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

By Guy B. Faguet, Julia F. Agee, and Joseph T. DiPiro

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-Kd glycoprotein antigen expressed selectively by malignant B cells in human CLL, hairy cell leukemia (HCL), and prolymphocytic leukemia. Immunoreactive ¹²⁵I-cCLLa (5μg/mouse, specific activity 4.3 μCi/μg) 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 μg/mouse (specific activity 50.1 μCi/μg) of ¹²⁵I-cCLLa. Distinct ¹²⁵I-cCLLa blood kinetics were observed in EH engrafted compared with tumor-free mice including: a longer ¹²⁵I-cCLLa T½ (153 hours vs 72 hours), and a considerably greater blood clearance (173 mg/h vs 54.7 mg/h) with biexponential rather than monoexponential configuration; and a greater volume of antibody distribution (31,483 mg vs 5,729 mg). These data suggest more rapid tissue uptake by grafted tumors. Preferential ¹²⁵I-cCLLa uptake by EH tumors relative to normal tissues was observed beginning 24 hours postinjection (mean ratio, 4.2) with average peak tumor ¹²⁵I-cCLLa levels of 428.7 pg/mg. ¹²⁵I-cCLLa uptake selectivity by EH tumor cells was also supported by: (1) negligible ¹²⁵I-cCLLa uptake by cCLLa⁺ Molt-4 xenografts (average 29.1 pg/mg 24 hours postinjection); (2) background uptake of cCLLa-irrelevant MoAb ¹²⁵I-LEU1 by CD65 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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MONOCLONAL ANTIBODIES (MoAbs) reactive against human B and T lymphocytes have been developed,¹⁻⁵ and human B- and T-cell leukemia xenografts in the nude mouse model have recently been described.⁶⁻¹⁰ However, most MoAbs described to date react against normal differentiation antigens and therefore lack tumor specificity. Thus, while their contribution to the classification of leukemias and lymphomas is well-accepted,¹¹ their diagnostic and therapeutic role remains limited.¹²⁻¹⁴ In contrast, we have developed MoAbs against the cCLLa, an antigen common to B-derived chronic lymphatic leukemia (CLL) and its lineage variants (B-hairy cell and B-prolymphocytic leukemias) but unreactive against normal B or T lymphocytes and against a wide array of normal and tumoral tissues.¹⁵⁻¹⁶ The high specificity of anti-cCLLa MoAbs,¹⁷ the universal expression of the cCLLa by all malignant CLL cells,¹⁸ and its restricted expression to CLL and allied disorders facilitate the objective diagnosis of these diseases even in their preclinical evolutionary phase.¹¹ This, and the high affinity of anti-cCLLa MoAbs for the target antigen, also suggest an immunotherapeutic role for these antibodies in the management of leukemias expressing the cCLLa. To ascertain the immunotherapeutic potential of anti-cCLLa MoAbs, a xenotransplantation model was developed using human hairy cell leukemia (HCL) cell lines EH and HK,¹⁸ implanted in a tumor growth-promoting micro-environment of radiation-preconditioned, congenitally athymic mice.¹⁰ Data emanating from our laboratory demonstrated that this xenograft model parallels human HCL, including the circulation of transplanted human malignant cells in mouse blood. This and the appearance of widely disseminated metastases¹⁹ suggest the model to be appropriate for biometric studies of anti-cCLLa MoAbs. This study reports the in vivo blood kinetics and organ distribution of anti-cCLLa MoAb CLL₂ in mice grafted¹⁰ with cCLLa-bearing human HCL cell line EH.¹⁸

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,¹⁶ 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-1080,²⁰ 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 × 10³/μL, patients with advanced HCL, and from healthy controls were prepared by Ficoll-Hypaque gradient as previously described.¹⁵⁻¹⁷,¹⁹,²¹ Enriched T-lymphocyte suspensions ≥90% immunophenotypically homogeneous were prepared as previously described.¹¹ Extensive data on the immunophenotypic characterization of these cells have been published previously.¹⁵⁻¹⁷,²¹ 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 cCLLa and CD5, only ~50% of blood lymphocytes in patients with advanced B-HCL express cCLLa and ~2% express CD5; likewise, ~50% of EH and HK cells express the cCLLa but ~2% express the CD5. Finally, while ~70% of Molt-4 cells express the CD5, ~1% express the cCLLa.

