Blood Kinetics, Tissue Distribution, and Radioimaging of Anti-Common Chronic Lymphatic Leukemia Antigen (cCLLa) Monoclonal Antibody CLL2 in Mice Transplanted With cCLLa-Bearing Human Leukemia Cells

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The blood kinetics and biodistribution of anti-common chronic lymphatic leukemia antigen (cCLLa) monoclonal antibody (MoAb) CLL2 were assessed in mice bearing cCLLa+ tumors. The cCLLa is a 69-kDa glycoprotein antigen expressed selectively by malignant B cells in human CLL, hairy cell leukemia (HCL), and prolymphocytic leukemia. Immunoreactive 

\[ ^{125}I \text{-cCLLa} \] (5 Bq/mouse, specific activity 4.3 \( \muCi/\mu g \)) was injected intravenously in mice bearing HCL-derived EH xenografts, and blood kinetics and biodistribution were ascertained up to 16 days postinjection. Radioimages were also obtained up to 72 hours after injecting 10 \( \mu g/mouse \) (specific activity 50.1 \( \mu Ci/\mu g \)) of \( ^{125}I \text{-cCLLa} \). Distinct \( ^{125}I \text{-cCLLa} \) blood kinetics were observed in EH engrafted compared with tumor-free mice including: a longer \( ^{125}I \text{-cCLLa} \) clearance (173 mg/h v 54.7 mg/h) with biexponential rather than monoexponential configuration; and a greater volume of antibody distribution (31,483 mg ± 7,529 mg). These data suggest more rapid tissue uptake by grafted tumors. Preferential \( ^{125}I \text{-cCLLa} \) uptake by EH tumors relative to normal tissues was observed beginning 24 hours postinjection (mean ratio, 4.2) with average peak tumor \( ^{125}I \text{-cCLLa} \) levels of 428.7 pg/mg. \( ^{125}I \text{-cCLLa} \) uptake selectivity by EH tumor cells was also supported by: (1) negligible \( ^{125}I \text{-cCLLa} \) uptake by cCLLa Molt-4 xenografts (average 29.1 pg/mg 24 hours postinjection); (2) background uptake of cCLLa-relevant MoAb J1-LEU, by CDS-EH xenografts (average 31.4 pg/mg 48 hours postinjection); and (3) by scintigraphy. The EH xenograft mouse model might be useful to ascertain preclinically the anti-tumor effect of anti-cCLLa MoAbs and of their conjugated derivatives.

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Materials and Methods

Cell Lines and Lymphocyte Suspensions

Hairy cell lines EH and HK, developed in our laboratory from blood of two patients with HCL,10 were grown in suspension cultures in a medium (Flow Laboratories, Inglewood, CA) supplemented with 15% fetal calf serum (FCS). Molt-4, a human T-cell line,19 and HT-1080,26 a fibrosarcoma cell line, were obtained from the American Type Culture Collection (Rockville, MD), and grown in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% FCS as suspension and monolayer cultures, respectively. All cultures were propagated in 75-cm² tissue culture flasks (Corning Glass Works, Corning, NY) in a 5% CO₂, 95% humidity incubator (Forma Scientific, Marietta, OH) with medium replacement twice weekly. Lymphocyte suspensions derived from blood of patients with CLL with lymphocytosis ≥ 30 × 10⁹/μL, patients with advanced HCL, and from healthy controls were prepared by Ficoll-Hypaque gradient as previously described.15,17,23 Enriched T-lymphocyte suspensions ≥ 90% immunophenotypically homogeneous were prepared as previously described.13 Extensive data on the immunophenotypic characterization of these cells have been published previously.15,17,23 Briefly, the following observations are pertinent to the current studies: while ≥ 80% of blood lymphocytes in patients with advanced

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B-CLL express cCLLα and CD5, only 50% of blood lymphocytes in patients with advanced B-HCL express cCLLα and 2% express CD5; likewise, 50% of EH and HK cells express the cCLLα but 2% express the CD5. Finally, while 70% of Molt-4 cells express the CD5, 1% express the cCLLα.

**The Xenograft Model**

Athymic mice, BABL/c (nu/nu) male, 6 weeks of age, weighing 20 to 25 g, were supplied by the Office of Mammalian Genetics and Animal Production, National Cancer Institute, Frederick Cancer Research Center, Frederick, MD. Animals were preconditioned by irradiation (200 R total body irradiation, weekly times three), before implanting HCL EH or Molt-4 cell lines subcutaneously (2 x 10⁶/mouse) along with pre-irradiated transplantation-promoting HT-1080 fibrosarcoma cell line (2 x 10⁶/mouse) as co-implants, as previously described. Mice bearing Molt-4 or EH and Molt-4 tumors implanted at different sites, and irradiated mice bearing no tumors served as controls. Tumor volume was calculated twice weekly from three-dimensional diameter measurements assessed with vernier calipers. Kinetic and radioligand studies were begun when tumor volumes of EH xenografts reached 2,000 mm³, corresponding to approximately 2 g of weight. Mice were kept under sterile conditions as were their diet and water, and all procedures. Drinking water was supplemented with potassium iodide starting 48 hours before administration of labeled antibody and for the duration of the experiment to reduce thyroid uptake of radioidide.

