ABSTRACT

We have shown previously that $^{131}$I-labeled antibodies against the Thy-1.1 differentiation antigen can cure AKR/Cum (Thy-1.2+) mice bearing AKR (Thy-1.1+) SL2 T-cell lymphoma. In the present study we have extended these studies to the therapy of SL2 lymphoma in AKR/J mice, where $^{131}$I-anti-Thy-1.1 antibodies react with both tumor and normal T-lymphocytes.

A single 25-$\mu$g bolus of $^{131}$I-labeled anti-Thy-1.1 antibody was rapidly cleared from serum by binding to spleen cells (t1/2 < 3 h) and only low concentrations (<2% injected dose/g) were present in tumor 24 h after infusion. Doses of 0.5–5.0 mg antibody saturated cells in the spleen but only slightly increased the proportion of antibody in tumor. In contrast, pretreatment of mice with 1.0 mg of unlabeled anti-Thy-1.1 antibody 24 h prior to $^{131}$I-labeled antibody resulted in a tumor concentration of 9.7% injected dose/g 24 h after infusion of the radiolabeled antibody. With this latter regimen, biodistribution approximated that seen in AKR/Cum mice, and infusion of 1000 $\mu$Ci would result in delivery of 16 Gy to tumor. Therapy of AKR/J mice bearing established s.c. lymphoma nodules with 1500 $\mu$Ci of $^{131}$I-anti-Thy-1.1 antibody given in this latter regimen resulted in complete regression of the nodule in 70% of animals and had a greater antitumor effect (27% complete regression, P < 0.001) than 750 $\mu$Ci of $^{131}$I-labeled irrelevant antibody, a dose that would deliver equivalent radiation to normal organs (liver, kidney, and lung). The anti-Thy-1.1 antibody had only a slightly greater antitumor effect than an equivalent $\mu$Ci dose (1500 $\mu$Ci) of $^{131}$I-labeled control antibody (42% complete regression, P = 0.12). Both antibodies were marrow toxic and all animals treated with 1500 $\mu$Ci died of marrow aplasia. These studies suggest that radiolabeled antibodies against differentiation antigens may be useful for therapy in spite of binding to normal cell populations but curative therapy may require infusion of unirradiated bone marrow.

INTRODUCTION

One of the critical factors in the use of radionuclide-conjugated monoclonal antibodies against tumor-associated antigens for either diagnosis or therapy is the extent to which these antibodies react with normal cell populations. Although it is possible in selected cases to generate monoclonal antibodies that are truly tumor specific for clinical therapy, e.g., anti-idiotypic for B-cell lymphomas (1, 2), the majority of antibodies reactive with tumors are directed against normal differentiation antigens expressed by at least some normal cell populations. Infusion of radiolabeled antibodies against these differentiation antigens will result in targeting of normal tissues as well as tumor. Thus, monoclonal antibodies against lymphoid differentiation antigens have been shown to be capable of penetrating solid lymphoma masses (3–5) and saturating tumor cell surface binding sites (3) but also to localize to normal lymphoid tissues (6, 7). However, it may be possible to use antibodies against differentiation anti-
iodination ratio (mol iodine/mol antibody) of ≤0.2. Immunoreactivity (percentage of counts able to bind at antigen excess) and avidity were determined by binding to viable tumor cells or AKR/J thymocytes. Approximately 60% of labeled 31E6.4 bound to target cells with an avidity of 1 x 10^4 liters/mol (8). Less than 0.1% of labeled 31E6.4 bound to SLI (Thy-1.2) cells. Iodinated G3G6 did not bind to SL2 or SL1 cells (≤0.1%).

Biodistribution. Biodistribution studies were performed by the double isotope labeling method of Pressman (11). SL2 lymphoma cells (3 x 10^6) were implanted s.c. in the flank of AKR/J mice. When a 0.5-1-cm-diameter tumor nodule was present (7-8 days after inoculation) a mixture of 131I-labeled anti-Thy-1.1 antibody and 131I-labeled control antibody was infused i.v. via tail vein. At various times following infusion of the labeled antibodies a blood sample was obtained and mice were sacrificed with ether anesthesia. Tissues were excised, weighed, and counted in a multiple channel gamma counter (Auto-Gamma spectrometer, Model 5330; Packard Instruments, Downers Grove, IL) to determine 131I and 131I activity. 131I counts were adjusted for cross-over from the 131I channel by subtracting 14% of the 131I channel counts from the 131I channel counts. Data were not corrected for decay of either 131I or 131I.

