Marrow Ablative and Immunosuppressive Effects of 131I-Anti-CD45 Antibody in Congenic and H2-Mismatched Murine Transplant Models

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Targeted hematopoietic irradiation delivered by 131I-anti-CD45 antibody has been combined with conventional marrow transplant preparative regimens in an effort to decrease relapse. Before increasing the pressure of therapy delivered by radiolabeled antibody, the myeloablative and immunosuppressive effects of such low dose rate irradiation must be quantified. We have examined the ability of 131I-anti-CD45 antibody to facilitate engraftment in Ly5-congenic and H2-mismatched murine marrow transplant models. Recipient B6-Ly5 mice were treated with 30F11 antibody labeled with 0.1 to 1.5 mCi 131I and/or total body irradiation (TBI), followed by T-cell-depleted marrow from Ly5-congenic (C57BL/6) or H2-mismatched (BALB/c) donors. Engraftment was achieved readily in the Ly5-congenic setting, with greater than 80% donor granulocytes and T cells after 0.5 mCi 131I (estimated 17 Gy to marrow) or 8 Gy TBI. A higher TBI dose (14 Gy) was required to achieve engraftment of H2-mismatched marrow, and engraftment occurred in only 3 of 11 mice receiving 1.5 mCi 131I delivered by anti-CD45 antibody. Engraftment of H2-mismatched marrow was achieved in 22 of 23 animals receiving 0.75 mCi 131I delivered by anti-CD45 antibody combined with 8 Gy TBI. Thus, targeted radiation delivered via 131I-anti-CD45 antibody can enable engraftment of congenic marrow and can partially replace TBI when transplanting T-cell-depleted H2-mismatched marrow.

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ents were transplanted with marrow from congenic Ly5<sup>+</sup> donors. In this model, the donor and recipient disparity is limited to CD45 allotype and does not evoke a cellular immune response. In an approach designed to test the immunosuppressive effects of the regimen, the same recipients (H<sup>2</sup>d) were transplanted with T-cell–depleted marrow from H<sup>2</sup>-mismatched (H<sup>2</sup>b) BALB/c donors. Our results demonstrate that irradiation delivered solely by <sup>131</sup>I-anti-CD45 antibody allows engraftment of marrow in Ly5-congenic recipients but not in H2-mismatched recipients, where both natural killer (NK) cells and T cells are known to cause rejection. However, engraftment of T-depleted H2-mismatched marrow could be accomplished by adding low-dose external beam TBI to the radiation delivered by <sup>131</sup>I-anti-CD45 antibody.

**MATERIALS AND METHODS**

**Mice.** Male B6-Ly5<sup>+</sup> mice were bred at the Fred Hutchinson Cancer Research Center (Seattle, WA) and housed under specific pathogen-free conditions with acidified water and autoclaved chow. C57BL/6 and BALB/c donors were purchased from Jackson Laboratory (Bar Harbor, ME) and were 6 to 12 weeks old at the initiation of each experiment.

**MoAbs.** MoAbs GK1.5 (rat IgG<sub>2a</sub> anti-CD4), 30-H12 (rat IgG<sub>2a</sub> anti-thy-1.2), and 2.34 (rat IgG<sub>2b</sub> anti-CD8) were prepared from culture supernatants of cells lines obtained from ATCC (HK1.5 and 2.34) or Dr Jeffrey Ledbetter (Bristol Meyers Squibb, Seattle, WA; 30-H12). A hybridoma cell line secreting MoAb 30F11 (rat IgG<sub>2a</sub>), which recognizes all murine CD45 isoforms, was the gift of Dr Ledbetter. Hybridoma cell lines secreting MoAbs A20 (murine IgG<sub>1</sub>), which recognizes the Ly5.1 epitope encoded by the Ly5<sup>+</sup> allotype of murine CD45, and 104 (murine IgG<sub>2a</sub>), which recognizes the Ly5.2 epitope encoded by the Ly5<sup>+</sup> allotype of murine CD45, were the gift of Dr Shoji Kimura ( Sloan Kettering Institute, New York, NY). Ascites containing each MoAb was produced in BALB/c mice under specific pathogen-free conditions. Batch extraction and purification of antibodies from ascites was performed using ABX exchange resin (J.T. Baker, Phillipsburg, NJ) and high-pressure liquid chromatography (Bio-Rad 510 Beckman, Fullerton, CA).

