Radiotherapy in Mice with Yttrium-90-labeled Anti-Ly1 Monoclonal Antibody: Therapy of the T Cell Lymphoma EL4

Heinz Schmidberger, Donald J. Buchsbaum, Bruce R. Blazar, Paul Everson, and Daniel A. Vallera2

ABSTRACT

Yttrium-90 is a potent β-emitting radionuclide with potential for therapy of lymphoma. A monoclonal antibody against Ly1, the murine homologue of human CDS, was labeled with 90Y and found to selectively bind to Ly1-positive, radiation-sensitive, EL4 mouse lymphoma cells. When tested in this aggressive model of T cell lymphoma, in vivo studies in C57BL/6 mice showed that a single 140-μCi i.p. dose of 90Y-anti-Ly1, given 1 day after i.v. injection of a lethal dose of EL4 cells, resulted in significant but transient improvement in survival. Protection was selective, since a 90Y-labeled irrelevant control antibody did not prolong survival. Biodistribution studies showed that protection was likely limited by inadequate localization of labeled antibody to tumor. Importantly, labeled anti-Ly1 specifically localized in the immunological tissue (spleen and thymus) and lowered the WBC count, perhaps limiting the tolerated dose. Myelosuppression, which is considered one of the major side effects associated with 90Y usage, was not a lethal complication, since WBC counts recovered in mice given a 140-μCi dose of 90Y-anti-Ly1 without EL4 cells and 100% of these animals survived. The maximum tolerated dose was less than 200 μCi. Despite the high localization of 90Y-anti-Ly1 in spleen, splenectomies of tumor-injected mice did not improve the antitumor efficacy of radiolabeled antibody. Further evidence for inadequate delivery of radionuclide to tumor was shown when external total-body irradiation was given to mice given injections of a lethal dose of EL4 tumor cells. Comparison of internal and external irradiation studies indicated that the partially protective effect of 140 μCi 90Y-anti-Ly1 was equivalent to external irradiation of only 100–200 cGy. Because this model reflects the current clinical limitations of radiolabeled antibodies for therapy, including partial antitumor efficacy, delivery of labeled anti-T cell antibodies to the immune system, and low maximum tolerated dose, the model may be useful for examining strategies which could increase the tolerated dose and therapeutic efficacy.

INTRODUCTION

T cell lymphoma and leukemia are among the neoplastic diseases that present patients with an unfavorable prognosis (1). Our institution has observed that, despite purging bone marrow of residual tumor cells in vitro, relapse is common in lymphoma/leukemia patients, and additional methods of eliminating patients’ tumor cells in vivo are necessary (2). Promising clinical studies have suggested that radiolabeled antibodies may be useful for therapy of T cell lymphoma (3, 4). The choice of radionuclide for therapy is important. 131I linked to various antibodies has been used clinically; however, unfavorable γ emissions, an 8-day physical half-life, marrow toxicity (5, 6), and instability of the 131I label in serum (7) and tumor (8, 9) have been observed. 90Y may offer some advantages (10): (a) a variety of methods for attaching metal-chelating groups to proteins have been reported (11–15); (b) its 64.2-h half-life is long enough for tumor localization but short enough so that radiation damage might not be sustained; (c) it is a β emitter with a shorter path length than γ emitters (thus decreasing toxicity to distant organs as well as to clinical staff); (d) it decays to a stable daughter with no additional toxicities. T cell lymphomas and leukemias express normal T cell differentiation antigens, to which MoAb2 are already available. A MoAb against one T cell differentiation antigen, anti-CD5, has been used clinically for MoAb therapy of leukemia (16), and some clinical success has been reported with radiolabeled anti-CD5 in imaging and therapy of cutaneous T cell lymphoma (3, 4). The MoAb anti-Ly1 was chosen for our mouse studies since it recognizes the murine homologue to the human CD5 antigen (17).

This murine study was designed to evaluate the efficacy and limitations of using 90Y-labeled anti-Ly1 against a Ly1-positive EL4 lymphoma. Administration of this radiolabeled anti-T cell MoAb resulted in specific localization in tumor and lymphoid tissue. Our studies showed significant but transient protection and other problems that parallel the clinical experience. In a second investigation (18), we extended our observation that 90Y-anti-Ly1 reacts with lymphoid tissue by examining the effect of 90Y-anti-Ly1 on T cells and its potential for therapy of graft versus host disease.