All results were expressed as the percentage of ID/g, mean ± SD) to allow ready comparison of the proportion of administered dose in tissue when varying quantities of antibody were administered. Absolute concentration in tissues for a given infusion can be obtained by multiplying this value by the administered dose in µg or µCi to obtain µg antibody/g tissue or µCi/g tissue, respectively. Statistical analyses of antibody concentration in tissues (Figs. 1 and 3) were performed by selecting the best polynomial relationship between antibody dose and the antibody concentration (µg/g) after reviewing the regression of all possible subsets (12) of linear quadratic and cubic measures of antibody dose on antibody concentration. Quoted significance levels represent the significance with which selected parameters differ from zero. Effective half-lives were estimated by fitting the last 3 or 4 time points (whichever resulted in the best fit) on the biodistribution curves to monoexponential clearance using the method of least squares.

Radiation Dosimetry. Radiation doses to various tissues from infusion of 131I-labeled anti-Thy-1.1 and 131I-labeled IgG2a control antibody were calculated from the biodistribution of labeled antibody assuming uniform distribution of isotope within individual organs. The area under the biodistribution curve was estimated from the mean percentage of ID/g obtained for each antibody at 1 and 8 h and 1, 2, 4, and 6 days using the trapezoidal integration method. Values for 131I-control antibody were calculated by correcting the data obtained for 131I-control antibody to the values that would have been obtained if the control antibody was labeled with 131I. The initial concentration of radiolabeled antibody in all tissues except blood was assumed to be 0% ID/g. Initial antibody was labeled with 131I. The initial concentration of radiolabeled antibody to the values that would have been obtained if the control antibody was labeled with 131I and 131I activity. 131I counts were adjusted for cross-over from the 131I channel by subtracting 14% of the 131I channel counts from the 131I channel counts. Data were not corrected for decay of either 131I or 131I.

RESULTS

Biodistribution of Single Antibody Dose. The biodistribution of 131I-labeled anti-Thy-1.1 antibody was examined in AKR/J mice (Thy-1.1+). A single bolus of 25 µg was rapidly cleared from serum in a biphasic pattern (initial half-life, <3 h, terminal half-life, 34 h; data not shown). The rapid clearance appeared to result mainly from uptake by the spleen where high levels of antibody were present at 6 h after infusion [15.9 ± 4.8% (SD) ID/g], and only low concentrations were present in the s.c. tumor [1.5 ± 1.0% ID/g]. Antibody concentration in spleen rapidly decreased (t1/2 = 12 h). These results suggested that antibody access to spleen was much greater than to tumor. We therefore determined whether it was possible to saturate binding sites in the spleen by increasing the amount of antibody infused and thus increase the proportion of antibody localizing to tumor.

Influence of Antibody Dose on Biodistribution. To examine the influence of antibody dose on biodistribution, we infused a single bolus of 25-5000 µg per animal and determined the concentration of 131I-labeled anti-Thy-1.1 antibody in tissues 24 h after infusion. As the antibody dose increased from 25 µg to 5000 µg/animal, the percentage of ID/g in spleen at 24 h after infusion decreased from 4.8 ± 0.7 to 0.5 to 0.2% ID/g (Fig. 1, P < 0.001), presumably as a result of saturation of splenic binding sites. The proportion remaining in blood increased from 1.0 ± 0.1 to 5.3 ± 0.8 ID/g (P < 0.001). The proportion of 131I-labeled anti-Thy-1.1 antibody in tumor increased over the dose range of 25-500 µg (from 1.9 ± 1.4 to 6.5 ± 2.6% ID/g).