**Iodination and characterization.** Antibodies were iodinated with Na<sup>125</sup>I or Na<sup>131</sup>I (ICN, Irvine, CA) using the Iodogen method<sup>19</sup> for trace labeling and the chloramine T-labeling method for high specific activity. Antibodies were iodinated with Na<sup>125</sup>I or Na<sup>131</sup>I (ICN, Irvine, CA) using the Iodogen method<sup>19</sup> for trace labeling and the chloramine T-labeling method for high specific activity. Antibodies were iodinated with Na<sup>125</sup>I or Na<sup>131</sup>I (ICN, Irvine, CA) using the Iodogen method<sup>19</sup> for trace labeling and the chloramine T-labeling method for high specific activity. Antibodies were iodinated with Na<sup>125</sup>I or Na<sup>131</sup>I (ICN, Irvine, CA) using the Iodogen method<sup>19</sup> for trace labeling and the chloramine T-labeling method for high specific activity. Antibodies were iodinated with Na<sup>125</sup>I or Na<sup>131</sup>I (ICN, Irvine, CA) using the Iodogen method<sup>19</sup> for trace labeling and the chloramine T-labeling method for high specific activity.

**Antibody localization and estimation of radiation absorbed doses.** The double isotope labeling method of Pressman<sup>22</sup> was used to determine the biodistribution of 30F11 antibody as previously described.<sup>8</sup> Animals were injected via tail vein with 200 µL volume containing 100 µg of antibody labeled with <sup>131</sup>I (specific activity, 1 µCi/µg) and an equal amount of rat IgG (Sigma Immunochemical, St Louis, MO) labeled with <sup>125</sup>I. Groups of 5 mice were killed at multiple time points from 1 to 96 hours after injection, and multiple tissues were sampled. The <sup>125</sup>I and <sup>131</sup>I contents of weighed samples and standard samples of the injection mix were determined by multichannel gamma counting (Packard 5000 Autogamma counter; Packard Instrument Co, Downers Grove, IL). Counting data were adjusted for cross-over from the <sup>131</sup>I channel to the <sup>125</sup>I channel and were corrected for decay.

**RESULTS**

**Biodistribution of <sup>131</sup>I-anti-CD45 antibody and estimation of radiation absorbed doses.** B6-Ly5<sup>+</sup> mice were injected with a 100 µg dose of trace <sup>131</sup>I-labeled anti-CD45 MoAb 30F11 or <sup>125</sup>I-labeled rat IgG and the amount of isotope in major organs was determined. The resulting time-activity curves demonstrated greater uptake and retention of radiolabeled anti-CD45 antibody in spleen, lymph nodes, and marrow as compared with the lung, the normal nontarget organ with the highest concentration (Fig 1A). The corresponding time-activity curves for <sup>125</sup>I-labeled rat IgG (ie, IgG purified from sera of normal rats), used as a nonspecific control, did not show any specific retention in spleen, lymph nodes, or marrow (Fig 1B). Anti-
CD45 antibody was cleared from the blood far more rapidly than control rat IgG, presumably by rapid binding of circulating antibody to CD45 on readily accessible cells in spleen and marrow (Fig 1C).

Estimates of absorbed irradiation doses delivered to target and normal organs by 30F11 antibody or polyclonal rat IgG, assuming they were labeled with $^{131}$I, were calculated using the time-activity curves for each tissue (Table 1). The estimated absorbed irradiation delivered to spleen, lymph nodes, and bone marrow by a 100 µg dose of antibody 30F11 labeled with 1 mCi $^{131}$I was, respectively, 6.5-, 4.0-, and 2.1-fold greater than that delivered to lung. When $^{131}$I was delivered from polyclonal rat IgG, the spleen, bone marrow, and lymph nodes all absorbed less irradiation than the lung and absorbed approximately the same amount of irradiation as the liver and kidney. The higher estimated irradiation absorbed by nontarget organs after administration of $^{131}$I-rat IgG as compared with $^{131}$I-30F11 antibody reflects the slower clearance of $^{131}$I-rat IgG from the blood.

Estimated absorbed irradiation rates varied between tissues and over time for a given tissue, as shown in Fig 2 for 0.5, 1.0, and 1.5 mCi of $^{131}$I. For antibody labeled with 1.0 mCi of $^{131}$I, the highest irradiation rate for a hematopoietic tissue was 3 cGy/min in the spleen between 4 and 24 hours after infusion. Maximum lymph node dose rates ranged from 1.3 to 1.5 cGy/min. For brachial lymph nodes, 75% of the total estimated irradiation was absorbed at a rate of at least 1 cGy/min. For marrow, 86% of the total dose was absorbed at an estimated rate of at least 0.6 cGy/min.