**Native and Radioiodinated MoAbs**

CLL₂ is an immunoglobulin (Ig) G₂,κ murine MoAb with specificity against the cCLLα, a 69-Kd glycoprotein which is expressed on the surface of malignant cells in CLL and lineage-related disorders, and on EH cells. The IgG fraction of anti-cCLLα MoAb CLL₁ was obtained by passage of CLL₁, ascites through a Staph protein A-Sepharose column (Pharmacia, Piscataway, NJ). The protein content and antigenic specificity of the effluent was assessed by spectral absorbance at 280 nm and by indirect immunofluorescence assay (IFA) against CLL₁ and normal B lymphocytes, respectively. Affinity-purified IgG of anti-CD5 MoAb LEU₁, (a generous gift from Becton Dickinson, Mountain View, CA), was selected as control antibody because EH cells do not express CD5 while Molt-4 cells do. The IgG fractions of MoAbs CLL₁ and LEU₁ were iodinated with reductant-free radioiodide.

**Antigenic Specificity of 125I-CLL₁**

The immunoreactivity of 125I-CLL₁ and 131I-LEU₁ preparations was assessed in vitro by IFA and binding studies. IFA studies, which included assessment of binding to cCLLα (CLL and HCL) and cCLLα- cells (T lymphocytes), and abrogation of binding by purified cCLLα were conducted as previously described for native MoAbs. Binding studies were conducted at low (4.3 μCi/μg) and high (21 μCi/μg) specific activities as previously described. Briefly, 1 ng to 10 μg of 125I-CLL₁ were added to triplicate aliquots of 10⁶ cells suspended in 0.1 mL of RPMI-1640 culture medium containing 0.5% bovine serum albumin (BSA), pH 7.2. The reaction mixture was incubated for 60 minutes at 22°C in polystyrene plastic tubes (Falcon Plastic, Oxnard, CA) that had been precoated overnight in phosphate-buffered saline (PBS) containing 3% BSA, pH 7.2, layered over 4:1 mixture of dibutyl phthalate and bis-2-ethylhexyl phthalate (Eastman Kodak Co, Rochester, NY) in microsedimentation tubes (Sarstedt, Inc, Princeton, NJ), and centrifuged at 10,000 x g for 2 minutes in a microcentrifugation tube (Beckman Instruments, Fullerton, CA). The microsedimentation tube tips containing the cell pellets were cut off, and cell-bound radioactivity was assessed in a γ counter (Gamma 4,000, Beckman Instruments).

**Kinetics and Biodistribution of 125I-CLL₁**

Pharmacokinetic and biodistribution studies were conducted in EH engrafted and tumor-free control mice after intravenous (IV) administration of 5 μg of 125I-CLL₁, (mean specific activity 4.3 μCi/μg, SEM 0.56) in 100 μL of PBS. Blood samples were obtained from the tail vein 15 minutes, 2 hours, 4 hours, 8 hours, and 1, 2, 3, 5, 7, 9, 11, 13, and 16 days after antibody injection. Animals were killed at 6 hours, 24 hours, and 16 days postinfusion and all organs were removed, rinsed in saline solution, weighed, and counted separately. All remaining tissues (carcass) were processed similarly except for homogenization and counting of aliquots. Total blood weight was calculated as 7% of body weight at autopsy. Blood samples and aliquots of tissue homogenates were counted in triplicate in a γ counter (Beckman Instruments). Additional biodistribution control experiments were conducted in mice engrafted with Molt-4 cell lines injected with 125I-CLL₁ and in mice bearing both EH and Molt-4 tumors at different sites injected with 125I-CLL₁ or 131I-LEU₁. Results of antibody biodistribution were expressed as a percentage of the total MoAb dose injected, as picogram of antibody per milligram of tissue, and as blood/tissue or tumor/tissue ratios at the various sampling times. Average 125I-CLL₁ and 131I-LEU₁ uptake in all tissues was calculated from total radioactivity divided by total weight at autopsy. From these values, uptakes by nontumoral tissues (mean cpm – tumor cpm/weight – tumor weight) and by all tissues combined after subtracting the blood contribution (mean total cpm – blood cpm/total weight – blood weight) were also estimated, as were the tissue/blood and tumor/tissue ratios. Pharmacokinetic parameters for blood 125I-CLL₁ were calculated using polyexponential least squares curve fitting (RSTRIPIII, MicroMath, Salt Lake City, UT). The number of exponentials used for pharmacokinetic determinations were chosen by optimal model selection criteria (modified Akaike Information Criteria). A weighting of one/concentration was used. The following parameters were determined: terminal elimination (A), apparent volume of distribution (V), elimination half-life (T₁/₂); area under the blood concentration curve versus time curve to infinity (AUC); area under the first moment curve to infinity (AUMC); antibody clearance from blood (CL, calculated from antibody dose/AUC); and apparent volume distribution (V, calculated from AUMC x dose/AUC²). For statistical comparisons of the control and xenografted mice, a nonparametric test (Kolmogrov-Smirnov) was used. A P value of 0.05 was considered significant.