MATERIALS AND METHODS

Antibodies. Anti-Ly1 (from clone 53-7.3) is an IgG2a rat anti-mouse MoAb recognizing a M, 67,000 glycoprotein on mouse T cells (19). H65 and T101 are IgG2a mouse MoAb that recognize CD5, a M, 67,000 glycoprotein on human T lymphocytes. H65 was provided by Xoma (Berkeley, CA) and T101 was provided by Hybritech (La Jolla, CA). RlgG was obtained from Sigma Chemical Co. (St. Louis, MO).

Mice and Tumors. Female C57BL/6 mice, 5–8 weeks old, were obtained from The Jackson Laboratory (Bar Harbor, ME) and used for all experiments. The chemically induced EL4 lymphoma was of C57BL/6 origin (20). The cells were obtained from American Type Culture Collection (Rockville, MD) and propagated as suspension cultures, in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, and 100 units/ml penicillin-streptomycin, at 37°C in a 5% CO2/95% air atmosphere. EL4 cells (103) given i.v. killed 100% of inoculated mice within 60 days.

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2 To whom requests for reprints should be addressed, at Box 367 UMHC, 420 Delaware Street S.E., Minneapolis, MN 55455.
Radiolabeling of Antibodies with ³¹¹In or ¹²⁵I. Purified antibodies were labeled with ³¹¹In or ¹²⁵I by incubating with ¹²⁵I or ³¹¹In. The chemical anhydride method was used for antibody labeling with ¹²⁵I or ³¹¹In. Briefly, 200 µg of antibody was incubated with ³¹¹In or ¹²⁵I in a 0.5 ml reaction solution. The solution was incubated at 37°C for 30 min, and the activity was counted. The specific activity of the labeled antibody was calculated as described previously (24, 25).

Clonogenic Assay. A highly sensitive limiting dilution assay (31) was used to determine the radiosensitivity of EL4 tumor cells. Cells were cultured in 96-well plates containing 10^4 cells/well and 5% FCS. The cells were allowed to attach for 24 h, and then 25 µCi of labeled antibody was added to each well. The plates were incubated for 72 h, and then the medium was removed and the wells were washed with sterile water. The remaining cells were stained with 0.4% crystal violet in 10% ethanol. The number of colonies was counted, and the clonogenic survival was calculated as described previously (26).

RESULTS

Labeling Efficiency, Specific Activity, and Immunoreactive Fraction of Anti-LY1 MoAb Labeled with ³¹¹In. Anti-LY1 was labeled with ³¹¹In by the cyclic anhydride labeling procedure. In
Fraction values were improved by using larger separation columns specifically bound to EL4 cells in culture. Immunoreactive fractions were 13.7, 30.1, and 17.5%, respectively, showing that 48 to 82% of the radioactivity were labeled on EL4 cells and modulation of the Lyl antigen was measured on human negative human leukemia CEM cells. Cells were centrifuged, and activity was measured in the pellet and supernatant. Percentage of binding was calculated as cpm pellet/cpm total + cpm supernatant. All points were measured in duplicate.

Biodistribution of Labeled Antibodies in 124 Tumor-bearing Mice. Groups of C57BL/6 mice were given i.v. injections of EL4 cells and treated with 90Y-labeled antibodies, as described in "Materials and Methods." Animals were bled at four time points, ranging from 16 to 200 h following injection of 150-200 μCi of radiolabeled MoAb. Biological half-lives were calculated from regression lines of the log percentage of injected dose/ml versus time. Correlation coefficients were always higher than 0.97. In all three experiments, the half-lives for anti-Lyl ranged from 22.1 to 28.6 h, as shown in Table 4. A radiolabeled mouse anti-human CDS MoAb (H65) was administered in an identical manner. The half-lives of the labeled mouse anti-human CDS MoAb ranged from 47.1 to 55.3 h, nearly twice the half-life of labeled anti-Lyl. Since anti-Lyl is a rat antibody, labeled RlgG was also used as a control. Labeled RlgG had a half-life of 27.8 h, similar to that of labeled anti-Lyl. Splenectomy of a group of animals in experiment 3 had no effect on the biological half-lives of labeled anti-Lyl or control antibodies.