Engraftment of congenic marrow after $^{131}$I-30F11 antibody or TBI. In the initial experiments with C57BL/6 donors and congenic B6-Ly5$^a$ recipients, we administered a 100 µg dose of 30F11 antibody labeled with 0 (ie, unlabeled antibody), 0.5, 1.0, 1.25, and 1.5 mCi of $^{131}$I, followed 4 days later by injection of T-cell–depleted marrow or medium alone. All mice receiving marrow survived, whereas 2 of 6 mice treated with 1.25 mCi $^{131}$I and 4 of 6 treated with 1.5 mCi $^{131}$I and no marrow died between 11 and 15 days after radiolabeled antibody injection (data not shown). Although postmortem examinations were not performed, death at these time points after delivery of radiation is consistent with death from marrow aplasia. All mice receiving at least 0.5 mCi $^{131}$I-anti-CD45 antibody demonstrated successful engraftment (Table 2). Myeloid engraftment occurred within 4 weeks posttransplant, but full T-cell engraftment...
did not occur until 12 weeks, presumably reflecting delayed disappearance of recipient T cells.

In subsequent experiments, mice received varying doses of TBI or a broader range of $^{131}$I doses before infusion of T-cell–depleted congenic marrow. A near-linear relationship between radiation dose and engraftment of donor marrow was found for $^{131}$I doses of up to 0.5 mCi (Fig 3) and TBI doses of up to 8 Gy (Fig 4). Fifty percent engraftment of granulocytes, B cells, and T cells was observed after treatment with 0.2 to 0.3 mCi of $^{131}$I-labeled antibody, which corresponded to an estimated marrow radiation dose of 7 to 10 Gy. Engraftment after 8 Gy TBI (dose rate, 20 cGy/min) was equivalent to engraftment after 0.5 mCi of $^{131}$I delivered via 30F11 antibody, with 80% donor cells. The estimated irradiation absorbed by marrow after treatment with 0.5 mCi of $^{131}$I on 100 µg 30F11 was approximately 17 Gy, more than twice the 8 Gy required when the irradiation was delivered by external beam.

Engraftment of T-cell–depleted C57BL/6 marrow in B6-Ly5$^a$ recipients. Values are the mean for 3 (antibody only) to 5 or 6 mice per group.

<table>
<thead>
<tr>
<th>Administered $^{131}$I Activity</th>
<th>28 d After BMT</th>
<th>113 d After BMT</th>
<th>28 d After BMT</th>
<th>113 d After BMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Ab only)</td>
<td>4.9 ± 5.6</td>
<td>1.6 ± 0.9</td>
<td>0 ± 0</td>
<td>0.1 ± 2</td>
</tr>
<tr>
<td>0.5 mCi</td>
<td>81.3 ± 7.5</td>
<td>86.6 ± 7.0</td>
<td>34.7 ± 8.1</td>
<td>80.4 ± 7.6</td>
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<tr>
<td>1.0 mCi</td>
<td>92.7 ± 3.5</td>
<td>91.5 ± 3.7</td>
<td>38.5 ± 9.4</td>
<td>87.6 ± 3.5</td>
</tr>
<tr>
<td>1.25 mCi</td>
<td>96.0 ± 2.9</td>
<td>95.8 ± 3.2</td>
<td>44.9 ± 9.8</td>
<td>89.1 ± 4.9</td>
</tr>
<tr>
<td>1.5 mCi</td>
<td>98.3 ± 1.4</td>
<td>94.9 ± 7.7</td>
<td>38.1 ± 14.8</td>
<td>89.8 ± 4.3</td>
</tr>
</tbody>
</table>

Table 2. Engraftment of Congenic Marrow in Mice After $^{131}$I-Anti-CD45 Antibody

Engraftment of T-cell–depleted H2-mismatched marrow after $^{131}$I-anti-CD45 antibody alone or with external beam TBI. In the initial experiments with BALB/c donors and MHC-mismatched B6-Ly5$^a$ recipients, we administered either a 100 µg dose of antibody 30F11 labeled with 0.5 to 1.5 mCi of $^{131}$I on
Fig 3. Engraftment of T-cell–depleted C57BL/6 marrow in B6-Ly5<sup>a</sup> recipients treated with 100 μg of 30F11 antibody labeled with 0.1 to 1.0 mCi <sup>131</sup>I. Proportion of granulocytes (A), B cells (B), and T cells (C) of donor origin (mean ± SD) 3 months after transplantation.