**Radioimmunoscinography**

Mice bearing EH tumors ≥ 3 g of calculated weight were lightly anesthetized by intraperitoneal (IP) injection of ketamine at 35 μg/g of body weight before MoAb injection and scintigraphy. After injection of 10 μg of 125I-CLL₁ (specific activity 50.1 μCi/μg to 77.6 μCi/μg) in 100 μL of PBS, IP or IV (tail vein), serial scintigrams were obtained at 4, 24, and 72 hours. Scintigrams were obtained with a Picker 415 LFOV gamma camera (½ inch NaI, 61PMT) (Picker International, Charlotte, NC) using a 20% energy window centered about 30 keV, with a medium energy parallel-hole collimator.
positioned approximately 10 cm from the target. At least 50,000 cpm or 20 minutes were accumulated for each image and the data recorded and analyzed on a Medical Data Systems imaging computer (Medical Data Systems, Ann Arbor, MI). Radioimages of mice bearing Molt-4 or EH and Molt-4 tumors were not attempted.

RESULTS

Tumor Growth

As previously reported,\textsuperscript{6,10} subcutaneously (SC) implanted EH cells gave rise to SC tumors in virtually all animals. EH tumors appeared on the average 7 days postimplant and progressed at an approximate rate of 0.3 g per week. Animals with tumors 1,000 mm\textsuperscript{3} to 2,000 mm\textsuperscript{3} in volume were chosen for study. This choice was made to ensure adequacy of tumor-derived data while minimizing tumor necrosis and excessive attrition during long-term pharmacokinetic studies. Indeed, our data have shown that necrosis and attrition are more prevalent in mice bearing larger HK xenografts.\textsuperscript{10} Average EH tumor and total body weight at autopsy 6 hours, 24 hours, and 16 days post-MoAb injection were 2.60 g and 28.43 g (n = 6), 2.81 g and 28.31 g (n = 4), and 5.87 g and 29.36 g (n = 4), respectively, for mice with EH grafts. For Molt-4 tumor-bearing animals killed 24 hours post-MoAb injection (n = 7), these measurements were 2.02 g and 28.23 g, respectively. Mice bearing EH and Molt-4 tumors concomitantly autopsied 48 hours postinjection (n = 2) exhibited tumor and total body weights of 1.98 g and 0.74 g, and 26.29 and 28.59 g, respectively. Control mice bearing no tumors (n = 6) weighed 30.8 g at autopsy 16 days after injection. Scintigraphs were obtained from animals (n = 4) with EH tumors weighing 3.0 g.

Specific Activity and Immunoreactivity of \( ^{125}\text{I}-\text{CLL}_2 \)

Aliquots of 50 \( \mu \text{g} \) to 100 \( \mu \text{g} \) of the IgG fraction of \( \text{CLL}_2 \) were radioiodinated for in vivo kinetic studies and exhibited specific activities ranging from 2.7 \( \mu \text{Ci}/\mu\text{g} \) to 14.4 \( \mu \text{Ci}/\mu\text{g} \) (mean 4.3 \( \mu \text{Ci}/\mu\text{g} \)) corresponding to a 45.1% average \( ^{125}\text{I} \) incorporation. For scintigraphy, a much higher specific activity was obtained ranging from 50.1 \( \mu \text{Ci}/\mu\text{g} \) to 77.6 \( \mu \text{Ci}/\mu\text{g} \) (mean 60.5 \( \mu \text{Ci}/\mu\text{g} \)), representing a 74% average \( ^{125}\text{I} \) incorporation efficiency. The immunoreactivity of \( ^{125}\text{I}-\text{CLL}_2 \) (specific activity up to 10.2 \( \mu \text{Ci}/\mu\text{g} \)) was assessed by binding to cCLLa\textsuperscript{+} and cCLLa\textsuperscript{−} cells in the presence and absence of purified cCLLa. EH cells and normal T lymphocytes were used as reciprocal controls for \( \text{CLL}_2 \) and LEU\textsubscript{1}, binding studies, because while \( \leq 2\% \) and \( \approx 50\% \) of EH cells exhibit the CD5 and the cCLLa\textsuperscript{−} \( \approx 90\% \) and \( \leq 2\% \) of T lymphocytes express these antigens, respectively.\textsuperscript{15,17} \( ^{125}\text{I}-\text{CLL}_2 \) binding to cCLLa-bearing EH and EH cells assessed by IFA was 89.5% and 42.0%, comparable with that of native \( \text{CLL}_2 \) (92.3% and 51.4%, respectively, Table 1). In addition, adsorption of either labeled or unlabeled antibody with affinity column-purified cCLLa abrogated binding to CLL and EH cells by greater than 96% and greater than 90%, respectively. In contrast, binding of irrelevant MoAb LEU\textsubscript{1} to either CLL cells or normal T lymphocytes was not affected appreciably by preadsorption with purified cCLLa (95.4% \( \pm \) 95.2% and 89.2% \( \pm \) 97.7%, respectively). Equilibrium binding of \( ^{125}\text{I}-\text{CLL}_2 \) (specific activity 4.3 \( \mu \text{Ci}/\mu\text{g} \)) to cCLLa-bearing EH cells was proportional to MoAb concentration, but with progressively decreasing binding gains toward the end of the concentration spectrum leading to a plateau when approaching saturation binding.\textsuperscript{21} Mathematical analysis of the data using the Ligand\textsuperscript{12,23} program indicated a high-affinity, low-density component (\( K_a = 300 \times 10^{3}/\text{cell} \), \( K_s = 10^{3} \text{mol}/\text{L}^{-1} \)) and a low-affinity, high-density component (\( R_2 = 900 \times 10^{3}/\text{cell} \), \( K_s = 10^{7} \text{mol}/\text{L}^{-1} \)). The goodness of fit of the estimated parameters was visually confirmed by Scatchard and semilogarithmic plots (Fig 1 and inset, [ ]). These data are consistent with heterogeneous cCLLa sites also reported for CLL cells,\textsuperscript{1,23} and as observed in most other receptor-ligand binding studies.\textsuperscript{21,22} In contrast, \( ^{125}\text{I}-\text{CLL}_2 \) binding to cCLLa-absorbed EH and EH cells was not affected

