Biological Properties of Chimeric Domain-deleted Anticarcinoma Immunoglobulins

Dale C. Slavin-Chiorini, Syed V. S. Kashmiri, Jeffrey Schlom, Benjamin Calvo, Lee Ming Shu, Margaret E. Schott, Diane E. Milenic, Philip Snyo, Jorge Carrasquillo, Kerr Anderson, and Patricia Horan Hand

Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, Maryland 20892 [D. C.-S., J. C.]; Bureau of Biologics, Food and Drug Administration, Bethesda, Maryland 20892 [P. S.]; Clinical Center, Nuclear Medicine, Bethesda, Maryland 20892 [J. C.]; and The Dow Chemical Company, Midland, Michigan 48674 [K. A.]

Abstract

CC49 is a second-generation monoclonal antibody (MAb) that has high affinity for the tumor-associated pancarcinoma antigen tumor-associated glycoprotein-72. In clinical trials using gamma scanning, radiolabeled CC49 has facilitated the detection of more than 90% of carcinomas. We report here the development of a constant heavy-chain 2 (CH2) domain-deleted chimeric (c) CC49 MAb by transfecting an expression construct consisting of the CC49 murine variable region and a CH2 domain-deleted human IgG1 constant region into cCC49κ producing SP2/0 murine myeloma cells. As determined by SDS-PAGE, the intact cCC49ΔCH2 has a molecular weight of 153,000 and, under reducing conditions, molecular weights of 43,000 and 27,000. The plasma clearance and tumor-targeting properties of cCC49ΔCH2 were evaluated and compared with those of mouse/human chimeric forms cCC49ΔCH1 and intact cCC49. Previous studies have shown that the in vitro antigen-binding properties of cCC49ΔCH1 are similar to those of cCC49. Biodistribution studies reported here, using 125I-labeled cCC49ΔCH1 and 125I-labeled CC49 in athymic mice bearing human colon carcinoma xenografts, demonstrated that both cMAbs localized to the tumor and cleared from the normal tissues similarly. However, in comparison with 125I-labeled CC49, 125I-labeled cCC49ΔCH2 localized to tumors earlier and had a significantly lower percentage of the injected dose of MAb/g (%ID/g) in normal tissues than CC49. Immunoscintigraphy of 125I-labeled cCC49ΔCH2 and 125I-labeled CC49 in athymic mice bearing human tumor xenografts demonstrated a clear image of the tumor by 24 h after i.v. administration of the ΔCH2 cMAb versus the 72 h required for cCC49. Biodistribution studies using 177Lu-conjugated cCC49ΔCH1 and cCC49 showed no significant difference between the radiolocalization indices (%ID/g in tumor divided by %ID/g in normal tissue). 177Lu-conjugated cCC49ΔCH2, however, had lower %ID/g values in tumor xenografts and lower radiolocalization indices than either 177Lu-conjugated cCC49ΔCH1 or 177Lu-conjugated cCC49. Pharmacokinetic studies in non-tumor-bearing athymic mice using cCC49ΔCH1 and cCC49 revealed no significant difference between these cMAbs. However, the plasma clearance of cCC49ΔCH2 in non-tumor-bearing mice was significantly faster than that of cCC49. These results were similar when the cMAbs were labeled with either iodine or lutetium. In nonhuman primates, 125I-labeled cCC49ΔCH2 cleared significantly faster than 125I-labeled cCC49. The similar plasma clearance and tumor localization of cCC49 and cCC49ΔCH1 suggest that these two cMAbs may be used in similar clinical settings. However, because of the unique pharmacokinetics and tumor targeting of cCC49ΔCH2 versus cCC49 or cCC49ΔCH1, this chimeric immunoglobulin form may be useful in clinical settings that require efficient tumor targeting and rapid serum and whole-body clearance.

Introduction

MAbs are used currently in the diagnosis and treatment of cancer. The antibodies used may be unlabeled (1), linked with a toxin or drug (2, 3), or labeled with a radionuclide (4). Radionuclides such as 131I and 125I or heavy metals such as 90Y, 111In, and 177Lu, when bound to a MAb, may provide not only diagnostic but also therapeutic applications (5–9).

Currently, however, radiolabeled MAbs have shortcomings in their clinical applications. When murine MAbs are used, there is the potential for HAMA formation (10, 11). By human antibodies complexing with the administered MAb, the HAMA response in a patient may decrease MAb localization to the tumor site. If the MAb is cleared slowly from the blood pool, there is potential for toxicity to normal tissue, with bone marrow toxicity being the dose-limiting factor. Also, a prolonged immunoglobulin clearance necessitates an increase in the length of time between administration of a radiolabeled MAb and radioimmunoguided surgery (12) or diagnostic imaging (13).

