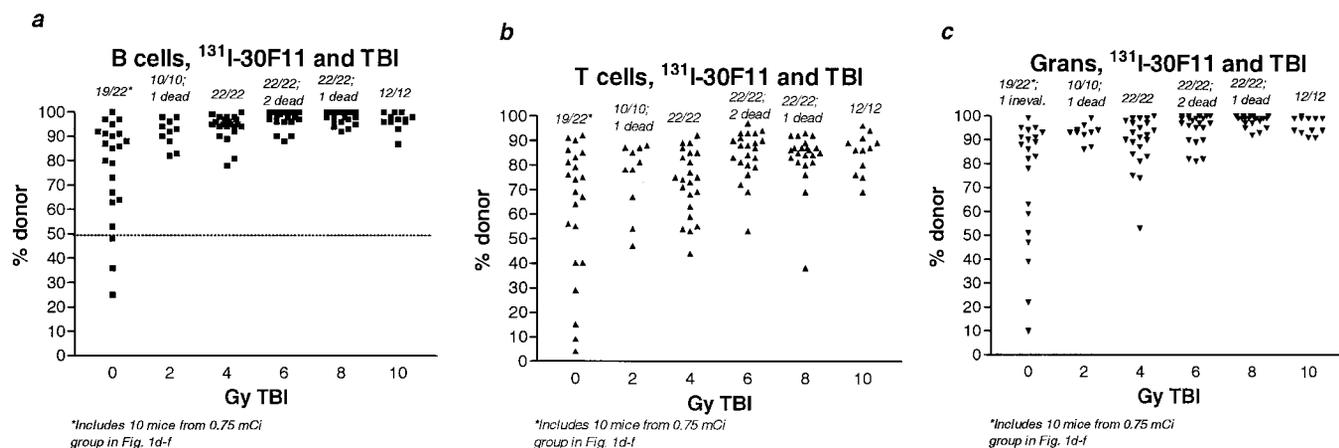


Fig. 2. Engraftment of B cells (a), T cells (b), and granulocytes (c) after 0.75 mCi of  $^{131}\text{I}$ -30F11 antibody and 0–10 Gy of TBI. Data points on or above the 50% line in a represent animals meeting the criteria for donor engraftment. The number of mice meeting the criteria for engraftment relative to the number of mice surviving to 3 months posttransplant and the number of deaths are indicated for each treatment group. Data represent the combined results of four experiments.

body counts of each mouse were measured using a radioisotope calibrator (Capintec Industries, Inc., Ramsey, NJ). Cages were changed every other day for the first week after antibody administration to reduce the nonspecific irradiation from urinary  $^{131}\text{I}$  in the cage bedding. External beam TBI was delivered on the day of marrow infusion at 20 cGy/min from either dual  $^{60}\text{Co}$  sources (J. L. Shepard Co., San Francisco, CA) or from a linear accelerator (J. M. Co., San Jose, CA).

Bone marrow cells were isolated from femora of BALB.B donors. Marrow cells were washed with hemolytic buffer, and T-cell depletion was carried out using complement-mediated lysis with antibodies against CD4, CD8, and Thy-1, which routinely depleted 97–99% of T cells (data not shown). A cell dose of  $1.0 \times 10^7$  nucleated cells, counted before T-cell depletion, was given as a 200- $\mu\text{l}$  tail vein injection. A high cell dose was used to ensure that cell dose was not a limiting factor in engraftment, especially given that some radiolabeled antibody remained in hematopoietic tissues at the time of marrow infusion (96 h after antibody injection; Ref. 3). T-cell depletion was carried out before marrow infusion in all experiments to allow comparison to previous studies of H2-mismatched transplantation and to prevent GVHD.