Table 1. Inhibition of \( ^{125}\text{I}-\text{CLL}_2 \) Binding by cCLLa

<table>
<thead>
<tr>
<th>Reagents</th>
<th>CLL Cells</th>
<th>HCL Cells</th>
<th>T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL\textsubscript{2}</td>
<td>Unabsorbed</td>
<td>92.3</td>
<td>51.4</td>
</tr>
<tr>
<td></td>
<td>cCLLa-absorbed</td>
<td>2.9</td>
<td>3.6</td>
</tr>
<tr>
<td>(Inhibition)</td>
<td>96.66</td>
<td>93.00</td>
<td>13.64</td>
</tr>
<tr>
<td>( ^{125}\text{I}-\text{CLL}_2 )</td>
<td>Unabsorbed</td>
<td>89.5</td>
<td>42.0</td>
</tr>
<tr>
<td></td>
<td>cCLLa-absorbed</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>(Inhibition)</td>
<td>96.65</td>
<td>90.48</td>
<td>ND</td>
</tr>
<tr>
<td>LEU\textsubscript{2}</td>
<td>Unabsorbed</td>
<td>95.4</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>cCLLa-absorbed</td>
<td>95.2</td>
<td>3.7</td>
</tr>
<tr>
<td>(Inhibition)</td>
<td>0.21</td>
<td>-12.12</td>
<td>9.53</td>
</tr>
</tbody>
</table>

Cell aliquots (10\textsuperscript{5}/well) were incubated for 60 minutes at 22°C with 5 \( \mu \text{g}/\text{cell} \) of CLL\textsubscript{2}, \( ^{125}\text{I}-\text{CLL}_2 \) (specific activity 1 \( \mu \text{Ci}/\mu\text{g} \)), or LEU\textsubscript{2} in RPMI-1640 before and after absorption with column-purified cCLLa (10:1 wt:wt ratio). After washing three times, FITC-conjugated rabbit anti-mouse IgG (10 \( \mu \text{g}/\text{well} \)) was added, incubated for 60 minutes at 22°C, and reactive cells were assessed by IFA. CLL and HCL cells were used as cCLLa\textsuperscript{+} target cells. MoAb LEU\textsubscript{1} and cCLLa\textsuperscript{−} T cells were used as controls. All assays were done in triplicate. Results are expressed as percent reactive cells or as percent inhibition.

Fig 1. In vitro binding of \( ^{125}\text{I}-\text{CLL}_2 \) to EH cells. Aliquots of 10\textsuperscript{5} cells were incubated in triplicate with 1 ng to 10 \( \mu \text{g} \) of \( ^{125}\text{I}-\text{CLL}_2 \) (specific activity 4.3 \( \mu \text{Ci}/\mu\text{g} \), line [ ], or 21.0 \( \mu \text{Ci}/\mu\text{g} \), line [ ][ ] in RPMI-1640 containing 5% BSA, pH 7.2 for 60 minutes at 22°C, and processed as described in the text to assess cell-bound radioactivity. Graphs show Scatchard and semilogarithmic (inset) plots of binding data.
binding to cCLLa− normal lymphocytes was negligible and linearly related to MoAb concentration (not shown), as expected for nonspecific binding.23 Equilibrium 125I-cCLLa binding experiments at high specific activity (21 μCi/μg) produced comparable mathematical binding parameters and similar graphical plots (Fig 1 and inset, [■]).