Fig. 1. Effect of antibody (Ab) dose on localization to tumor. Concentrations of 131I-labeled anti-Thy-1.1 antibody [%ID/g, mean ± SD (bars)] present in blood, spleen, and tumor 24 h after a single bolus of 25-5000 µg are shown for 3-5 animals/group.

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4.2 ± 3.9% ID/g) and then decreased from 500 to 5000 μg (from 4.2 ± 3.9 to 1.3 ± 0.3% ID/g) (P = 0.04). Thus, infusion of a dose of 500 μg 131I-labeled anti-Thy-1.1 antibody led to a modestly greater concentration of the radiolabeled antibody in tumor than either higher or lower doses.

We therefore infused a bolus of 500 μg of 131I-labeled anti-Thy-1.1 antibody and, as a control, 200 μg of 125I-labeled antibody of irrelevant specificity and examined their biodistribution over time (Fig. 2). Infusion at 500 μg/animal of 131I-labeled anti-Thy-1.1 antibody resulted in a slight prolongation of the initial half-life (t1/2 = 6 h) compared to a 25-μg dose with a similar terminal half-life (t1/2 = 34 h). The initial concentrations within the tumor were low (2.0 ± 1.4% ID/g) and decreased exponentially over time (t1/2 = 90 h). Clearance from other organs, e.g., liver, lung, and kidney, corresponded to the blood clearance (t1/2 = 34-60 h). In contrast, clearance of the 125I-labeled control antibody from blood was monoeXponential and much slower (t1/2 = 58 h) than the 131I-labeled anti-Thy-1.1 antibody. Clearance of the control antibody from tumor (t1/2 = 100 h) and normal organs (t1/2 = 70-130 h) was similarly prolonged.

There was considerable animal to animal variation in the tumor concentration of both 131I-labeled anti-Thy-1.1 and 125I-labeled control antibodies. A comparison of absolute antibody concentrations in individual tumors 24 h after infusion of antibody revealed that, in 10 of 11 animals receiving a single infusion of antibody, the concentration of 131I-labeled anti-Thy-1.1 exceeded that of the 125I-labeled control antibody in spite of the former's much more rapid clearance from serum (Fig. 3). As had been observed previously in AKR/Cum mice, there was a correlation between the tumor concentration of the two antibodies (R2 = 0.91, P = 0.0001).

Thus, infusion of an optimal single dose of labeled anti-Thy-1.1 antibody led to only a small increase in the concentration of antibody in tumor compared to an irrelevant antibody. The poor uptake in tumor appeared to be a result, in part, of the rapid serum clearance due to removal of antibody by binding to spleen cells. We therefore determined whether it was possible to infuse unlabeled antibody, partially block splenic uptake, and enable a subsequent infusion of 131I-labeled antibody to bind in greater concentration to the tumor.

Pretreatment with Unlabeled Antibody. To examine pretreatment, we infused varying doses of unlabeled anti-Thy-1.1 antibody followed 24 h later by a mixture of 500 μg 131I-labeled anti-Thy-1.1 antibody and 200 μg 125I-labeled irrelevant antibody. Concentrations of 131I-labeled anti-Thy-1.1 antibody [%ID/g, mean ± SD (bars)] in blood and tumor 24 h after infusion of labeled antibody are shown for 3–5 animals/group. Concentrations of 125I-labeled irrelevant antibody in tumor or other tissues did not vary significantly among groups (not shown).

Fig. 2. Biodistribution of 131I-labeled anti-Thy-1.1 antibody in AKR/J mice. Concentrations [%ID/g, mean ± SD (bars) over time in various tissues following a single bolus of a mixture of 500 μg of 131I-labeled anti-Thy-1.1 (— — —) and 200 μg of 125I-labeled irrelevant antibody (— — —) are shown for 4–5 animals/time point.

Fig. 3. Antibody concentration in individual tumors. Antibody concentrations (%ID/g) in individual tumors 24 h after a single infusion of 500 μg of 131I-labeled anti-Thy-1.1 antibody (y-axis) and 200 μg of 125I-labeled control antibody (x-axis). Data are from the same animals shown in Figs. 1 and 2. Regression line (%ID/g 131I) = 0.6 + 1.3 (%ID/g 125I); R2 = 0.9, P = 0.0001. Animal represented by O was excluded from the regression.