Radiosensitivity of EL4 Cells Measured in Clonogenic Assays. To determine whether EL4 cells could be killed by ionizing irradiation, EL4 cells were exposed in vitro to increasing doses of irradiation from a 220-KeV X-ray unit (Table 5). Log kill measured 6 days after X-ray treatment showed that EL4 tumors were irradiated with 220-KeV X-rays. After treatment, cells were washed, serially diluted, and incubated for 6 days. The number of wells showing clonogenic growth was counted. Data are expressed as the most probable number of clonogenic units (CU) and log kill. Log kill was calculated using the formula: log kill = log(CUcontrol/CUtreated).

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Labeling efficiency (%)</th>
<th>Specific activity (mCi/mg)</th>
<th>Immunoreactive fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.7</td>
<td>0.73</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>30.1</td>
<td>0.74</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>17.5</td>
<td>0.49</td>
<td>74</td>
</tr>
</tbody>
</table>

Table 2 Selective binding of 90Y-antil to the EL4 tumor cell line
In a representative experiment, 90Y-anti-Lyl (approximately 2500 cpm) was added to increasing concentrations of either Lyl-positive EL4 cells or Lyl-negative human leukemia CEM cells. Cells were centrifuged, and activity was measured in the pellet and supernatant. Percentage of binding was calculated as cpm pellet/cpm total + cpm supernatant. All points were measured in duplicate.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell number (x 10^6)</th>
<th>cpm pellet</th>
<th>cpm supernatant</th>
<th>Binding (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL4</td>
<td>8</td>
<td>590</td>
<td>1822</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>535</td>
<td>2103</td>
<td>20.3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>135</td>
<td>2771</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>41</td>
<td>2719</td>
<td>1.5</td>
</tr>
<tr>
<td>CEM</td>
<td>8</td>
<td>38</td>
<td>2537</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>31</td>
<td>2225</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>17</td>
<td>2551</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>10</td>
<td>2436</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 3 Modulation of the Lyl antigen on EL4 tumor cells
Cells were incubated with 0.1, 1, or 10 μg/ml MoAb for 30 min at 4°C and for 2 h at 37°C. Cells were washed, incubated with the same MoAb again for 30 min at 4°C to bind any re-expressed antigen, washed again, incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG, and analyzed by fluorescence-activated cell sorting. Percentage of modulation was calculated as described in "Materials and Methods." Modulation of Lyl antigen was measured on EL4 cells and modulation of the CD5 antigen was measured on human peripheral blood mononuclear cells (PBMC).

<table>
<thead>
<tr>
<th>MoAb (μg/ml)</th>
<th>Cell line</th>
<th>Modulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Lyl</td>
<td>0.1</td>
<td>EL4: 0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>EL4: 5.6</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>EL4: 53.3</td>
</tr>
<tr>
<td>Anti-CD5</td>
<td>0.1</td>
<td>PBMC: 36.4</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>PBMC: 46.2</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>PBMC: 48.2</td>
</tr>
</tbody>
</table>

Table 4 Biological half-lives of 90Y-labeled antibodies
Groups of C57BL/6 mice were given 10^3 to 10^6 EL4 tumor cells i.v. on the previous day. Animals were bled at four time points, ranging from 16 to 200 h following injection of 150-200 μCi of radio-labeled MoAb. Biological half-lives were calculated from regression lines of the log percentage of injected dose/ml versus time. Correlation coefficients were always higher than 0.97. In all three experiments, the half-lives for anti-Lyl ranged from 22.1 to 28.6 h, as shown in Table 4. A radiolabeled mouse anti-human CDS MoAb (H65) was administered in an identical manner. The half-lives of the labeled mouse anti-human CDS MoAb ranged from 47.1 to 55.3 h, nearly twice the half-life of labeled anti-Lyl. Since anti-Lyl is a rat antibody, labeled RlgG was also used as a control. Labeled RlgG had a half-life of 27.8 h, similar to that of labeled anti-Lyl. Splenectomy of a group of animals in experiment 3 had no effect on the biological half-lives of labeled anti-Lyl or control antibodies.