MAbs have been manipulated in several ways to eliminate their above-mentioned limitations. To decrease the HAMA response and possibly the antidiotypic antibody response in patients, mouse/human chimeric forms have been generated. cMAbs have variable regions derived from their native molecules and human constant regions. Thus, cMAbs maintain the antigenic specificity of their native antibody molecules, although there is decreased potential for antigenicity in patients.

One approach to decrease the clearance time of a radiolabeled antibody and thus minimize normal tissue radiation exposure would be to reduce the size of a MAb. Antibody fragments such as F(ab')2 and Fab' have been generated and showed faster clearance rates than the intact MAb (14). However, when radiolabeled with either iodine or heavy metals, these fragments show increased liver, spleen, and kidney uptake in comparison with the whole MAb (14–18). Genetically engineered sFv antibodies have also been developed in an attempt to increase the clearance rate. The sFv genes generated thus far clearly more rapidly from the blood pool of mice than either a Fab' or F(ab')2 fragment (14). sFvs also exhibit faster tumor localization and better signal/noise ratios (19) at earlier time points than Fab', F(ab')2, or intact MAb. However, the actual %ID/g delivered to the tumor is reduced significantly in comparison with that of an intact MAb (14).

Another approach to increase the rate of MAb clearance and to maintain MAb tumor targeting has been to generate domain-deleted chimeric immunoglobulins. Iodine-labeled cMAbs, in which the CH2 domain is deleted, have been generated and have displayed a faster clearance rate, more rapid tumor targeting, and a lack of metabolic uptake in normal organs compared with intact chimeric immunoglobulins (20–23). The unique properties observed for the domain-deleted cMAbs suggest that these two cMAbs may be used in similar clinical settings. However, the unique pharmacokinetics and tumor targeting of cCC49ΔCH2 versus cCC49 or cCC49ΔCH1, this chimeric immunoglobulin form may be useful in clinical settings that require efficient tumor targeting and rapid serum and whole-body clearance.

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2 In partial fulfillment of the Ph.D. requirements in the Graduate Genetics Program at The George Washington University.

3 To whom requests for reprints should be addressed, Laboratory of Tumor Immunology and Biology, National Cancer Institute, NIH, Building 10, Room 8B07, Bethesda, MD 20892.

4 The abbreviations used are: MAb, monoclonal antibody; HAMA, human antimouse antibody; c, chimeric; sFv, single-chain Fv; ID, injected dose; TAg, tumor-associated glycoprotein; RI, radiolocalization index; BSM, bovine submaxillary mucin; Vκ, variable heavy chain; Cκ, constant heavy chain; Cλ, constant light chain; PA-DOTA, 1,4,7,10-tetraazadodecane-1,4,7,10-tetraacetic acid.
deleted cMAbs may be due to not only size, but also the loss of the C\textsubscript{4}2 glycosylation site. The C\textsubscript{4}2 glycosylation site may facilitate receptor-mediated clearance (24), and changes in the C\textsubscript{4}2 sugar moieties affect the clearance of the cMAb from murine plasma (25). It is possible that total ablation of the C\textsubscript{4}2 domain and its associated carbohydrate may affect the pharmacokinetics, normal tissue metabolism, and tumor targeting of a cMAb.

CC49 is a second-generation anti-TAG-72 MAb, which binds an epitope on the TAG-72 molecule distinct from the B72.3 epitope (26). And the relative affinity of CC49 is greater than that of B72.3 (26). In Phase I clinical trials, CC49 exhibited higher tumor:serum ratios than B72.3, leading to more efficient tumor targeting (28). Because of its high affinity and tumor localization, CC49 is undergoing evaluation in Phase II clinical trials.

We have developed recently a C\textsubscript{4}1-deleted version of CC49, designated cCC49\textsubscript{i@CH1}, and characterized its in vitro biochemical and antigen-binding properties (29). cCC49\textsubscript{i@CH1} is a single-gene construct in which both the C\textsubscript{1} and C\textsubscript{4}1 domains are deleted from the gene. cCC49\textsubscript{i@CH1} has an approximate M\textsubscript{r} of 120,000 under nonreducing conditions, and on reduction with β-mercaptoethanol, the cMAb reduces to one band of approximately M\textsubscript{r} 60,000 (29). The observation of one band of reduced cCC49\textsubscript{i@CH1} was consistent with the peptide linker linking the variable light-chain region to the V\textsubscript{H} region. In competition assays, the slopes of the cCC49\textsubscript{i@CH1} competition curves were similar to those of cCC49.