Fig. 4. Effect of pretreatment with unlabeled anti-Thy-1.1. Groups of tumor-bearing animals received 0–1000 μg of unlabeled anti-Thy-1.1 antibody (Ab) followed 24 h later by a mixture of 500 μg 131I-labeled anti-Thy-1.1 antibody and 200 μg 125I-labeled irrelevant antibody. Concentrations of 131I-labeled anti-Thy-1.1 antibody [%ID/g, mean ± SD (bars)] in blood and tumor 24 h after infusion of labeled antibody are shown for 3–5 animals/group. Concentrations of 125I-labeled irrelevant antibody in tumor or other tissues did not vary significantly among groups (not shown).
in mice that were not pretreated to 3.7 ± 0.2 %ID/g in mice following pretreatment with 1000 μg (P < 0.001). The anti-Thy-1.1 antibody concentrations in spleen did not vary significantly among doses (P = 0.5) presumably as a result of the fact that the 500-μg dose of 131I-labeled antibody saturated splenic binding sites regardless of pretreatment.

We therefore examined antibody biodistribution over time following infusion of the combination of 1000 μg of unlabeled anti-Thy-1.1 antibody followed 24 h later by a mixture of 500 μg of 131I-labeled anti-Thy-1.1 antibody and 125I-labeled control antibody. The biodistribution of the 131I-labeled and anti-Thy-1.1 antibody is shown in Fig. 5. Antibody concentration in tumor rose over the first 24 h to a maximum of 4.4 ± 4.2% ID/g and then decreased exponentially (t1/2 = 47 h). Antibody clearance from blood was prolonged only modestly compared to a single infusion and concentrations in lung, liver, and kidney increased slightly corresponding to the slower clearance from serum. The biodistribution of the 125I-control antibody was similar to that in mice without pretreatment (data not shown).

Radiation Dosimetry. Radiation to tissues that would be delivered over the initial 168 h following infusion of 1000 μCi of 131I-labeled antibody was estimated for mice receiving a single 500-μg infusion of 131I-labeled anti-Thy-1.1 antibody, a single infusion of 131I-labeled control antibody, or pretreatment with 1000 μg unlabeled anti-Thy-1.1 antibody followed by 500 μg of 131I-labeled anti-Thy-1.1 antibody (Table 1). Absorbed radiation doses following a single infusion of 131I-labeled anti-Thy-1.1 antibody were equivalent or slightly greater for tumor (450 cGy) than for normal tissues (300–450 cGy). Pretreatment of mice with 1000 μg of unlabeled antibody resulted in an approximately 4-fold increase in the dose to tumor (1600 cGy) compared to a single infusion with only a doubling of radiation doses to normal tissues. Infusion of 1000 μCi of an 131I-labeled irrelevant antibody would result in 750 cGy to tumor and 1000–1500 cGy to normal tissues (kidney, liver, and lung).

Of note, the estimated radiation doses to marrow from both 131I-labeled anti-Thy-1.1 antibody and 131I-labeled control antibody were relatively low (110 and 75 cGy, respectively). These doses were estimated from the average isotope concentration in the small amount of marrow able to be expressed from the

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**Table 1** Radiation dosimetry

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total dose (cGy)</th>
<th>Absorbed radiation dose (cGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1200</td>
<td>200</td>
</tr>
<tr>
<td>Tumor</td>
<td>1400</td>
<td>200</td>
</tr>
<tr>
<td>Spleen</td>
<td>3200</td>
<td>450</td>
</tr>
<tr>
<td>Kidney</td>
<td>1600</td>
<td>240</td>
</tr>
<tr>
<td>Liver</td>
<td>1000</td>
<td>150</td>
</tr>
<tr>
<td>Lung</td>
<td>600</td>
<td>90</td>
</tr>
<tr>
<td>Marrow</td>
<td>80</td>
<td>72</td>
</tr>
</tbody>
</table>

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**Table 2** Therapy of established lymphoma

Animals with palpable tumor (0.5–1.0 cm in diameter) were treated 7 days after implantation of 2 × 10⁶ SL2 lymphoma cells. All antibody-treated animals received 1000 μg unlabeled anti-Thy-1.1 antibody 24 h prior to labeled antibody.