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<table>
<thead>
<tr>
<th>cGy</th>
<th>Clonogenic units</th>
<th>Log kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.5 x 10^3</td>
<td>1.28</td>
</tr>
<tr>
<td>250</td>
<td>3.9 x 10^4</td>
<td>1.28</td>
</tr>
<tr>
<td>500</td>
<td>2.1 x 10^5</td>
<td>2.56</td>
</tr>
<tr>
<td>750</td>
<td>1.6 x 10^6</td>
<td>2.67</td>
</tr>
<tr>
<td>1000</td>
<td>3.1 x 10^7</td>
<td>3.37</td>
</tr>
<tr>
<td>3000</td>
<td>6.2 x 10^8</td>
<td>4.07</td>
</tr>
</tbody>
</table>

changed only slightly when measured 1 week later (46%), indicating that the 90Y label did not adversely affect the specificity of the labeled antibody over time and that 90Y-labeled conjugates were quite stable. Table 2 shows that 90Y-anti-Lyl selectively bound to Lyl-positive EL4 cells but not to Lyl-negative CEM cells.

Lyl Antigen on EL4 Cells. Anti-Lyl bound to EL4 cells with an association constant (K_a) of 4.4 x 10^8 M^-1. Scatchard analysis showed about 35,000 Lyl receptors/EL4 cell. The r^2 value for regression analysis was 0.84. As shown in Table 3, the Lyl antigen was modulated from the surface of EL4 cells 2 h after treatment with anti-Lyl. However, modulation was dose dependent, and significant modulation occurred only at the highest concentration tested (10 μg/ml). In contrast, marked modulation of human CD5 from the surface of human peripheral blood mononuclear cells occurred even at 100-fold lower antibody concentrations. Identical results were obtained from 4-h modulation experiments (data not shown).

Biological Half-lives of 90Y-labeled Antibodies. The 90Y-labeled MoAb shown in Table 1 were injected i.v. into groups of C57BL/6 mice given 10^3 to 10^6 EL4 tumor cells i.v. on the previous day. Animals were bled at four time points, ranging from 16 to 200 h following injection of 150-200 μCi of radiolabeled MoAb. Biological half-lives were calculated from regression lines of the log percentage of injected dose/ml versus time. Correlation coefficients were always higher than 0.97. In all three experiments, the half-lives for anti-Lyl ranged from 22.1 to 28.6 h, as shown in Table 4. A radiolabeled mouse anti-human CDS MoAb (H65) was administered in an identical manner. The half-lives of the labeled mouse anti-human CDS MoAb ranged from 47.1 to 55.3 h, nearly twice the half-life of labeled anti-Lyl. Since anti-Lyl is a rat antibody, labeled RlgG was also used as a control. Labeled RlgG had a half-life of 27.8 h, similar to that of labeled anti-Lyl. Splenectomy of a group of animals in experiment 3 had no effect on the biological half-lives of labeled anti-Lyl or control antibodies.

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Biodistribution of Labeled Antibodies in 124 Tumor-bearing Mice. Groups of mice bearing small s.c. EL4 tumors (<0.5 g) were given anti-Lyl MoAb that were labeled with 111In by the same procedure used to label with 90Y. Mice were sacrificed 5 days following injection of labeled antibody, and the percentage of injected dose/g of tissue was measured. As shown in Fig. 1A, more labeled anti-Lyl accumulated in the spleen and thymus.
RADIOLABELED ANTIBODY THERAPY FOR LYMPHOMA

A.

111In-anti-Ly1
125I-anti-Ly1

Fig. 1. Biodistribution of anti-Ly1 antibodies in EL4-tumor-bearing C57BL/6 mice. Mice (four/group) were inoculated s.c. with 10^6 EL4 cells. Nine to 14 days after tumor inoculation, the animals received radiolabeled antibodies. A, the tissue distribution of 111In-anti-Ly1 MoAb, in comparison to nonspecific 111In-RlgG, 5 days after i.p. injection. The activity (percentage of injected dose/g tissue) is shown for various tissues. B, the distribution of 125I-anti-Ly1, in comparison to 111In-anti-Ly1, 5 days after injection.