Based on the faster plasma clearance and lower metabolic uptake of previously generated C\textsubscript{4}2 domain-deleted cMAbs, and in light of the reported effects of C\textsubscript{4}2 glycosylation on MAb clearance (25, 30), one might expect that cCC49\textsubscript{i@CH1} would be advantageous for certain diagnostic and therapeutic applications. Furthermore, cCC49\textsubscript{i@CH1}, which lacks the C\textsubscript{4}1 domain, although maintaining an intact Fe region and C\textsubscript{4}2 glycosylation site, may have promise in defined clinical protocols. Therefore, the goals of this study were: (a) to generate and determine the antigen-binding and affinity characteristics of a C\textsubscript{4}2 domain-deleted cCC49 MAb (cCC49\textsubscript{i@CH2}); (b) to evaluate and compare, in a murine model, the pharmacokinetic, tumor localization, and metabolic patterns of iodine-labeled and heavy metal chelate-conjugated cCC49\textsubscript{i@CH2}, cCC49\textsubscript{i@CH1}, and intact cCC49 MAb; and (c) in nonhuman primates, to compare the plasma clearance of cCC49\textsubscript{i@CH2} versus that of cCC49.

Materials and Methods

Cell Lines. The LS-174T human colon adenocarcinoma cell line (31) was obtained from the American Type Culture Collection (Rockville, MD). The SP20-Ag14 cell line was received from Dr. S. Morrison (UCLA, Los Angeles, CA).

Eukaryotic Expression Vectors. The eukaryotic expression vector pEHCMygph has been described previously (22).

Development of Domain-deleted Variants. The generation of a cDNA clone of the chimeric heavy chain of MAb B72.3 (30), its C\textsubscript{4}2 domain-deleted variant (23), and the expression construct pECCMygB72.3HuG1\textsubscript{CH2} (22) have been reported previously. Construction of a single-gene SGA\textsubscript{CH1} encoding CC49 V\textsubscript{H} and variable light-chain regions and the entire Fc region of the gene was described previously. Construction of a single-gene SGA\textsubscript{CH1} has been described previously (29).

The expression construct of SGA\textsubscript{CH1} has been described previously (29). Isolation of cCC49\textsubscript{i@CH1} and cCC49\textsubscript{i@CH2}, and purification of cCC49\textsubscript{i@CH1} and cCC49\textsubscript{i@CH2}, cCC49 was purified from a bioreactor supernatant supplied by The Dow Chemical Co. Briefly, the supernatant was filtered, and the buffer was exchanged for 0.01 M sodium phosphate (pH 6.8). The supernatant was loaded on a Zorbax semi-preparative weak anion exchange column (MAC-MOD Analytical, Inc., Chadds Ford, PA). The column was washed until spectrophotometer readings were zero. The cMAb was eluted from the column by a NaCl step gradient. Fractions that had high chimeric immunoglobulin content as determined by SDS-PAGE and A\textsubscript{280} nm were pooled and concentrated.

cCC49\textsubscript{i@CH1} was purified using protein G chromatography as described previously (29).

C\textsubscript{4}2 domain-deleted cCC49 was purified from murine ascites fluid. The ascites were clarified and delipidated using Clean Ascite (Affinity Technology, Tustin, CA) and diluted 1:1.5 with 0.1 M Tris-HCl (pH 7.2). An antibody against CC49, designated AI49-3, was coupled to Reacti-gel resin (Pierce Chemical Co., Rockford, IL) according to the manufacturer's instructions. The ascites was passed over the column, and the column was washed with each of the following buffers in this order: 0.1 M Tris-HCl (pH 7.2); 0.1 M Tris-HCl (pH 7.2) with 0.5 M NaCl; 0.1 M Tris-HCl (pH 9.0) with 0.5 M sodium chloride; and, finally, sodium carbonate (pH 10.0). Bound cCC49\textsubscript{i@CH1} was eluted with 0.1 M sodium carbonate (pH 11.5), buffered, and concentrated with a Cx 30 microconcentrator (Polysciences, Inc., Warrington, PA). Concentrated eluent was flowed over a goat antimouse IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD) Reacti-gel column. The flowthrough was concentrated using a Cx 30 microconcentrator and a Centricon-10 (Amicon, Beverly, MA). The concentrate was loaded onto a GF250 column (MAC-MOD Analytical, Inc.). Peak fractions were collected, concentrated, and dialyzed using a Centricon-10.