<table>
<thead>
<tr>
<th>Antibody dose</th>
<th>Total no.</th>
<th>PR + CR*</th>
<th>PR</th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>131I-Anti-Thy-1.1 (1500 μCi antibody)</td>
<td>17</td>
<td>14 (82)*</td>
<td>3 (15)</td>
<td>12 (71)</td>
</tr>
<tr>
<td>131I-Control (750 μCi antibody)</td>
<td>21</td>
<td>5 (23)*</td>
<td>0 (0)</td>
<td>5 (23)*</td>
</tr>
<tr>
<td>131I-Control (1500 μCi antibody)</td>
<td>12</td>
<td>9 (75)</td>
<td>4 (33)</td>
<td>5 (42)*</td>
</tr>
<tr>
<td>Anti-Thy-1.1 (unlabeled antibody)</td>
<td>11</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* Partial regression (PR) was defined as a decrease in tumor volume on two successive measurements. Complete regression (CR) was defined as absence of palpable tumor.

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femur (3–5 mg) without attempting to correct for inhomogeneity, e.g., from marrow fat or bony spicules. Thus, the true radiation dose to the cellular marrow was probably underestimated.

**Therapy of Established Tumor.** The estimated radiation doses suggested that it was possible to deliver therapeutic doses of radiation to tumor in AKR/J mice. To examine the therapeutic effect of 131I-labeled anti-Thy-1.1 antibodies in AKR/J mice directly, we treated groups of AKR/J mice with established s.c. lymphoma nodules (0.5–1.0 cm in diameter) 1 week after tumor implantation (Table 2). Animals were first pretreated with 1000 μg unlabeled anti-Thy-1.1 antibody 24 h prior to labeled antibody. Groups of animals were then treated with 1500 μCi/500 μg of 131I-labeled anti-Thy-1.1 antibody, 750 μCi/200 μg of 131I-labeled irrelevant antibody, 1500 μCi/200 μg of 131I-labeled irrelevant antibody, or 500 μg of unlabeled anti-Thy-1.1 antibody. A control group remained untreated. The lower dose (750 μCi) of 131I-labeled control antibody was selected to achieve approximately equivalent concentration of 131I in blood over time for the 131I-labeled anti-Thy-1.1 and 131I-labeled control antibodies. Given the biodistribution and faster clearance of the 131I-labeled anti-Thy-1.1 antibody observed in the biodistribution studies a dose of 750 μCi of control antibody would deliver approximately equivalent doses of radiation to normal organs while 1500 μCi of 131I-labeled control antibody would deliver higher doses of radiation to critical normal organs (liver, kidney, and lung) (see Table 1).
Infusion of the unlabeled antibody followed by 1500 µCi of 131I-labeled anti-Thy-1.1 antibody resulted in complete regression of the tumor in 71% (12 of 17) of the animals. However, in 10 of 12 of these animals these tumors subsequently regrew. Indirect immunofluorescence demonstrated that the s.c. tumors continued to express the Thy-1.1 antigen (not shown). Infusion of 131I-labeled anti-Thy-1.1 antibody had a significantly greater antitumor effect than infusion of 750 µCi of 131I-labeled control antibody or unlabeled anti-Thy-1.1 antibody which led to complete regression in 26% (5 of 19) of animals (P < 0.001). Infusion of 1500 µCi of 131I-labeled anti-Thy-1.1 antibody had an equivalent or greater antitumor effect than infusion of 1500 µCi of 131I-control antibody [50% (6 of 12) complete regression; \( P = 0.12 \)] in spite of the former’s more rapid clearance from blood and normal tissues. Infusion of unlabeled anti-Thy-1.1 antibody had no effect on tumor growth.