**Assessment of Engraftment.** Mice were examined daily for survival and for signs of illness and/or GVHD. At 30, 60, and 90 days posttransplant, orbital blood samples were stained with 50  $\mu\text{l}$  of biotinylated 104 (anti- $\text{Ly}5^b$ ) or biotinylated A20 (anti- $\text{Ly}5^a$ ) antibody at 100  $\mu\text{g}/\text{ml}$  for 30 min followed by phycoerythrin-streptavidin (PharMingen, San Diego, CA) and FITC-conjugated anti-CD3 antibody (PharMingen). Control samples were obtained from unmanipulated mice of both donor and recipient strains. Granulocyte and lymphocyte populations were defined by both forward scatter and 90° side scatter parameters. To distinguish B cells from T cells, the lymphocyte population was analyzed separately using two colors to distinguish between  $\text{CD}3^-$  and  $\text{CD}3^+$  subpopulations. Engraftment was defined as  $\geq 50\%$  of the B-cell fraction being of donor origin at 3 months after the marrow infusion.

## RESULTS

**Engraftment after TBI.** To determine the amount of TBI required for engraftment of B6- $\text{Ly}5^a$  recipients with BALB.B donor marrow,

Table 1 Estimated Gy delivered to tissues by 30F11 antibody labeled with varying doses of  $^{131}\text{I}$  [extrapolated from previously published data (Ref. 3)]

	Dose of $^{131}\text{I}$ (mCi)				
	0.5	0.75	1.0	1.25	1.5
Spleen	54	81	107	135	162
Marrow	17	26	35	44	52
Brachial lymph node	35	53	70	88	105
Thymus	6	10	13	16	19
Lung	8	13	17	21	25
Liver	5	8	11	13	16
Kidney	6	9	13	16	19
Stomach	5	8	10	13	16
Total body	3	4	6	7	9

groups of six recipient mice were treated with 1–12 Gy of TBI followed by marrow infusion (Fig. 1a–c; summary of five experiments). All mice receiving 10 Gy demonstrated engraftment by 3 months posttransplant (Fig. 1a), as did 32 of 35 mice (91%) receiving 8 Gy and 22 of 28 mice (79%) receiving 6 Gy. Myeloid and B-cell engraftment occurred within 4 weeks after marrow infusion, but T-cell engraftment did not occur until 8–12 weeks after marrow infusion, presumably reflecting the delayed elimination of recipient T cells in the absence of GVHD. After TBI doses between 2 and 5 Gy, the percentage of granulocytes of donor origin at 3 months was higher than the percentage of either B or T cells of donor origin (Fig. 1, a–c). In these groups, a donor granulocyte percentage as high as 97% was seen in the absence of any detectable donor T cells and in the presence of only 0–28% donor B cells, suggesting that some of these mice may be stable mixed chimeras. In three experiments where engraftment was reevaluated 4 months after transplant, the levels of T-cell, B-cell, and granulocyte engraftment were unchanged as compared with 3 month data (data not shown). Although there was some variability in the minimum dose of TBI required for engraftment between experiments, there was a consistent increase in the percentage of B and T cells of donor origin at TBI doses of 6 Gy and higher. Thus, these experiments demonstrate that TBI doses of 8–10 Gy are necessary to enable engraftment of H2-matched, minor antigen-mismatched, T-cell-depleted marrow in recipients that have not been primed against donor alloantigens.

**Engraftment after  $^{131}\text{I}$ -30F11 Antibody.** To determine the amount of  $^{131}\text{I}$  delivered as an anti-CD45 radioimmunoconjugate required for engraftment in this donor/recipient combination, groups of six mice were treated with 0.5–1.5 mCi of  $^{131}\text{I}$ -30F11 antibody followed by marrow infusion (Fig. 1, d–f; summary of two experiments). Controls consisted of groups of three mice treated with sham injections of RPMI 1640 followed by marrow infusion. Ten of 12 mice (83%) receiving 0.5 mCi of  $^{131}\text{I}$ -30F11 antibody engrafted, and all mice receiving at least 0.75 mCi of  $^{131}\text{I}$ -30F11 engrafted (Fig. 1d). When the data from subsequent experiments involving mice treated with 0.75 mCi of  $^{131}\text{I}$ -30F11 antibody and no TBI are included, engraftment was seen in 19 of 22 (86%) mice (Fig. 2a), which is comparable to the engraftment seen in 32 of 35 (91%) mice treated with 8 Gy of TBI alone (Fig. 1a). Thus, 0.75 mCi of  $^{131}\text{I}$ -30F11 antibody provides a biological effect equivalent to 8 Gy of TBI delivered at 20 cGy/min in B6- $\text{Ly}5^a$  mice. The estimated radiation absorbed doses to target organs from 0.75 mCi of  $^{131}\text{I}$ -30F11 antibody are 26 Gy to marrow, 53 Gy to brachial lymph nodes, and 81 Gy to