The amount of protein was determined by the method of Lowry et al. (32). The purity was determined by discontinuous SDS-PAGE using a gradient of 4%–20% acrylamide (Novex, San Diego, CA) according to the method of Laemmli (33). cCC49 and cCC49\textsubscript{i@CH2} were analyzed with and without reduction by β-mercaptoethanol. All samples were boiled at 100°C. The proteins were visualized using Coomassie blue R250.

ELISA. The ELISA used for the detection of cCC49\textsubscript{i@CH1} was performed as described by Slavin-Chiorini et al. (22).

Radiolabeling of cMAbs. Purified cCC49, cCC49\textsubscript{i@CH1}, and cCC49\textsubscript{i@CH2} were labeled with either Na\textsuperscript{125I} or Na\textsuperscript{131I} using the Iodo-Gen (Pierce) method (34). For all three cMAbs, the iodination protocol yielded specific activities of 2 × 10⁶ cpm/µg.

cMAbs cCC49, cCC49\textsubscript{i@CH1}, and cCC49\textsubscript{i@CH2} were labeled with the lutetium complex of the macrocyclic bifunctional coordinator PA-DOTA as reported previously (19). In brief, cMAb samples were equilibrated in 20 mM sodium carbonate (pH 9.5) and diluted to 80 µl in the same buffer. To each cMAb sample was added ⁹⁷⁷⁰Lu-isothyocyanato-Pa-DOTA solution (~20 µl). Molar ratios of ⁹⁷⁷⁰Lu chelate to MAb in the three reaction mixtures ranged from 3.0 to 3.5. Following a 37°C incubation, the radiolabeled antibodies were isolated by passage over a PD-10 gel filtration column (Pharmacia LKB, Piscataway, NJ). Fractions with the highest activity were pooled and quanti-
tated for protein by the method of Lowry et al. (32). The specific activities for $^{177}$Lu-conjugated cCC49, $^{177}$Lu-conjugated cCC49@CH1, and $^{177}$Lu-conjugated cCC49@CH2 were 3.1, 4.0, and 2.8 pCi/tg, respectively.

**RIAs.** Radiolabeled MAb s were tested for the retention of immunoreactivity using a solid-phase RIA with BSM (positive for the cCC49 epitope) or BSA (negative for the cCC49 epitope), as described (19).

An affinity resin assay, BSM bound to Reacti-gel resin, also was used to determine the retention of immunoreactivity (19) with these modifications: 100 μl resin slurry were placed in microcentrifuge tubes, and 1 ml 75,000 cpm radiolabeled cMAb in 2.5 ml 1% BSA/PBS was added to the resin. The tube was incubated at room temperature with end-over-end rotation for 5 min and then centrifuged. The supernatant was transferred to a fresh tube. The resin beads were washed with 1 ml 1% BSA/PBS and centrifuged, and the wash was discarded. The percentage of bound immunoreactivity was calculated as: (bead cpm + supernatant tube cpm)/(starting cpm) × 100.

For iodine-labeled cCC49 and cCC49@CH2, the average immunoreactivity was 40 and 44%, respectively, for the solid-phase RIA and 90% for both cCC49 and cCC49@CH2 using the resin assay.

When cCC49, cCC49@CH1, and cCC49@CH2 were labeled with $^{177}$Lu, the percentages of immunoreactivity were 47, 40, and 44%, respectively, in the solid-phase RIA. An earlier $^{177}$Lu labeling of the three MAb s in which the molar ratio of $^{177}$Lu/MAb was less than 1 gave results for the solid-phase and resin RIAs, respectively, as follows: $^{177}$Lu-labeled cCC49, 82 and 78%; $^{177}$Lu-labeled cCC49@CH1, 60 and 68%; and $^{177}$Lu-labeled cCC49@CH2, 84 and 71%.

**Competition RIAs.** Solid-phase competition RIAs were done as described by Horan Hand et al. (30), with the exception that 10 ng BSM/well were used instead of LS-174T xenograft extract. In each RIA, $^{125}$I-labeled cCC49 or $^{131}$I-labeled cCC49 was used as the radiolabeled antibody. The percentage of radiolabeled MAb bound to antigen compared with a buffer control was calculated. To normalize for the differences in molecular weight between the two cMAbs, the results were plotted on a nomogram scale.