Fig 4. Engraftment of T-cell–depleted C57BL/6 marrow in B6-Ly5<sup>a</sup> recipients treated with 1 to 12 Gy TBI. Proportion of granulocytes (A), B cells (B), and T cells (C) of donor origin (mean ± SD) 3 months after transplantation.
day −4 or 2 to 16 Gy TBI on day 0, followed by infusion of T-cell–depleted marrow or medium alone. With external beam TBI and no marrow infusion, the LD$_{50}$ was approximately 10 Gy, and no recipients survived exposures of ≥14 Gy (data not shown). With infusion of T-cell–depleted H2-incompatible marrow, engraftment (>80% donor T cells at 3 months posttransplant) was observed in all recipients prepared with ≥14 Gy TBI delivered by external beam (Fig 5). With $^{131}$I-labeled antibody and no marrow infusion, the LD$_{50}$ was between 0.5 and 1.0 mCi, and no recipients survived after administration of 1.5 mCi (data not shown). With infusion of T-cell–depleted H2-incompatible marrow, 1.5 mCi of $^{131}$I-labeled antibody was not sufficient to permit engraftment. Only 1 of 6 mice receiving the highest isotope dose had more than 80% T cells of donor origin (Fig 6A).

It was not possible to deliver more irradiation with the use of $^{131}$I-30F11 antibody alone, because labeling the antibody to a specific activity higher than 15 mCi/mg impaired immunoreactivity of the antibody, and antibody doses higher than 100 µg delayed clearance from both blood and marrow, causing the infused donor marrow to be damaged by isotope persisting for more than 4 days (data not shown). To circumvent this problem, we tested the immunosuppressive effects of radiation from $^{131}$I-30F11 antibody combined with 4 Gy external beam TBI. This combined preparative regimen resulted in engraftment in most recipients treated at $^{131}$I doses of at least 1.0 mCi $^{131}$I (Fig 6B). In two experiments, 5 of 12 mice receiving 1.5 mCi survived and demonstrated donor engraftment, but the remaining 7 mice treated with 1.5 mCi died between 9 and 14 days after marrow transplant. These results suggest that the margin between immunosuppressive and toxic doses of radiation is small when high doses of radioisotope are combined with 4 Gy TBI.

In two experiments we combined 0.75 mCi of $^{131}$I-anti-CD45 antibody with TBI doses varying between 2 and 10 Gy (Fig 7). With 6 Gy TBI, engraftment was observed in 9 of 11 recipients (experiment no. 1) and 4 of 11 recipients (experiment no. 2), and with 8 Gy TBI, engraftment was observed in 12 of 12 recipients (experiment no. 1) and 10 of 11 recipients (experiment no. 2). These results suggest that the immunosuppressive effect of $^{131}$I-anti-CD45 antibody labeled with 0.75 mCi $^{131}$I can replace 6 to 8 Gy TBI in producing engraftment equivalent to that seen with 14 Gy TBI alone. A dose of 0.75 mCi $^{131}$I is estimated to deliver approximately 25 Gy to marrow, 50 Gy to lymph nodes, and 80 Gy to spleen.

**DISCUSSION**

We asked if radiation delivered by $^{131}$I-labeled anti-CD45 antibody could enable engraftment of congenic marrow and of H2-incompatible marrow. Using congenic marrow, $^{131}$I-anti-CD45 antibody alone enabled engraftment, with ≥80% engraftment when antibody was labeled with ≥0.5 mCi $^{131}$I, and thus could replace TBI. When donor and recipient were H2-
with a much lower dose of $^{131}$I delivered by anti-CD45 antibody (0.3 mCi) had 60% T-cell, 70% B-cell, and 45% myeloid engraftment after infusion of Ly5-congenic marrow. Eighty percent engraftment required 8 Gy TBI or 0.5 mCi of $^{131}$I. The 17 Gy estimated irradiation absorbed by marrow delivered by antibody labeled with 0.5 mCi $^{131}$I supports the concept that radiation delivered at the low continuous dose rate and low linear energy transfer of $\beta$-particles such as $^{131}$I has a lower relative biological efficacy (RBE) than radiation delivered at the much more rapid rate used for TBI.