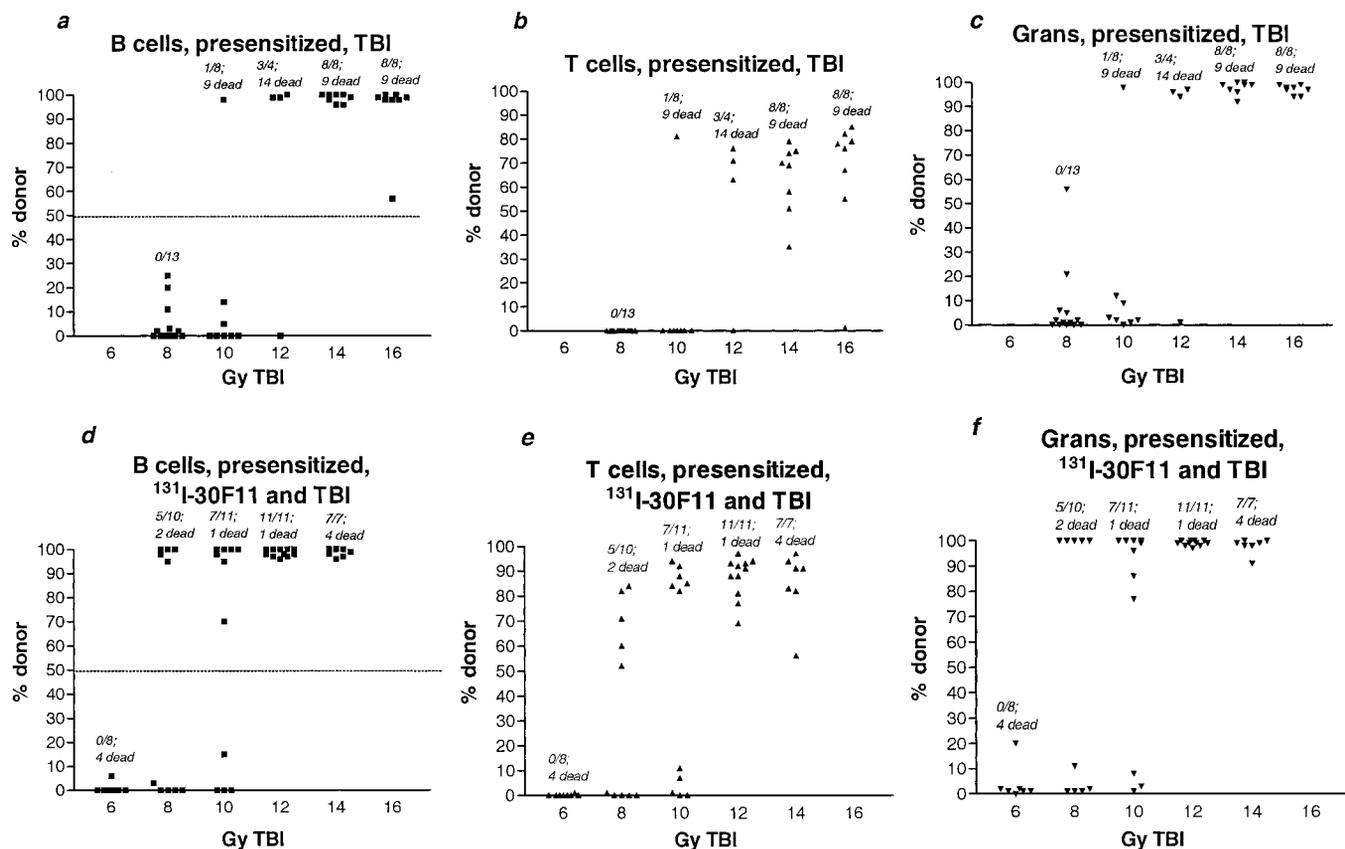


Fig. 3. Engraftment of B cells (a and d), T cells (b and e), and granulocytes (c and f) in presensitized recipient mice after 6–16 Gy of TBI alone (a–c), or 0.75 mCi of <sup>131</sup>I-30F11 antibody plus 6–14 Gy of TBI (d–f). Data points on or above the 50% line in a and d represent animals meeting the criteria for donor engraftment. The number of mice meeting the criteria for engraftment relative to the number of mice surviving to 3 months posttransplant and the number of deaths are indicated for each treatment group. The data in a–c are the combined results of three experiments, and the data in d–f are the combined results of two experiments.

spleen (Table 1), based on previously published antibody biodistribution data (3).

At doses higher than 0.75 mCi of <sup>131</sup>I-30F11 antibody, 14 deaths occurred among 36 mice treated (39%), with the highest death rate (8 of 12 mice, 67%) in the 1.5 mCi-treated group. The majority of deaths (11 of 14) occurred by day 10 posttransplant. Because mice engrafted after <sup>131</sup>I-antibody doses of 0.75–1.0 mCi, these early deaths at higher dose levels presumably reflect radiation toxicity rather than graft rejection.

**Engraftment after 30F11 Antibody Labeled with 0.75 mCi of <sup>131</sup>I Combined with Varying Doses of TBI.** To test the additive immunosuppression of <sup>131</sup>I-30F11 antibody combined with nonablative doses of TBI in this donor/recipient pair, groups of five or six B6-Ly5<sup>a</sup> mice were injected with 30F11 antibody labeled with 0.75 mCi of <sup>131</sup>I, followed by TBI at doses ranging from 0 (*i.e.