2). A profound reduction of absolute WBC, lymphocyte, and neutrophil counts occurred during the entire 21 days following treatment. In mice given 90Y-RlgG, there was a similar hematological inhibition. In non-tumor-bearing animals, profound myelosuppression was also observed during the first 21 days with 90Y-anti-Ly1. The longer term studies without EL4 showed that lymphocytes had recovered to 50% of control levels by day 28, while neutrophils had fully recovered. Treatment of mice with similar doses of unlabeled anti-Ly1 showed no effect on WBC counts (data not shown). These results suggest a profound effect of 90Y-labeled MoAb on hematopoiesis.

Antitumor Effect of 90Y-Anti-Ly1. Having established that radiolabeled Ly1 localized to tumor, we tested its efficacy for in vivo therapy of EL4 tumor. Groups of 12 C57BL/6 mice were given a lethal dose (10^5) of EL4 cells i.v. Postmortem studies of mice dying 20–40 days after injection revealed tumor presence in kidney, liver, spleen, lymph node, brain, peritoneum, and bone marrow. Large tumors were occasionally observed on the flank, shoulder, or face. Animals were treated with 140 μCi of 90Y-anti-Ly1 on the day following a lethal dose of EL4 cells. Survival was plotted in an actuarial manner over 100 days. We observed transient yet significant protection (P < 0.04) from tumor-related mortality, compared to mice given control 90Y-labeled anti-human CDS MoAb (Fig. 3A) or PBS (Fig. 3B). Mice that were not given EL4 cells but that received (8.9 and 4.2%, respectively), compared to labeled RlgG (4.7 and 1.6%, respectively), probably due to the presence of Ly1-expressing T cells in these tissues. Although more labeled anti-Ly1 accumulated in tumor, the difference was small. The tumor/blood ratios for labeled anti-Ly1 and labeled RlgG were 2.17 and 1.37, respectively. Others have reported a high nonspecific uptake of labeled immunoglobulin in tumors (33, 34). Notably, more labeled RlgG (11.1%) concentrated in the liver, compared to labeled anti-Ly1 (6.4%). 111In-labeled MoAb and RlgG had an equally high accumulation in the kidney.

For comparison, we evaluated the accumulation of anti-Ly1 labeled with 125I, in a separate experiment (Fig. 2B). 125I-Anti-Ly1 also accumulated primarily in the spleen (19.8%), in even greater concentrations than 111In-anti-Ly1. Less 125I-anti-Ly1 accumulated in the liver and kidney than 111In-anti-Ly1. An equal amount of 125I-anti-Ly1 accumulated in tumor, compared to 111In-anti-Ly1. The biodistribution of 90Y-anti-Ly1 was discussed in the accompanying paper (18). The results were similar, except that localization was higher in bone.

Hematological Effects of 90Y-labeled Antibodies. Myeloablative side effects have been reported in therapeutic studies using 90Y-labeled antibodies (10, 35). Thus, we studied the effects of labeled antibody on WBC, lymphocyte, and neutrophil recovery. Groups of mice were given 150 μCi 90Y-anti-Ly1 i.p. on the day following i.v. administration of EL4 tumor cells (Fig.
RADIOLABELED ANTIBODY THERAPY FOR LYMPHOMA

Days Post Treatment

Fig. 3. Antitumor effect of \( {^{90}}Y \)-anti-Lyl MoAb in C57BL/6 mice given EL4 tumor cells. In A, 24 h after i.v. administration of 10^6 EL4 cells, mice were given 140 \( \mu \)Ci \( {^{90}}Y \)-anti-Lyl or \( {^{90}}Y \)-anti-human CDS MoAb i.p. One group of mice with no EL4 cells was given \( {^{90}}Y \)-anti-Lyl. In B, 24 h after i.v. administration of 10^5 EL4 cells, mice were given 140 \( \mu \)Ci \( {^{90}}Y \)-anti-Lyl or PBS. In C, 24 h after i.v. administration of EL4 cells, mice were given PBS, 200 \( \mu \)Ci \( {^{90}}Y \)-RlgG, 200 \( \mu \)Ci \( {^{90}}Y \)-anti-Lyl, or unlabeled Lyl. Survival data are plotted in an actuarial manner. The same 140 \( \mu \)Ci dose of \( {^{90}}Y \)-anti-Lyl did not die, even after 100 days after therapy. When groups of tumor-bearing mice were treated with 200 \( \mu \)Ci \( {^{90}}Y \)-anti-Lyl, all mice died before day 10 (Fig. 3C). These early deaths were likely toxicity related rather than tumor related. Similar results were obtained using labeled RlgG. The earlier deaths from \( {^{90}}Y \)-Lyl treatment suggest that the animals may have died from myelosuppression and enhanced immunosuppression due to a localization of labeled antibody in lymphoid tissue.