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 × 10⁶/mouse) along with pre-irradiated transplantation-promoting HT-1080 fibrosarcoma cell line (2 × 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 radioimaging 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 radioiodide.

Native and Radioiodinated MoAbs

CLL2 is an immunoglobulin (Ig) G₂ₙₖ murine MoAb with specificity against the cCLLa, 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-cCLLa MoAb CLL2 was obtained by passage of CLL2 ascites through a Staph protein A-Sepharose column (Pharmacia, Piscataway, NJ). The protein content and antigenic specificity of the IgG fractions of MoAbs anti-cCLLa MoAb CLL, was obtained by passage of CLL, ascites by affinity-purified IgG of anti-CDS Life Sciences Group, Irvine, CA), by the chloramine-T method. Radioiodide incorporation was sequentially monitored to achieve the desired specific activities. Free radioiodine was removed by passage of the mixture was incubated for 60 minutes at 22°C in polystyrene plastic tubes (Falcon Plastic, Oxnard, CA) that had been presoaked overnight in phosphate-buffered saline (PBS) containing 3% BSA, pH 7.2, layered over 4:1 mixture of dibutylyl phthalate and bis-2-ethylhexyl phthalate (Eastman Kodak Co, Rochester, NY) in microcentrifugation tubes (Sarstedt, Inc, Princeton, NJ), and centrifuged at 10,000 × g for 2 minutes in a microcentrifugation tube (Beckman Instruments, Fullerton, CA). The microcentrifugation 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-CLL2

Pharmacokinetic and biodistribution studies were conducted in EH engrafted and in tumor-free control mice after intravenous (IV) administration of 5 µg of 125I-CLL2, (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 % 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-CLL2 and in mice bearing both EH and Molt-4 tumors at different sites injected with 125I-CLL2, or 125I-LEU2. 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-CLL2 and 125I-LEU2 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/mean weight – tumor weight) and by all tissues combined after subtracting the blood contribution (mean total cpm – blood cpm/mean total weight – blood weight) were also estimated, as were the tissue/blood and tumor/tissue ratios. Pharmacokinetic parameters for blood 125I-CLL2 were calculated using polyexponential least squares curve fitting (RSTRIIP, 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 (λz), 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.

Radioimmunoscintrigraphy

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-CLL2 (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 mouse bearing Molt-4 or EH and Molt-4 tumors were not attempted.

**RESULTS**

**Tumor Growth**

As previously reported, 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 mm³ to 2,000 mm³ 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. 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 125I-CLL₂**

Aliquots of 50 μg to 100 μg of the IgG fraction of CLL₂ were radioiodinated for in vivo kinetic studies and exhibited specific activities ranging from 2.7 μCi/μg to 14.4 μCi/μg (mean 4.3 μCi/μg) corresponding to a 45.1% average 125I incorporation. For scintigraphy, a much higher specific activity was obtained ranging from 50.1 μCi/μg to 77.6 μCi/μg (mean 60.5 μCi/μg), representing a 74% average 125I incorporation efficiency. The immunoreactivity of 125I-CLL₂ (specific activity up to 10.2 μCi/μg) was assessed by binding to cCLLa⁺ and cCLLA⁻ cells in the presence and absence of purified cCLLa. EH cells and normal T lymphocytes were used as reciprocal controls for CLL₂ and LEU₁, binding studies, because while ≤2% and ∼50% of EH cells exhibit the CD5 and the cCLLa, ∼90% and ≤2% of T lymphocytes express these antigens, respectively. The 125I-CLL₂ binding to cCLLa-bearing EH and EH cells assessed by IFA was 89.5% and 42.0%, comparable with that of native CLL₂ (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 LEU₁ to either CLL cells or normal T lymphocytes was not affected appreciably by preadsorption with purified cCLLa (95.4% v 95.2% and 89.2% v 97.7%, respectively). Equilibrium binding of 125I-CLL₂ (specific activity 4.3 μCi/μg) 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. Mathematical analysis of the data using the Ligand program indicated a high-affinity, low-density component (R₁ = 300 × 10⁶/cell, Kᵣ = 10⁻⁷ mol/L⁻¹) and a low-affinity, high-density component (R₂ = 900 × 10⁶/cell, Kᵣ = 10⁻² mol/L⁻¹). The goodness of fit of the estimated parameters was visually confirmed by Scatchard and semilogarithmic plots (Fig 1A, inset, [ ]). These data are consistent with heterogeneous cCLLa sites also reported for CLL cells, and as observed in most other receptor-ligand binding studies. In contrast, 125I-CLL₂