Although the 131I-labeled antibody resulted in regression of the s.c. nodule, all animals died. Autopsy examination revealed bone marrow aplasia and marked splenic atrophy with pneumonia or other infection as the proximate cause of death in all 131I-labeled anti-Thy-1.1-treated animals. Importantly, none of these mice had metastatic tumors. In contrast, control animals, receiving 750 µCi of 131I-labeled control antibody, had extensive diffuse metastatic disease with lymphoma involving spleen, bone marrow, liver, kidneys, and lungs, and bone marrow and spleen showed relatively normal hematopoiesis in areas that were not replaced with tumor. Animals receiving 1500 µCi of 131I-labeled control antibody showed marrow aplasia similar to those receiving 1500 µCi of 131I-labeled anti-Thy-1.1 antibody.

**DISCUSSION**

The extent to which antibodies against tumor-associated antigen react with normal cells is one important determinant in the selection of antibodies for use as carriers of radioisotopes for the therapy of malignant disease. We (8) and others (14–17) have shown that 131I-labeled antibodies against antigens that are absolutely or relatively restricted to tumor cells can selectively deliver radiation to tumors. The present report represents an extension of our previously reported studies of radiolabeled antibodies in a setting where these antibodies were absolutely tumor specific (8). In those studies we used 131I-labeled anti-Thy-1.1 antibodies to treat SL2 (Thy-1.1*) lymphoma nodules growing in congenic AKR/Cum (Thy-1.2*) mice. 131I-labeled antibody was able to deliver curative amounts of radiation to an established s.c. nodule, a situation where unmodified antibody was ineffective. The present studies used the same anti-Thy-1.1 antibody (31E6.4) and tumor (SL2) but differ in the host animal (AKR/J, Thy-1.1*).

In our studies in which antibody reacted with tumor but not normal cells, the biodistribution of 131I-labeled anti-Thy-1.1 antibody was independent of antibody dose until saturation of antigenic sites in the tumor occurred (see Ref. 8, Chart 3). In contrast, in the present study where the antibody used reacted with both the malignant and normal T-cells the quantity of antibody that was infused had a major influence on clearance and biodistribution. Small doses of antibody (25–100 µg) were rapidly cleared as a result of binding to spleen and presumably lymph nodes (6), and only low concentrations were present in solid s.c. tumors (see Fig. 4). Increasing the antibody dose up to 500 µg/animal resulted in an increase in tumor antibody concentration but even with this optimal single dose the cumulative radiation dose to tumor, relative to normal organs, would result in little if any advantage for radiolabeled antibodies compared to total body irradiation. Moreover, a further increase in antibody dose up to 5000 µg/animal resulted in a decrease in the proportion of antibody in tumor presumably as a result of saturation of antigenic sites in the tumor.

It was possible to significantly increase the concentration of antibody in tumor by pretreating animals with unlabeled, anti-Thy-1.1 antibody 24 h prior to the infusion of 131I-labeled antibody. The increase in tumor concentration following two infusions was presumably due to blocking of 131I-labeled anti-Thy-1.1 antibody binding to normal T-lymphocytes or elimination of these cells (18) with resulting prolongation of serum clearance of the labeled antibody. Relatively large doses (500–1000 µg/animal, 20–40 mg/kg) of unlabeled antibody were needed to achieve this increase suggesting that near saturation of binding sites in normal tissues by the unlabeled antibody was required for increased tumor binding.

We examined two infusions 24 h apart since the majority of unlabeled antibody was cleared from the blood at this time while there was persistent antibody in the spleen. A maximum pretreatment dose of 1000 µg was examined since higher doses resulted in saturation of binding sites in the tumor. It is possible, however, that neither the dose of antibody nor the time interval between doses was optimal and a further increase in tumor concentration could have been achieved. Optimal doses of labeled and unlabeled antibody may vary and presumably depend upon the mass of antigen and the relative rates of antibody uptake and clearance in tumor versus normal tissues. Thus, these factors will presumably be different when studying other tumor-antibody systems.