*, no TBI) to 10 Gy and infusion of BALB.B marrow (Fig. 2, a–c; summary of four experiments). Control mice received 6–10 Gy of TBI followed by marrow infusion (Fig. 1, a–c) or 8–10 Gy of TBI and no marrow. Although there was some variation between experiments in the dose of TBI required in addition to 0.75 mCi of <sup>131</sup>I-30F11 antibody for uniform engraftment of all recipients, all mice (10 of 10) receiving at least 2 Gy of TBI plus 0.75 mCi of <sup>131</sup>I-antibody engrafted (Fig. 2a). Thus, these experiments demonstrate that treatment with 0.75 mCi of <sup>131</sup>I-antibody plus 2 Gy of TBI provides a biological effect equivalent to that of 10 Gy of TBI administered at 20 cGy/min to B6-Ly5<sup>a</sup> mice.

**Engraftment in Presensitized Recipient Mice after TBI Alone or TBI Combined with 0.75 mCi of <sup>131</sup>I-30F11 Antibody.** To determine the amount of TBI required for engraftment of donor marrow in mice presensitized to donor antigens, groups of six mice

underwent injection of donor splenocytes, followed 4 weeks later by 10–16 Gy of TBI and donor marrow infusion (Fig. 3a–c; summary of three experiments). Engraftment occurred in almost all (19 of 20) surviving mice receiving at least 12 Gy (Fig. 3a), but most mice (42 of 69, 59%) receiving at least 10 Gy of TBI died. Thirty-eight of the 42 deaths (90%) occurred before day 30 posttransplant, suggesting failure of engraftment of donor marrow with ablation of autologous stem cells. This is consistent with previous studies demonstrating that the LD<sub>50</sub> of TBI given to B6.Ly5<sup>a</sup> mice at 20 cGy/min without subsequent marrow rescue is between 10 and 12 Gy, with no mice surviving after 12 Gy (3). The death rate was highest (14 of 18 mice) in mice receiving 12 Gy of TBI, suggesting that the dose required to achieve adequate immunosuppression was higher than the myeloablative dose in this presensitized recipient setting.