Fig. 4A represents mice treated after 10^6 EL4 cells were administered i.v. Again, treatment with \( {^{90}}Y \)-anti-Lyl resulted in a transient but significant (P < 0.02) antitumor effect, compared to PBS-treated controls. Treatment with irrelevant labeled antibody protected slightly, but not as effectively as treatment with \( {^{90}}Y \)-anti-Lyl (P < 0.02). Because of localization of labeled anti-Lyl in the spleen (Fig. 1), three groups of mice were splenectomized 4 weeks before treatment with EL4 and were then given labeled antibodies (Fig. 4B). The survival of splenectomized mice was similar to the survival of nonsplenectomized mice in all groups.

Estimate of Irradiation Dose Delivered in Vivo to EL4 Tumor by Radiolabeled Antibodies, Based on External TBI. Since EL4 cells were injected i.v., the distribution of tumor and the dose of radiolabeled antibody reaching the tumor were undetermined. In order to estimate the radiobiological effect of \( {^{90}}Y \)-anti-Lyl on tumors cells following i.v. injection, EL4-injected C57BL/6 mice (n = 10/group) were given increasing doses of external-beam TBI, using an X-ray source. Fig. 5 shows that protection comparable to protection observed in mice given \( {^{90}}Y \)-anti-Lyl was achieved at a dose between 100 and 200 cGy of TBI. Mice given 400–500 cGy TBI were cured. Early deaths, most likely related to hematological toxicity, resulted in groups of mice given 600-cGy TBI.

DISCUSSION

The goal of our study was to evaluate the role of anti-Lyl MoAb, a murine homologue of anti-human CDS MoAb, as a radionuclide carrier for targeting and killing established EL4 lymphoma cells in C57BL/6 mice. Investigators (3, 4, 36–41) are currently interested in therapy of lymphoma with radiolabeled antibodies because these tumors are usually radiosensitive and accessible. Anti-Lyl was chosen because Lyl and CDS have a similar distribution on mouse and human lymphocytes, respectively (17, 19). Also, anti-human CDS (30) and anti-Lyl (in this study) each trigger antigenic modulation. EL4, a murine lymphoma (20), was chosen because it is highly invasive, is non-virally induced, is radiation sensitive, and forms micrometastases in vivo. Postmortem studies have shown widespread infiltration of EL4 tumors into all internal organs examined (42). Yttrium-90 was chosen because of its favorable energy, half-life, and radiochemistry and because anti-human CDS an-
tibodies labeled with \(^{90}\text{Y}\) have been used for therapy of lymphoma (4). Our \textit{in vitro} and \textit{in vivo} studies with \(^{90}\text{Y}\)-anti-Ly 1 showed that the radiolabeled antibody was active and selective. \textit{In vitro} studies suggested that the conjugate was stable, since IRF values were unchanged even after 1 week. It will be important to evaluate the stability of \(^{90}\text{Y}\)-labeled antibody conjugates \textit{in vivo} in future studies.

We evaluated the therapeutic efficacy of \(^{90}\text{Y}\)-labeled anti-Ly 1 day after EL4 injection. At this stage, the disease likely consisted of disseminated micrometastases accessible to antibody (43). Since actual uptake of radioactivity in micrometastatic lesions could not be measured, we monitored survival. We observed a significant, but transient, increase in survival with \(^{90}\text{Y}\)-anti-Lyl treatment. A slight but not significant ther

tibodies labeled with \(^{111}\text{In}\) have been used for therapy of lymphoma (4). Our \textit{in vitro} and \textit{in vivo} studies with \(^{111}\text{In}\)-anti-Ly 1 showed that the radiolabeled antibody was active and selective. \textit{In vitro} studies suggested that the conjugate was stable, since IRF values were unchanged even after 1 week. It will be important to evaluate the stability of \(^{111}\text{In}\)-labeled antibody conjugates \textit{in vivo} in future studies.