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**Table 1. Inhibition of CLL₂ and 125I-CLL₂ 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>Unabsorbed</td>
<td>92.3</td>
<td>51.4</td>
<td>2.2</td>
</tr>
<tr>
<td>(Inhibition)</td>
<td>96.66</td>
<td>93.00</td>
<td>13.64</td>
</tr>
<tr>
<td>cCLLa-absorbed</td>
<td>2.9</td>
<td>3.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Unabsorbed</td>
<td>89.5</td>
<td>42.0</td>
<td>ND</td>
</tr>
<tr>
<td>(Inhibition)</td>
<td>96.65</td>
<td>90.48</td>
<td>ND</td>
</tr>
<tr>
<td>LEU₁: Unabsorbed</td>
<td>95.4</td>
<td>3.3</td>
<td>89.2</td>
</tr>
<tr>
<td>(Inhibition)</td>
<td>0.21</td>
<td>-12.12</td>
<td>-9.53</td>
</tr>
</tbody>
</table>

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**Fig 1.** In vitro binding of 125I-CLL₂ to EH cells. Aliquots of 10⁶ cells were incubated in triplicate with 1 ng to 10 μg of 125I-CLL₂ (specific activity 4.3 μCi/μg, line [ ]), or 21.0 μCi/μg, 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.\(^3\) Equilibrium \(^{125}\text{I}}\text{-CLL,} binding experiments at high specific activity (21 \(\mu\text{Ci}/\mu\text{g}\)) produced comparable mathematical binding parameters and similar graphical plots (Fig 1 and inset, [■]).

**Radioimmunoscintigraphy**

In a preliminary experiment where 10 \(\mu\text{g} \text{^{125}I}}\text{-CLL,} 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 \(^{125}\text{I}}\text{-CLL,} uptake by nontumoral tissues, and the self-absorption of \(^{125}\text{I}}\text{ 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 \(\mu\text{g} \text{^{125}I}}\text{-CLL,} (specific activity 50.1 \(\mu\text{Ci}/\mu\text{g}\)) is shown in Fig 2 superimposed to the mouse outline (right) adjacent 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 \(^{125}\text{I}}\text{-CLL,} (10 \(\mu\text{g/mouse}) IV resulted in nonvisualization of the tumors 24 hours postinjection (not shown).

**Blood Kinetics of \(^{125}\text{I}}\text{-CLL,}**

Blood \(^{125}\text{I}}\text{-CLL,} 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 \(^{125}\text{I}}\text{-CLL,} 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 \(^{125}\text{I}}\text{-CLL,} was detectable in their respective blood compartments. Assumptions of blood and tissue data were analyzed by monoexponential equations for control mice and by biexponential equations for EH grafted mice. Mean pharmacokinetic parameters are given in Table 2. Mean values for \(V\) and \(\text{CL}\) were approximately three and five times greater in the xenografted \((173.2 \text{ mg/h and } 3,148 \text{ mg/h}, \text{respectively})\) than in control mice \((54.7 \text{ mg/h and } 5,729 \text{ mg/h}, \text{respectively})\). \(^{125}\text{I}}\text{-CLL,} \frac{T}{2} \text{ was shorter in controls (72.4 hours) than in EH xenografted mice (152.6 hours).}

**Biodistribution of \(^{125}\text{I}}\text{-CLL,}**

As picograms of \(^{125}\text{I}}\text{-CLL,} 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 \(^{125}\text{I}}\text{-CLL,} 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 \(^{125}\text{I}}\text{-CLL,} was highlighted by the distinctly higher average radioactivity in tumors than in...
BIDISTRIBUTION OF ANTI-cCLL ANTIBODY CLL

Fig 3. Blood \(^{125}\text{-CLL}_2\) 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.