In additional experiments, presensitized mice were treated with 0.75 mCi of <sup>131</sup>I-30F11 antibody combined with 6–14 Gy of TBI (Fig. 3, d–f; summary of two experiments). In contrast to mice treated with TBI alone, most mice (29 of 35 mice, 83%) receiving radio-labeled antibody and at least 10 Gy of TBI survived, and only three of these deaths occurred before day 30 posttransplant. Furthermore, all surviving mice receiving antibody and at least 12 Gy of TBI engrafted (Fig. 3d). These experiments indicate that 0.75 mCi of <sup>131</sup>I-30F11 antibody given in conjunction with 12 Gy of TBI, which together provide the biological effect of 20 Gy of TBI, causes sufficient immunosuppression for survival and donor marrow engraftment in the majority of presensitized recipients. The delivery of targeted hematopoietic radiation, particularly to lymphoid tissues, resulted in uniform engraftment with excellent survival (11 of 12 mice, 92%) in this dose group.

## DISCUSSION

We have demonstrated that radiation delivered by  $^{131}\text{I}$ -anti-CD45 antibody provides sufficient immunosuppression to enable engraftment of T-cell-depleted marrow that is matched for major histocompatibility antigens but mismatched for multiple minor histocompatibility antigens. Engraftment occurred in 86% of all animals treated with 0.75 mCi of  $^{131}\text{I}$ -30F11 antibody alone and in 100% of animals treated with either 10 Gy of TBI alone or 0.75 mCi of  $^{131}\text{I}$ -antibody and 2 Gy of TBI, demonstrating that the radiation delivered by 0.75 mCi of  $^{131}\text{I}$ -anti-CD45 antibody provides a biological effect equivalent to approximately 8 Gy of TBI. These results suggest the potential for  $^{131}\text{I}$ -anti-CD45 antibody to substitute for most or all of the systemic TBI delivered in conventional transplant regimens used to condition recipients of HLA-matched sibling HSCs. When this donor-host murine model was manipulated to favor rejection by the presensitization of recipient mice against donor alloantigens 4 weeks before transplant, engraftment was seen in surviving animals receiving 12–16 Gy of TBI, but most mice receiving at least 10 Gy of TBI alone died in the first month posttransplant, presumably because of rejection of donor marrow and ablation of host hematopoiesis. However, when TBI was combined with antibody labeled with 0.75 mCi of  $^{131}\text{I}$ , however, 83% of presensitized mice receiving both antibody and at least 10 Gy of TBI survived, and the TBI dose required for engraftment in at least a portion of surviving animals decreased to 8–10 Gy. These results again demonstrate an appreciable contribution of  $^{131}\text{I}$ -anti-CD45 antibody to the immunosuppression provided by the combined preparative regimen.

The immunosuppressive effects of external beam radiation are strongly dose rate dependent, with multiple studies demonstrating that a higher total radiation dose is required for engraftment when delivered at a lower dose rate (6–10). This suggests that the host cells responsible for rejection of donor HSCs have significant ability to repair sublethal irradiation damage. The dose rate of radiation provided by  $^{131}\text{I}$ -30F11 antibody is much lower than conventional dose rates used for TBI, with a dose of 1.0 mCi of  $^{131}\text{I}$ -30F11 estimated to deliver a maximum dose rate of 3 cGy/min to spleen, 1.3–1.5 cGy/min to lymph nodes, and 0.6 cGy/min to marrow between 4 and 24 h after antibody injection (3). The mice engrafting after treatment with 0.75 mCi of  $^{131}\text{I}$  received estimated radiation doses of 81 Gy to spleen, 53 Gy to lymph nodes, and 26 Gy to marrow (Table 1). These doses are higher than the 8-Gy external beam TBI at 20 cGy/min required for equivalent engraftment in unsensitized recipients, supporting the premise that radiation delivered by radioisotopes with low continuous dose rates has a much lower relative biological efficacy than external beam radiation administered at the rates conventionally used for TBI.