Radioimmunoaustigraphy

In a preliminary experiment where 10 μg 125I-cCLLa was injected IP in EH xenografted animals, the following scintigraphic observations were made: most radioactivity remained in the abdominal cavity 6 hours postinjection; most radioactivity had located in SC tumors 24 hours postinjection with a significant amount detected in the bladder; approximately 20% of the radioactivity counted at 24 hours remained in tumors 72 hours postinjection; and virtually no radioactivity was detected at any time either in nontumoral tissues or as background. The latter observation was expected given the low level of nonspecific 125I-cCLLa uptake by nontumoral tissues, and the self-absorption of 125I counts by interposing tissues, which is proportional to the distance square between the source of the counts and the collimator. A representative scintigraph of EH-tumor bearing mice obtained 24 hours after IV injection of 10 μg of 125I-cCLLa (specific activity 50.1 μCi/μg) is shown in Fig 2 superimposed to a photograph of the MoAb-recipient mouse (left). As shown, large amounts of radioactivity were detected in the tumor. Total body scintigraphy of two mice bearing Molt-4 tumors (3.2 g average weight) injected 125I-cCLLa (10 μg/mouse) IV resulted in nonvisualization of the tumors 24 hours postinjection (not shown).

Blood Kinetics of 125I-cCLLa

Blood 125I-cCLLa concentrations at various times postinjection in EH grafted mice (n = 4) and nontumor-bearing control mice (n = 6) are shown in Fig 3, lines (■) and (○), respectively. One-half hour after injection, blood concentrations of 125I-cCLLa in control and EH grafted mice were 1,040.5 pg/mg and 440.5 pg/mg, respectively. From these blood levels it was calculated that 43.7% and 18.5% of the injected 125I-cCLLa was detectable in their reperfused blood compartments. And 125I-cCLLa concentration data were fit by monoexponential equations for control mice and by biexponential equations for EH grafted mice. Mann pharmpokinetic parameters are given in Table 2. Mean pharmpokinetic parameters were approximately three and five times greater in the xenografted (173.2 mg/h and 3.1,483 mg, respectively) than in control mice (54.7 mg/h and 5,729 mg, respectively). 125I-cCLLa T1/2 was shorter in controls (72.4 hours) than in EH xenografted mice (152.6 hours).

Biodistribution of 125I-cCLLa

As picograms of 125I-cCLLa per milligram of tissue. Of the total radioactivity injected, 81.7%, 76.5% and 5.6% was recovered in EH grafted mice killed at 6 hours, 24 hours, and 16 days, respectively. While the highest concentration of 125I-cCLLa 6 hours postinjection was found in blood (376.2 pg/mg), bladder (333.8 pg/mg), heart (282.1 pg/mg), and lungs (257.6 pg/mg), preferential uptake by tumors (428.7 pg/mg) and lungs (423.9 pg/mg) began to appear at 24 hours and lasted for the duration of the study (Fig 4). Preferential tumor uptake of 125I-cCLLa was highlighted by the distinctly higher average radioactivity in tumors than in
Biodistribution of Anti-CLL Antibody CLL2

Fig 3. Blood 125I-CLL2 concentrations in EH-tumor bearing grafted and control mice were assessed at the postinjection times shown and analyzed as described in the text. Results expressed as picograms of MoAb per milligram of blood are plotted as a function of time.

Table 2. Pharmacokinetic Parameters for Xenografted and Control Mice (± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Xenografts</th>
<th>Controls</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ₀ (h⁻¹) (× 10⁻³)</td>
<td>4.55 (0.22)</td>
<td>9.61 (0.54)</td>
<td>.036</td>
</tr>
<tr>
<td>T¹/₂ (h)</td>
<td>152.6 (7.8)</td>
<td>72.4 (4.1)</td>
<td>.036</td>
</tr>
<tr>
<td>AUC (pg × h/mg) (× 10⁴)</td>
<td>2.89 (0.049)</td>
<td>9.22 (0.89)</td>
<td>.036</td>
</tr>
<tr>
<td>AUMC (pg × h²/mg) (× 10⁴)</td>
<td>5.25 (8.5)</td>
<td>9.61 (0.84)</td>
<td>.036</td>
</tr>
<tr>
<td>Cl (mg of blood/h)</td>
<td>173.2 (2.9)</td>
<td>54.7 (6.8)</td>
<td>.036</td>
</tr>
<tr>
<td>V (mg of blood) (× 10⁵)</td>
<td>31.48 (0.57)</td>
<td>57.3 (0.87)</td>
<td>.036</td>
</tr>
</tbody>
</table>

Fig 4. MoAb biodistribution of tissues of EH-tumor bearing mice assessed 6 hours (■) and 24 hours (□) after a single IV injection (5 µg/mouse) of 125I-CLL2. Radioactivity is expressed as mean picograms of MoAb per milligram of tissue for each tissue. Ht, heart; Lu, lung; Li, liver; Sp, spleen; Ki, kidney; Bd, bladder; Gi, gastrointestinal tract; Tu, tumor; Cs, carcass; Bl, blood.

nontumoral tissues of engrafted mice 24 hours (428.7 pg/mg v 102.7 pg/mg) and 16 days postinjection (32.0 pg/mg v 4.2 pg/mg). At 16 days postinjection, mean radioactivity in nontumoral tissues of engrafted mice (4.2 pg/mg) was comparable with that of tumor-free control mice (4.0 pg/mg). The high bladder radioactivity (demonstrably in the urine) 6 hours (333.8 pg/mg) and 24 hours (440.5 pg/mg) postinjection decreased at 16 days to levels observed in other normal tissues.