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 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) despite somewhat higher uptake by lungs and blood of engrafted mice (15.2 pg/mg v 9.6 pg/mg and 39.9 pg/mg v 24.9 pg/mg, respectively, Fig 5). 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 \(^{125}\text{-CLL}_2\) 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 \(10 \mu\)g/mouse of \(^{125}\text{-CLL}_2\) or \(^{131}\text{-LEU}_1\) (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 \(^{125}\text{-CLL}_2\) 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

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>(\lambda) (h(^{-1}) (x10(^{-5}))</td>
<td>4.55 (0.22)</td>
<td>9.61 (0.54)</td>
<td>.036</td>
</tr>
<tr>
<td>T(1/2) (h)</td>
<td>152.6 (7.8)</td>
<td>72.4 (4.1)</td>
<td>.036</td>
</tr>
<tr>
<td>AUC (pg x h/mg) (x10(^4))</td>
<td>2.89 (0.049)</td>
<td>9.22 (0.89)</td>
<td>.036</td>
</tr>
<tr>
<td>AUMC (pg x h(^2)/mg) (x10(^4))</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 (2.8)</td>
<td>54.7 (6.8)</td>
<td>.036</td>
</tr>
<tr>
<td>V (mg of blood) (x10(^3))</td>
<td>31.48 (0.57)</td>
<td>5.73 (0.87)</td>
<td>.036</td>
</tr>
</tbody>
</table>

Abbreviations: \(\lambda\), terminal elimination rate constant; T\(1/2\), terminal elimination half-life; AUC, area under the blood concentration versus time curve to infinity; AUMC, area under the first moment curve; CL, clearance of antibody from blood; V, apparent volume of antibody distribution.

Fig 4. MoAb biodistribution of tissues of EH-tumor bearing mice assessed 6 hours (■), 24 hours (□), and 16 days (■) after a single IV injection (5 \(\mu\)g/mouse) of \(^{125}\text{-CLL}_2\). Radioactivity is expressed as mean picograms of MoAb per milligram of tissue for each tissue. Tissues are coded as in Fig 4.
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 $^{125}$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.

**DISCUSSION**

Preferential uptake of antibodies by tumors bearing the specific target antigen has been reported in several experimental models.9,25,28 Our studies indicate that $^{125}$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 $^{125}$I-CLL$_2$ uptake in mice bearing xenogeneic EH tumors was 1.06, 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, $^{125}$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 $^{131}$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.

**As tumor/tissue ratio.** $^{125}$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, $^{125}$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 $^{131}$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.

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including: (5) biexponential blood $^{125}$I-CLL$_2$ clearance in EH grafted mice compared with monoexponential clearance in tumor-free control mice (indicating more rapid initial antibody uptake from blood of EH grafted mice); (6) shorter $\lambda_2$ and $T_1/2$ 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 $^{125}$I-CLL$_2$ and EH tumor uptake of $^{125}$I-LEU, equal or less than those of nontumoral tissues. These data strongly suggest that $^{125}$I-CLL$_2$ accumulation in tumors represents in vivo binding to EH tumor cells and was therefore cCLLa-specific. The specificity of in vitro $^{125}$I-CLL$_2$ binding and its abrogation by purified cCLLa or by anti-cCLLa MoAb CLL$_2$ have been amply documented in this and previous studies.\textsuperscript{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) negligible $^{125}$I-CLL$_2$ uptake by Molt-4 tumor tissue; (2) background $^{125}$I-LEU$_1$ uptake by EH tumor tissue; and (3) reciprocal $^{125}$I-CLL$_2$ and $^{125}$I-LEU$_1$ 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 $^{125}$I-CLL$_2$ biodistribution rather than for detection of metastases. The relatively low $^{125}$I-CLL$_2$ 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, sniffagraphic imaging using $^{125}$I 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 $\geq 2 \mu$Ci.\textsuperscript{10} However, radioimaging is being explored for detection of metastases in sites difficult to demonstrate otherwise, especially using stable, high efficiency nuclide-labeled MoAb.\textsuperscript{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.\textsuperscript{5,21,27} Radioiodination of CLL$_2$ to a specific activity up to 21 $\mu$Ci/$\mu$g 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 $\geq 50.1 \mu$Ci/$\mu$g. This view is supported by the high urinary content of $^{125}$I-CLL$_2$, 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\textsuperscript{23} bound in circulation to infused $^{125}$I-CLL$_2$. 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.\textsuperscript{10} In addition, cell-free cCLLa, like other lymphoid cell antigen, circulates after spontaneously shed tumor fragments.\textsuperscript{21} Moreover, antibody-complex dissociation or $^{125}$I-CLL$_2$ deiodination might also occur as epiphenomena of antigenic modulation.\textsuperscript{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 $^{125}$I-CLL$_2$. The total $^{125}$I-CLL$_2$ MoAb protein and radioactivity injected were 5 $\mu$g and 25 $\mu$Ci to 40 $\mu$Ci, respectively, amounts much smaller than needed to