Previous studies have shown that when the external beam TBI dose rate is decreased from 25 to 1 cGy/min, the LD$_{50}$ for early gastrointestinal toxicity is increased from 12 to 21 Gy and the LD$_{50}$ for late nonhematopoietic deaths occurring by 1 year is increased from 10 to 21 Gy in BALB/c mice supported with syngeneic marrow transplants. In that study, no mice treated with 20 Gy at 1 cGy/min had detectable histologic changes in the lung or kidney. In CBA mice treated with thoracic irradiation at high dose rate (180 cGy/min), the minimum dose required to produce a detectable elevation in breathing rate at 28 weeks after treatment increased from 13.8 Gy rad when the irradiation was delivered as a single fraction to 30.4 Gy when the irradiation was delivered in 7 fractions. In a study with $\beta$-emitting radionuclide $^{90}$Y administered as inhaled fused aluminosilicate particles, estimated lung radiation doses up to 27 Gy did not decrease survival as compared with untreated controls. These results are consistent with our observations of lower RBE of radiation delivered by $^{131}$I-labeled antibody compared with equivalent doses delivered as conventional TBI.

In a transplant model demanding profound immunosuppression, engraftment of T-cell–depleted H2-mismatched marrow could not be achieved using $^{131}$I-CD45 antibody alone. Engraftment occurred in only a minority of mice receiving the highest $^{131}$I dose delivered (1.5 mCi). Several factors might explain the inability to achieve engraftment after treatment with radiolabeled antibody, despite the delivery of estimated radiation doses of almost 100 Gy to lymph nodes, 50 Gy to marrow, and 160 Gy to spleen. First, a blood-thymus barrier impeded antibody localization in the thymus, with a dose of approximately 19 Gy delivered by 1.5 mCi $^{131}$I. Second, the absorbed radiation actually delivered to cells that cause graft rejection might be less than estimated from the biodistribution studies. The estimates of absorbed dose in hematopoietic tissues represent the average for cells that are assumed to reside in that tissue for at least the first 96 hours after radiolabeled antibody infusion and do not account for heterogeneity within the tissue. T cells or NK effectors that circulate during some or all of the period of radiation delivery may thus receive a lower radiation dose. A cell that circulates during the entire period of radiation delivery will receive at least the radiation dose estimated for total body (5.9 Gy/mCi $^{131}$I). It is not possible to quantitate the additional radiation received by a circulating cell because it involves not only radiation delivered by antibody bound to the cell and to neighboring cells in circulation as well as that present in plasma (which depends on geometry of blood vessels in relationship to the path length of the isotope), but also radiation delivered during the time the cell traffics in tissues such as the spleen where greater radiation effects are present. Within lymph nodes, radiation delivery may be heterogeneous, and the amount of

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**Fig 7.** Engraftment of T-cell–depleted BALB/c marrow in mice treated with 100 $\mu$g 30F1 antibody labeled with 0.75 mCi $^{131}$I combined with 2 to 10 Gy TBI. Proportion of T cells of donor origin (mean ± SD) 3 months after transplantation. Data point labels indicate the number of mice with ≥80% donor T cells of total surviving mice (of 12 treated mice per group).
irradiation delivered to individual nodes could vary. Finally, cellular repair mechanisms might be able to keep pace with the rate of damage inflicted by the low radiation dose rate delivered from 131I, thereby avoiding cell death.

Our results with CD45-congenic donor/recipient pairs suggest that treatment with 131I-anti-CD45 antibody alone is sufficient to allow engraftment of donor cells at 131I doses that are well tolerated. This raises the possibility that 131I-labeled anti-CD45 antibody might provide a less toxic method for selective ablation of marrow and might enable reconstitution with autologous hematopoietic cells modified by gene therapy. Furthermore, the finding that engraftment of T-cell-depleted H2-mismatched marrow reliably occurred when 0.75 mCi of 131I-anti-CD45 antibody was combined with 8 Gy TBI suggests that targeted radiation may be able to replace nearly half of the TBI ordinarily administered in such transplants. Such an approach holds the potential for enhanced antileukemic efficacy while inducing less systemic toxicity, thereby improving the outcome after marrow transplantation for acute leukemia.

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