The relative affinity constants were calculated from the competition RIAs, in which both the competitor and the radiolabeled cMAb were the same (30).

**Pharmacokinetic, Biodistribution, and Imaging Studies in Athymic Mice.** Female athymic (nu/nu) mice bearing TAG-72-positive tumors were obtained as described previously (30). The iodine pharmacokinetics study was performed as described previously (22), with the following exceptions: seven mice were killed at specified time intervals, and specific organs were removed. The biodistribution studies, three separate groups of mice were injected iv. with $^{125}$I-labeled cCC49 or $^{131}$I-labeled cCC49@CH1 and killed at the indicated times.

<table>
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<tr>
<th>Tissue</th>
<th>cCC49@CH1</th>
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<th>cCC49@CH1</th>
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<tr>
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<td>19.1</td>
<td>42.0</td>
<td>27.3</td>
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<td>17.8</td>
<td>15.6</td>
<td>12.5</td>
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<tr>
<td>72 h</td>
<td>11.4</td>
<td>12.1</td>
<td>9.9</td>
<td>10.0</td>
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<tr>
<td>168 h</td>
<td>7.9</td>
<td>6.0</td>
<td>5.8</td>
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**Results**

**Biodistribution of Iodine-labeled cCC49@CH1 and cCC49**

The generation and in vitro characterization of cCC49ΔCH1 and cCC49 has been described previously (29). In brief, the molecular weights of the nonreduced and reduced cCC49ΔCH1 are 120,000 and 60,000, respectively. Competition RIAs demonstrated similar levels of binding of both cMAbs to TAG-72.

To compare the in vivo tumor-targeting properties and metabolism of the domain-deleted cCC49ΔCH1 versus cCC49, biodistribution studies were done in which $^{131}$I-labeled cCC49ΔCH1 was coinjected i.v. with $^{125}$I-labeled cCC49 in athymic mice bearing human colon carcinoma xenografts (Table 1). At all time intervals, the %ID/g of $^{131}$I-labeled cCC49ΔCH1 was approximately 1.4–1.6-fold higher than the %ID/g of $^{125}$I-labeled cCC49 in the tumor (0.1 $\pm$ 0.001); however, $^{131}$I-labeled cCC49ΔCH1 levels were also higher than $^{125}$I-labeled cCC49 in the blood and all other normal tissues (0.2 $\geq$ %ID/g $\geq$ 0.01).

Table 1 Biodistribution of iodine-labeled cCC49@CH1 and cCC49 in athymic mice bearing LS-174T tumors

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<tr>
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**Discussion**

The results show that $^{131}$I-labeled cCC49ΔCH1 and $^{125}$I-labeled cCC49 have similar biodistribution profiles in athymic mice. These results suggest that the domain-deleted cCC49ΔCH1 may be a suitable candidate for further preclinical and clinical studies.

**Conclusion**

The data presented in this study indicate that $^{131}$I-labeled cCC49ΔCH1 and $^{125}$I-labeled cCC49 have similar biodistribution and pharmacokinetic profiles in athymic mice. These results support the further development of cCC49ΔCH1 as a potential diagnostic and therapeutic agent for the treatment of colorectal cancer.
PROPERTIES OF CHIMERIC DOMAIN-DELETED ANTIBODIES

Fig. 1. SDS-PAGE analysis of cCC49ΔCH2 and cCC49. A, nonreduced samples of cCC49 (lane 1) and cCC49ΔCH2 (lane 2). B, reduced samples of cCC49 (lane 1) and cCC49ΔCH2 (lane 2). M, molecular weight (in thousands); kD, kilodalton.

0.001), with the exception of the spleen, in which there was a minimal difference. The Rls (%ID/g in tumor/%ID/g in normal tissue) reflect how rapidly a cMAb clears from normal tissues and how well it remains within the tumor. As shown in Table 1, at 72 and 168 h, when the %ID/g of cMAb in the tumor was at a high level in comparison with levels in the normal tissues, the Rls for 131I-labeled cCC49ΔCH2 and 125I-labeled cCC49 showed no significant difference for any tissue. The exception of the spleen, in which 131I-labeled cCC49ΔCH2 had a significantly higher RI value than that of 125I-labeled cCC49 (0.01 ≤ P ≤ 0.002). Surprisingly, despite the size difference between cCC49ΔCH2 and cCC49, these biodistribution studies demonstrated similar Rls for iodine-labeled cCC49ΔCH2 and cCC49 (with the exception of tumor:spleen).