We evaluated the therapeutic efficacy of \(^{111}\text{In}\)-labeled anti-Ly 1 day after EL4 injection. At this stage, the disease likely consisted of disseminated micrometastases accessible to antibody (43). Since actual uptake of radioactivity in micrometastatic lesions could not be measured, we monitored survival. We observed a significant, but transient, increase in survival with \(^{111}\text{In}\)-anti-Lyl treatment. A slight but not significant therapeutic effect could be seen with \(^{111}\text{In}\)-labeled irrelevant MoAb, supporting our contention that \(^{111}\text{In}\)-anti-Ly 1 is at least partially selective. Our ability to observe an effect on micrometastatic lesions using \(^{111}\text{In}\)-labeled antibody was somewhat surprising. The killing of cells by radiolabeled antibodies can occur at a distance from the binding site and relies in part on a “cross-fire” effect. In theory, a single day following i.v. administration, 10⁵ EL4 tumor cells (with a doubling time of about 12–24 h) might not have reached sufficient mass to effectively take advantage of the cross-fire effect.

We feel that prolongation of survival in mice given a lethal dose of EL4 cells was primarily limited by failure to deliver radiolabeled antibody to tumor cells, since mice died with widely disseminated tumors. Furthermore, in external-beam therapy experiments, protection similar to that observed with radiolabeled antibodies was produced by only 100–200-cGy TBI (and \textit{in vitro} clonogenic assay showed only 1.28 log EL4 inhibition at 250 cGy). Although such comparisons were used to estimate dose, it should be noted that external-beam doses and internal irradiation delivered by radiolabeled antibodies are not entirely equivalent. External beam delivers a homogeneous dose, while labeled antibody likely concentrates the dose in the region of antigen-expressing targets that are well vascularized. Biodistribution experiments did show that only a small quantity of radiolabeled anti-Ly 1 localized selectively in s.c. EL4 tumors, which might relate to the ability of anti-Ly 1 to induce antigenic modulation. Others have reported a poor cumulative concentration of radiolabeled antibody in murine B cell lymphoma (44).

The selective localization of \(^{90}\text{Y}\)-anti-Ly 1 in lymphoid tissue was impressive and undoubtedly related to the high Ly 1 expression on T cells and on some Ly 1-positive B cells. We suspect that the lymphohematopoietic reactivity of the antibody may have limited the tolerated dose, since earlier mortality was observed in groups of mice treated with 200 \(\mu\text{Ci}\) of \(^{90}\text{Y}\) anti-Ly 1, in comparison to those treated with the same dose of \(^{90}\text{Y}\)-RlgG. Our hematological studies showed a profound reduction in lymphocyte count for the first 21 days following treatment. Differential analysis showed that neutrophils had fully recovered by day 28, whereas lymphocytes had not fully recovered even 56 days after treatment. Perhaps myeloid precursors are more radioresistant than lymphoid progenitors, or maybe the microenvironment is more supportive of myeloid recovery in this system. Although the destruction of normal lymphocytes by \(^{90}\text{Y}\)-anti-Ly 1 in the EL4 model was likely disadvantageous, the selective depletion of Ly 1-expressing lymphocytes proved useful for treating lethal graft versus host disease, as shown in the accompanying study (18).

In our experiments, control \(^{111}\text{In}\)-RlgG accumulated in the liver more than did \(^{111}\text{In}\)-anti-Ly 1. Interestingly, evaluation of serum alanine aminotransferase levels (not shown) to assess liver damage in small groups of mice (\(n = 3\) /group) showed that \(^{90}\text{Y}\)-anti-Ly 1 did not significantly elevate enzyme levels (118 \(\pm\) 24 units/liter), compared to PBS-treated controls (89 \(\pm\) 68 units/liter). \(^{90}\text{Y}\)-labeled irrelevant RlgG did elevate serum alanine aminotransferase levels (230 \(\pm\) 108 units/liter), but the elevation was not significant, possibly due to small sample size. Both reagents had a high uptake in the kidney. We feel that these data indicate that our reagents were selective but selectivity was not absolute, perhaps due to Fc binding or reactivity with the reticuloendothelial system.