The time sequence of the mean tissue 125I-CLL2 biodistribution in grafted mice showed three groups of relatively homogeneous tissue compartments with high (bladder, lung, and tumor), moderate (blood and the highly vascularized kidney, spleen, liver, and heart), and low (gastrointestinal [GI] and carcass) antibody concentrations (Fig 6). Mice (n = 2) bearing both EH and Molt-4 tumors (average weights, 1.98 and 0.74 g, respectively) implanted at different sites exhibited the highest radioactivity in EH (747.3 pg/mg) and Molt-4 tumors (354.4 pg/mg) compared with 15 pg/mg to 276 pg/mg in nontumoral tissues 48 hours after receiving 10 µg/mouse of 125I-CLL2 or 131I-LEU2 (specific activity 12.8 µCi/µg). In these mice (Fig 8), most of the radioactivity (74%) was recovered in blood and the carcass, with Molt-4 tumors exhibiting uptakes (80.3 pg/mg) comparable with that of nontumoral tissues (108.6 pg/mg).

As blood/tissue ratio. Six hours after injection, selective uptake of 125I-CLL2 by EH tumors was not yet evident. Indeed, the mean blood to normal tissue ratio of radioactivity was 2.64 (range, 1.13 [bladder] to 7.70 [GI]), compared with a mean blood/tumor ratio of 2.49 (Table 3). By 24 hours, preferential uptake by bladders, EH tumors, and lungs resulted in blood/tissue ratios of 0.53, 0.54, and 0.55, respectively, compared with a mean ratio of 2.27 for all
nontumoral tissues (range, 0.94 [kidney] to 2.70 [GI]). At 16 days postinjection, radioactivity was much higher in blood than in nontumoral tissues (ratios ranging from 2.63 [lung] to 22.86 [GI] with a mean of 9.47) but was comparable with that of tumors (ratio of 1.25). Selective uptake of $^{125}\text{I-CLL}_2$ was also demonstrated in mice bearing Molt-4 grafts either as single tumors or as dual EH and Molt-4 tumors engrafted at different sites. In these mice, the mean blood/Molt-4 ratios were 5.57 and 6.06 24 hours and 48 hours postinjection, respectively, which was comparable with average blood/nontumoral tissue ratios of 4.11 and 2.50, respectively.

As tumor/tissue ratio. $^{125}\text{I-CLL}_2$ uptake by EH tumor tissue 24 hours postinjection was 4.17-fold and 8.22-fold greater than the average uptake by nontumoral tissues in mice bearing EH tumors (Table 3) or EH and Molt-4 tumors at different sites (not shown). EH tumors retained higher levels of radioactivity relative to all other tissues (except for blood) 16 days postinjection as indicated by ratios ranging from 2.11 (lung) to 18.35 (GI) with a mean tumor/nontumoral tissue ratio of 7.63. In contrast, $^{125}\text{I-CLL}_2$ uptake by Molt-4 tumors in mice bearing Molt-4 or EH and Molt-4 tumors was comparable with that of nontumoral tissues (0.42 or 0.74 ratios) 24 hours or 48 hours postinjection, respectively. Inversely, while the Molt-4 tumor/nontumoral tissues $^{125}\text{I-LEU}_1$ uptake ratio in mice bearing EH and Molt-4 tumors was highest (9.7-fold), it was negligible in the EH tumors (0.87-fold) of animals killed 48 hours postinjection.

**DISCUSSION**

Preferential uptake of antibodies by tumors bearing the specific target antigen has been reported in several experimental models. Our studies indicate that $^{125}\text{I-CLL}_2$ injected in athymic, radiation-preconditioned mice bearing cCLLa+ EH tumors is preferentially taken up by EH tumor cells. Indeed, the mean tumor to normal tissue ratio of $^{125}\text{I-CLL}_2$ uptake in mice bearing xenogeneic EH tumors was 1.06, 4.17, and 7.60 at 6 hours, 24 hours, and 16 days postinjection, respectively. Preferential uptake is also supported by the following observations: (1) nonspecific uptake by normal tissues appeared proportional to tissue vascularization, peaked shortly postinjection, and declined rapidly; (2) lower blood/tissue $^{125}\text{I-CLL}_2$ concentration ratios in EH tumors than in nontumoral tissues of EH grafted mice after 24 hours postinjection; (3) greater average $^{125}\text{I-CLL}_2$ concentrations in EH tumors than in highly vascularized organs such as kidney, heart, and liver after 24 hours postinjection; (4) preferential $^{125}\text{I-CLL}_2$ uptake by EH tumors but not by Molt-4 tumors as judged by scintigraphy. Distinct pharmacokinetics in EH grafted mice compared with tumor-free control mice also support preferential $^{125}\text{I-CLL}_2$ uptake.
observed in the lung, a site for micro- and macro-metastases. This, despite the relatively high tissue radioactivity in tumor-free control mice (indicating more rapid initial antibody uptake from blood of EH grafted mice); (6) shorter λz and T½ in EH grafted than in tumor-free control mice; (7) considerably greater CL and V in EH grafted than in tumor-free control mice supporting more extensive tissue distribution in the EH tumor-bearing mice; and (8) Molt-4 tumor uptake of 125I-CLL2 and EH tumor uptake of 125I-LEU, equal or less than those of nontumoral tissues. These data strongly suggest that 125I-CLL2 accumulation in tumors represents in vivo binding to EH tumor cells and was therefore cCLLA-specific. The specificity of in vitro 125I-CLL2 binding and its abrogation by purified cCLLA or by anti-cCLLA MoAb CLL2 have been amply documented in this and previous studies.16 It could be argued that a substantial portion of the apparent tumor uptake results from nonspecific accumulation of MoAb protein in the expanded extravascular space of the tumor. However, this view is contradicted by: (1) negligeable 125I-CLL2 uptake by Molt-4 tumor tissue; (2) background 125I-LEU uptake by EH tumor tissue; and (3) reciprocal 125I-CLL2 and 125I-LEU uptake specificity by EH and by Molt-4 tissues, respectively, in mice bearing both tumors. Taken together, the above data demonstrate the specificity of the antigen/antibody reaction in vivo and support the adequacy of our model for conducting blood kinetics, biodistribution, and other preclinical studies amenable to biometric measurements.