### Table 3. Organ Distribution of $^{125}$I-CLL$_2$ in EH Xenografts

<table>
<thead>
<tr>
<th>Days</th>
<th>Ht</th>
<th>Lu</th>
<th>Li</th>
<th>Sp</th>
<th>Ki</th>
<th>Bd</th>
<th>Gl</th>
<th>Tu</th>
<th>Cs</th>
<th>Bi</th>
<th>Mean</th>
<th>Mean-Tu</th>
<th>Mean-Bi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4</td>
<td>282.1</td>
<td>257.6</td>
<td>181.0</td>
<td>189.7</td>
<td>145.9</td>
<td>333.8</td>
<td>48.9</td>
<td>151.0</td>
<td>147.8</td>
<td>376.2</td>
<td>144.3</td>
<td>142.5</td>
<td>144.3</td>
</tr>
<tr>
<td></td>
<td>171.2</td>
<td>423.9</td>
<td>161.6</td>
<td>181.6</td>
<td>247.1</td>
<td>440.5</td>
<td>82.6</td>
<td>428.7</td>
<td>89.0</td>
<td>233.0</td>
<td>130.3</td>
<td>102.7</td>
<td>368.2</td>
</tr>
<tr>
<td>16</td>
<td>7.4</td>
<td>15.2</td>
<td>7.3</td>
<td>6.4</td>
<td>9.1</td>
<td>9.6</td>
<td>1.7</td>
<td>32.0</td>
<td>3.3</td>
<td>39.9</td>
<td>10.0</td>
<td>4.2</td>
<td>24.8</td>
</tr>
<tr>
<td>1/4</td>
<td>1.33</td>
<td>1.46</td>
<td>2.08</td>
<td>1.98</td>
<td>2.58</td>
<td>1.13</td>
<td>2.70</td>
<td>2.49</td>
<td>2.54</td>
<td>1.00</td>
<td>2.61</td>
<td>2.64</td>
<td>2.96</td>
</tr>
<tr>
<td>16</td>
<td>1.36</td>
<td>0.55</td>
<td>1.44</td>
<td>1.28</td>
<td>0.94</td>
<td>0.53</td>
<td>2.70</td>
<td>0.54</td>
<td>2.62</td>
<td>1.90</td>
<td>1.79</td>
<td>2.27</td>
<td>1.90</td>
</tr>
<tr>
<td>Tumor/Tissue Ratio</td>
<td>5.38</td>
<td>2.63</td>
<td>5.44</td>
<td>6.20</td>
<td>4.40</td>
<td>4.17</td>
<td>22.86</td>
<td>1.28</td>
<td>12.26</td>
<td>5.18</td>
<td>4.01</td>
<td>9.47</td>
<td>7.35</td>
</tr>
</tbody>
</table>

Distribution of $^{125}$I-CLL$_2$ 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.

Abbreviations: Mean, mean values for all tissues; mean-Tu, mean values minus tumor values; mean-BI, mean values minus blood values.
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 <10^5/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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REFERENCES

5. Royston I, Majda JA, Baird SM, Mersuev, Griffths JC: Human T-cell antigens defined by monoclonal antibodies: The 65,000 dalton antigen to T-cells (T65) is also found on chronic lymphocytic leukemia cells bearing surface immunoglobulins. J Immunol 125:725, 1980
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

GB Faguet, JF Agee and JT DiPiro