Non-specific binding might have resulted from the choice of radionuclide and/or chelation method, since liver and kidney accumulation were lower using \(^{125}\text{I}\)-anti-Ly 1, in comparison to \(^{111}\text{In}\)-anti-Ly 1. Others have reported high accumulation of \(^{111}\text{In}\)-labeled antibodies, compared to radiiodine-labeled antibodies, in liver and kidney (45, 46). Transcomplexation of \(^{111}\text{In}\) to transferrin might play a role in the observed differences between radiiodinated and \(^{111}\text{In}\)-labeled antibodies (45, 47). In our experiments, the localization of anti-Ly 1 MoAb to splenic cells was better when the antibody was labeled with \(^{125}\text{I}\), in comparison to \(^{111}\text{In}\).

It could be argued that the mortality seen in our model was partially due to the myelosuppressive effect of \(^{90}\text{Y}\)-labeled antibody, which has also been observed in clinical studies (48, 49). We observed hematological toxicity after treatment with \(^{90}\text{Y}\)-
anti-Ly1 or control 90Y-RiG, indicating that myelotoxicity was not due to choice of antibody. Yttrium-90 has a comparatively long range of penetration in tissue (maximum, 10 mm), and circulating antibodies carrying 90Y may result in a form of TBI. Furthermore, radiolabeled antibodies may accumulate in bone marrow. Free 90Y in bone was a major cause of the myelotoxicity observed with 90Y-labeled antibody therapy in mice and humans (35), and our biodistribution studies with 90Y-anti-Ly1 showed accumulated activity in bone (18). However, we doubt that myelotoxicity was the major contributor to high mortality at the 140-μCi dose of 90Y-anti-Ly1, since non-nontumor-bearing mice given this dose did not die but mice with EL4 tumor given the same dose died with disseminated disease. Myelosuppression was reversible in these animals with time.

A dose of 140–150 μCi of 90Y-labeled antibodies was tolerated by all injected C57BL/6 mice. Two hundred μCi was not. Other investigators have reported different maximum tolerated doses of 90Y-labeled anti-carcinoembryonic antigen MoAb (50 μCi) in nude mice (35, 50). In a BALB/c model in which mice were given injections of a Rauscher erythroleukemia, mice tolerated 100 μCi of 90Y-labeled antibody (10). Differences in radiosensitivity between mouse strains, in labeling procedures, in half-life and biodistribution, and in the quality of radioimmunoconjugate might be responsible for variations in tolerated dosages.

We observed a blood half-life between 22.1 and 28.6 h for 90Y-Ly1. Free 90Y was cleared much more rapidly in our system, with an estimated half-life of 3 h (data not shown). Thus, the radiolabel was probably not cleaved immediately from the carrier in vivo. Other investigators have shown that 90Y-labeled antibodies prepared with the same chelate as used in this study are stable in vivo (51). The biological half-lives of labeled immunoglobulins in our system are independent of their specificity but dependent on the species of their origin, as observed in other experimental systems (52).

In conclusion, this study of 90Y-anti-Ly1 in the EL4 lymphoma model has contributed new findings. (a) Intraperitoneal administration of radiolabeled antibody the day after an i.v. injection of lethal tumor resulted in prolonged and selective protection, albeit transient. (b) The selective localization of 90Y-anti-Ly1 in lymphoid tissue was striking and likely related to the presence of Ly1-expressing lymphocytes. (c) Following the administration of a therapeutically efficacious dose of 90Y-anti-Ly1, myelosuppression was observed. However, it was reversible with time. In our opinion, the murine model of T cell lymphoma will be useful for studying methods such as bone marrow transplantation or use of recombinant cytokines to augment lymphohematopoiesis after 90Y-labeled antibody therapy, as well as other approaches which might reduce toxicity and increase the tolerated dose and therapeutic response.

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RADIOLABELED ANTIBODY THERAPY FOR LYMPHOMA


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