The combination of 1000 µg of unlabeled anti-Thy-1.1 followed by 500 µg of 131I-labeled anti-Thy-1.1 antibody resulted in biodistribution of the labeled antibody that approximated that seen when antibody was tumor specific (see Ref. 8, Chart 1). This distribution resulted in estimated mean radiation doses of 1600 cGy to tumor as compared to 500–900 cGy to kidney, liver, and lung following infusion of 1000 µCi. However, there was a wide variation in the concentration of both anti-Thy-1.1 and control antibodies in the tumor among animals within single experiments as well as among experiments (see Fig. 3). This variation makes estimation of tumor radiation dose in an individual animal uncertain. The variability in antibody concentration noted in the biodistribution studies suggests that tumor radiation doses in individual animals may have been 2–3-fold higher or lower than the estimated mean for both the 131I-labeled anti-Thy-1.1 and 131I-labeled control antibodies. Similar animal to animal variation was observed in our previous studies of therapy in AKR/Cum mice (see Ref. 8, Chart 2) and presumably resulted from variations in tumor vascularity, vascular permeability, or other factors.

The marked difference in serum clearance of the 131I-labeled anti-Thy-1.1 antibody and control antibody and the fact that the 131I-labeled anti-Thy-1.1 antibody specifically localized to marrow makes comparison of the therapeutic effects of these two preparations difficult. Treatment with 1500 µCi of 131I-labeled anti-Thy-1.1 antibody had a significantly greater antitumor effect than 750 µCi of 131I-labeled control antibody. These two doses of 131I-labeled antibody would lead to approximately equivalent radiation to normal tissues, except for marrow, and thus equivalent non-marrow toxicity. Furthermore, treatment with 1500 µCi 131I-labeled anti-Thy-1.1 antibody had an equivalent, or possibly greater, antitumor effect than an equivalent µCi dose of 131I-labeled control antibody. Since the control antibody did not specifically bind to cells and remained in serum for a longer period, it would deliver greater radiation...
slow clearance of the labeled control antibody, combined with
would be expected to produce greater non-marrow toxicity. The
regressions in animals receiving 131I-labeled control antibody.
It is unlikely that these regressions were a result of total body
irradiation from the γ-emission of 131I since only a small
amount of this radiation is absorbed within the mouse. 131I-
labeled proteins have been shown previously to accumulate in
tumors in the absence of any binding activity (19).

The dose-limiting toxicity for both 131I-labeled and anti-Thy-
1.1 and 131I-labeled control antibodies was hematopoietic sup-
pression. Although estimated marrow radiation doses for infusion
of 1500 μCi of both 131I-labeled anti-Thy-1.1 and control antibodies were relatively low (165 and 112 cGy), animals died of
marrow aplasia. Death from marrow aplasia in AKR mice
occurs after doses of 500–600 cGy external beam irradiation,3
suggesting that estimated marrow doses were systematically
low. In spite of the more rapid clearance from serum, 1500 μCi of
131I-labeled anti-Thy-1.1 antibody had an equivalent or
greater effect on marrow than 1500 μCi of 131I-labeled control
antibody. The results of the biodistribution studies suggested that the bone marrow toxicity of the 131I-labeled anti-Thy-1.1 antibody was, in part, due to binding to antigen-positive cells
within the marrow since 131I-labeled anti-Thy-1.1 antibody con-
centrations in marrow were higher than those of 125I-labeled
control antibody. However, in previous studies doses of 1000–
1500 μCi of 131I-labeled anti-Thy-1.1 antibody were required to
achieve tumor responses in a majority of animals and were
similarly marrow toxic even when the antibody was absolutely
tumor specific (8). These results suggest that, unless the con-
centrations of antibody in tumor relative to marrow can be
improved from those observed in the present studies, it is
unlikely that it will be possible to infuse curative doses of radiolabeled antibody without the infusion of unirradiated bone
marrow. In situations where cells expressing the target antigen
are present in marrow such support will probably be required.
Fortunately, techniques for either allogeneic or autologous bone
marrow transplantation are well established (20, 21).

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Experimental Radioimmunotherapy of Murine Lymphoma with $^{131}I$-labeled Anti-T-Cell Antibodies

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