In our studies, radioimaging was used to confirm specificity of 125I-CLL2 biodistribution rather than for detection of metastases. The relatively low 125I-CLL2 uptake by nontumoral tissues and self-absorption by tissues between the source of the counts and the collimator explains discrepancies between well and camera counting of nontumoral tissues. Indeed, scintigraphic imaging using 123I tended to highlight SC tumors and reduce detection of all other tissue activity. This, despite the relatively high tissue radioactivity observed in the lung, a site for micro- and macro-metastases in 76% of mice bearing EH tumors ≥2 pg.10 However, radioimaging is being explored for detection of metastases in sites difficult to demonstrate otherwise, especially using stable, high efficiency nuclide-labeled MoAb.27 It must be noted that the use of labeled MoAb for the early diagnosis of metastases, staging, and for discriminating between inflammation or fibrosis and truly cancerous tissue is less valuable in liquid than in solid cancers.5,27,29

Radioiodination of CLL2 to a specific activity up to 21 μCi/μg had no apparent effect on the in vitro immunoreactivity of the antibody as judged by binding studies. However, some degree of radiation damage with partial protein denaturation must have occurred, particularly at a specific activity ≥50.1 μCi/μg. This view is supported by the high urinary content of 125I-CLL2 6 hours postinjection and by the heavy bladder radiolabel localization demonstrated by radioimaging 6 hours post-IP injection. Alternatively, the bladder activity might represent rapid antibody metabolism or excretion of antigen-antibody complexes derived from tumor-shed cCLLA bound in circulation to infused 125I-CLL2. Lungs and blood were the only tissues that exhibited consistently higher MoAb concentrations in EH grafted than in tumor-free control mice 16 days postinjection. This is not surprising given the observation that EH tumors metastasize extensively to lungs and that a sizable fraction of transplanted EH cells circulate.10 In addition, cell-free cCLLA, like other lymphoid cell antigen, circulates after spontaneously shed by the lymph node and modulates heavily in the presence of specific MoAb.21 Finally, antigen-antibody complex dissociation or 125I-CLL2 deiodination might also occur as epiphenomena of antigen modulation.23 These events might have contributed to the seemingly high concentrations of radioactivity in blood relative to normal tissues, especially during the first 24 hours postinjection.

The present studies were not designed to assess therapeutic effectiveness of 125I-CLL2. The total 125I-CLL2 MoAb protein and radioactivity injected were 5 μg and 25 μCi to 40 μCi, respectively, amounts much smaller than needed to