In addition to the lower dose rate at which radiation is delivered by radioimmunoconjugate, other factors may contribute to the apparent need to deliver higher radiation doses to target tissues as compared to TBI to achieve equivalent engraftment. First, all estimates of internal radiation-absorbed dose to organs have uncertainties. Radiation-absorbed doses were determined from the time-activity curves for organs of interest by direct measurement of  $^{131}\text{I}$  concentration in tissues after injection of 100  $\mu\text{g}$  of trace- $^{131}\text{I}$ -labeled 30F11 antibody, using a dosimetric model developed for the laboratory mouse (13, 14) that accounts for the size and position of organs and estimates the percentage of energy that is actually deposited within a tissue from  $^{131}\text{I}$  on antibody bound to cells in that tissue. Any T cell or natural killer cell that is in circulation during the 96 h after antibody injection will receive a smaller dose of radiation than those cells remaining in marrow, lymph nodes, or spleen. Furthermore,  $^{131}\text{I}$ -30F11 antibody does not

penetrate the thymus well, with an estimated radiation dose of 12.8 Gy/mCi  $^{131}\text{I}$  to this potential sanctuary site for T cells.

Our results demonstrate unequivocally that radiation delivered by  $^{131}\text{I}$ -anti-CD45 antibody can substitute for most or all of the TBI necessary to enable engraftment of H2-matched, minor antigen-mismatched marrow in recipients that were not presensitized against donor alloantigens. However, the ability of murine transplant models to accurately predict outcome in human HSCT is imprecise, in part because many patients are presensitized against donor alloantigens by previous exposure from transfused blood products. In the murine model studied here, presensitization against the donor minor histocompatibility antigens resulted in a marked increase in the TBI dose from 8 Gy to between 12 and 14 Gy to produce uniform engraftment in all surviving recipients. Furthermore, most presensitized recipients treated with at least 10 Gy of TBI did not survive to 3 months posttransplant, presumably because the graft was rejected at a TBI dose that ablated host hematopoiesis. This hypothesis is supported by the finding that TBI doses of 10–12 Gy were lethal in control mice not rescued with donor marrow (data not shown) and by the previously documented  $\text{LD}_{50}$  of 10–12 Gy of TBI in B6- $\text{Ly}5^a$  mice.

When presensitized recipients were given 0.75 mCi of  $^{131}\text{I}$ -30F11 antibody combined with varying doses of TBI, engraftment occurred in 7 of 11 (64%) surviving animals receiving 10 Gy of TBI and in 100% (18 of 18) of surviving animals receiving 12–14 Gy of TBI. This finding suggests that the addition of 12 Gy of TBI to 0.75 mCi of  $^{131}\text{I}$ -30F11 antibody, which has a combined biological effect equivalent to 20 Gy of TBI, provides effective immunosuppression without excessive toxicity. The increased immunosuppression provided by  $^{131}\text{I}$ -anti-CD45 antibody is probably due to enhanced irradiation of lymphoid tissue with estimated doses that are twice as high as those delivered to marrow. This improves the likelihood that immunosuppressive doses of radiation are delivered to lymphoid cells when myeloablative doses of radiation are delivered to HSCs, thus avoiding the situation of stem cell ablation with inadequate immunosuppression.

The roles of TBI in transplant preparative regimens include myeloablation, immunosuppression, and antileukemic activity. We have shown here that  $^{131}\text{I}$ -anti-CD45 antibody can provide immunosuppression and that the ability to deliver maximum doses of radiation using this radioimmunoconjugate can result in delivery of high doses of radiation to leukemic cells in hematopoietic tissues. Clinical studies are ongoing to evaluate the impact on disease response and disease-free survival provided by the addition of  $^{131}\text{I}$ -anti-CD45 antibody to conventional preparative regimens for allogeneic transplantation. Once the antileukemic activity of  $^{131}\text{I}$ -antibody is further elucidated, the potential for this radioimmunoconjugate to function as a less toxic, equally efficacious alternative to TBI in the treatment of acute leukemia will be better known.

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## Immunosuppressive Effects of $^{131}\text{I}$ -Anti-CD45 Antibody in Unsensitized and Donor Antigen-presensitized H2-matched, Minor Antigen-mismatched Murine Transplant Models

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*Cancer Res* 2001;61:5126-5131.

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