Table 3. Organ Distribution of 125I-CLL2 in EH Xenografts

<p>| Abbreviations: Mean, mean values for all tissues; mean-Tu, mean values minus tumor values; mean-BI, mean values minus blood values. |</p>
<table>
<thead>
<tr>
<th>Days</th>
<th>1/4</th>
<th>1</th>
<th>16</th>
<th>1/4</th>
<th>1</th>
<th>16</th>
<th>1/4</th>
<th>1</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ht</td>
<td>282.1</td>
<td>171.2</td>
<td>7.4</td>
<td>1.33</td>
<td>1.36</td>
<td>5.38</td>
<td>0.54</td>
<td>2.50</td>
<td>4.32</td>
</tr>
<tr>
<td>Lu</td>
<td>257.6</td>
<td>423.9</td>
<td>15.2</td>
<td>1.46</td>
<td>0.55</td>
<td>2.63</td>
<td>0.59</td>
<td>1.01</td>
<td>2.11</td>
</tr>
<tr>
<td>Li</td>
<td>181.0</td>
<td>161.6</td>
<td>7.3</td>
<td>2.08</td>
<td>1.44</td>
<td>5.44</td>
<td>0.83</td>
<td>2.65</td>
<td>4.36</td>
</tr>
<tr>
<td>Sp</td>
<td>189.7</td>
<td>181.6</td>
<td>6.4</td>
<td>1.98</td>
<td>1.28</td>
<td>6.20</td>
<td>0.80</td>
<td>2.36</td>
<td>4.98</td>
</tr>
<tr>
<td>Ki</td>
<td>145.9</td>
<td>247.1</td>
<td>9.1</td>
<td>2.58</td>
<td>0.94</td>
<td>4.40</td>
<td>1.03</td>
<td>1.73</td>
<td>3.53</td>
</tr>
<tr>
<td>Bd</td>
<td>333.8</td>
<td>440.5</td>
<td>9.6</td>
<td>1.13</td>
<td>0.53</td>
<td>4.17</td>
<td>0.45</td>
<td>0.97</td>
<td>3.35</td>
</tr>
<tr>
<td>Gl</td>
<td>48.9</td>
<td>86.2</td>
<td>1.7</td>
<td>7.70</td>
<td>2.70</td>
<td>22.86</td>
<td>3.09</td>
<td>4.97</td>
<td>18.35</td>
</tr>
<tr>
<td>Tu</td>
<td>151.0</td>
<td>428.7</td>
<td>32.0</td>
<td>2.49</td>
<td>0.54</td>
<td>1.25</td>
<td>1.00</td>
<td>1.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Cs</td>
<td>147.8</td>
<td>89.0</td>
<td>3.3</td>
<td>2.54</td>
<td>2.62</td>
<td>12.26</td>
<td>1.02</td>
<td>4.81</td>
<td>8.84</td>
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<tr>
<td>Bl</td>
<td>376.2</td>
<td>233.0</td>
<td>59.9</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.40</td>
<td>1.84</td>
<td>0.80</td>
</tr>
<tr>
<td>Mean</td>
<td>144.3</td>
<td>130.3</td>
<td>10.0</td>
<td>2.61</td>
<td>1.79</td>
<td>4.01</td>
<td>1.05</td>
<td>3.29</td>
<td>3.20</td>
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<tr>
<td>Mean-Tu</td>
<td>142.5</td>
<td>102.7</td>
<td>4.2</td>
<td>2.64</td>
<td>2.27</td>
<td>9.47</td>
<td>1.06</td>
<td>4.17</td>
<td>7.63</td>
</tr>
<tr>
<td>Mean-BI</td>
<td>132.8</td>
<td>368.2</td>
<td>24.8</td>
<td>2.96</td>
<td>1.90</td>
<td>5.18</td>
<td>1.14</td>
<td>1.16</td>
<td>1.29</td>
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</table>

Distribution of 125I-CLL2 was calculated for each organ listed as picograms per milligram of wet tissue. Tissue/blood and tumor/tissue concentration ratios were then calculated. Mean picograms per milligram values were obtained by dividing total radioactivity into total weight at autopsy.
demonstrate therapeutic efficacy. In addition, the bulk of neoplastic cells at the time of injection were of large SC tumors, which are relatively inaccessible to circulating antibody. However, the antigenic specificity of native and radio-labeled MoAbs suggests potential clinical applications in detecting metastases and in the treatment of tumors, particularly those with above average blood supply and radiosensitivity, such as lymphomas. The restricted expression of the cCLLa to CLL and its variants suggest the potential uniqueness of anti-cCLLa MoAbs in the management of these disorders. Despite the preferential homing of labeled Moabs, relatively high levels of radioactivity were detected in blood and other highly vascularized normal tissues in our study and those of others. This would result in appreciable irradiation of highly vascularized organs, thus curtailing the usefulness of labeled MoAb in the clinical setting. However, this general limitation to the clinical use of labeled MoAbs is not relevant to anti-cCLLa MoAbs. Indeed, given the nature of CLL and lineage-related diseases, anti-cCLLa MoAbs as carriers of radioactive labels appear less therapeutically desirable than as conjugates of plant toxins or chemotherapeutic agents. Limitations of concern to MoAb immunotherapy of CLL include anti-murine antibody formation, antigenic modulation, and circulating antigen/antibody complexes. Data from our laboratory have shown that in CLL patients, degree of modulation and titers of circulating cCLLa parallel tumor load. Patients with early-stage CLL and cCLLa+ lymphocyte counts ≥10⁴/μL of blood exhibit serum cCLLa titers ≥1 μg/mL. Thus, selection of CLL patients with limited disease would both enhance response rates and reduce potential side effects of MoAb immunotherapy. Selection of such patients for immunotherapy is also supported by the observation that immunologically mediated anti-tumor effects are subject to tumor mass threshold. Finally, the degree of radioiodination of MoAbs has been reported to affect antibody affinity for the antigen and to influence blood clearance. Thus, optimal iodination ratios might have a favorable impact on tumor uptake and retention of MoAb, and hence on tumor lysis. The uniqueness of the cCLLa with regard to its nearly exclusive expression by malignant cells in CLL and its variants; the affinity, complement binding properties, the kinetics and biodistribution of anti-cCLLa MoAbs; and our preliminary immunotherapy results using native CLL, in the EH xenograft model (unpublished data, August 1985) support the appropriateness of phase I/II clinical trials in CLL.

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Blood kinetics, tissue distribution, and radioimaging of anti-common chronic lymphatic leukemia antigen (cCLLa) monoclonal antibody CLL2 in mice transplanted with cCLLa-bearing human leukemia cells

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