Generation of cCC49ΔCH2

Because of the more efficient tumor-targeting properties observed with second-generation anti-TAG-72 MAb CC49 versus B72.3 (27), and the rapid clearance from normal tissues and the blood pool observed using cB72.3ΔCH2 compared with cB72.3 (22), a CH2 domain-deleted cCC49 MAb was generated. SP2/0 murine myeloma cells were transfected first with pLNCXIIneoC49HuK. The pECMgpC49HuG1ΔCH2 heavy-chain eukaryotic expression construct was then transfected via electroporation into the cCC49K, producing SP2/0 cells. A subclone producing the highest titer of TAG-72-immunoreactive chimeric immunoglobulin was designated cCC49ΔCH2 and used for the studies described below.

SDS-PAGE Analysis of cCC49ΔCH2

To compare the apparent molecular weight of cCC49ΔCH2 with that of cCC49, the domain-deleted and intact cMAbs were evaluated under nonreducing and reducing SDS-PAGE conditions. Under nonreducing conditions (Fig. 1A), cCC49 (lane 1) appeared as one major band with an apparent Mr of 169,000. When cCC49 was reduced with β-mercaptoethanol (Fig. 1B, lane 1), two major bands were observed with apparent molecular weights of 53,000 and 27,000.

Nonreduced cCC49ΔCH2 (Fig. 1A, lane 2) appeared as a major band with Mr 153,000 and a minor band doublet of Mr 73,000–80,000. Inasmuch as it is approximately one-half the size of the whole cCC49ΔCH2 molecule, the Mr 73,000 band may be a half molecule generated possibly by incomplete disulfide bonding of the heavy and light chains, which becomes apparent under denaturation with SDS. Gillies and Wesolowski (20) reported a similar phenomenon with the generation of c14.18ΔCH2 in which both whole and half molecules were observed by SDS-PAGE. The Mr 80,000 portion of the doublet may be formed by incomplete denaturation in SDS. Further analysis of cCC49ΔCH2 using high performance liquid chromatographic sizing under nondenaturing conditions yielded one major peak (>95%) at approximately Mr 166,000. A lower-molecular-weight peak relating to the Mr 73,000 band observed by SDS-PAGE was not apparent by high performance liquid chromatographic sizing. Finally, Western blotting studies demonstrated that only antibodies specific for human immunoglobulin reacted with the Mr 73,000 band, further suggesting that it is a fragment of the cCC49ΔCH2 molecule.

On reduction with β-mercaptoethanol (Fig. 1B), cCC49ΔCH2 (lane 2) resolved into two bands with approximate molecular weights of 43,000 and 27,000. The Mr 27,000 band of cCC49 and cCC49ΔCH2 was consistent with the molecular weight of an immunoglobulin light chain. The Mr 43,000 heavy-chain band of cCC49ΔCH2 seemed to be Mr 10,000 less than that of the intact cCC49 heavy chain, consistent with the loss of the CH2 domain.

Competitor RIAs and Determination of Relative Affinities for cCC49ΔCH2 and cCC49

Solid-phase competition RIAs were performed to compare the immunoreactivity and relative binding affinity of cCC49ΔCH2 to cCC49. cCC49ΔCH2 and cCC49 were used as competitors for the binding of 131I-labeled cCC49ΔCH2 to BSM, which is a rich source of the CC49 epitope (Fig. 2A). Approximately 1.5–2.0 nM of each unlabeled competitor was necessary for 50% competition with 131I-labeled cCC49ΔCH2. Both of the competitor cMAbs generated curves that had similar slopes and similar levels of competition.

Ccc49 and cCC49ΔCH2 were also used as competitors for the binding of 125I-labeled cCC49 to BSM (Fig. 2B). Again, approximately 50% competition was observed for cCC49ΔCH2 and cCC49 at a competitor concentration of 1.8–2.0 nM. When the competitor
cMAbs were compared with each other, the slope and levels of competition for both of cCC49ΔC₄₂ and cCC49 were similar in both competition RIAs (Fig. 2, A and B).

The relative affinity of cCC49ΔC₄₂, 8.38 × 10⁹ M⁻¹, was calculated from the curve in which cCC49ΔC₄₂ was the radiolabeled cMAb. The relative affinity of cCC49, determined from the curve in which unlabeled cCC49 competed with ¹³¹I-labeled cCC49ΔC₄₂, was 9.12 × 10⁹ M⁻¹.

### Biodistribution of Iodine-labeled cCC49ΔC₄₂ and cCC49

To investigate the tumor-targeting and metabolic patterns of cCC49ΔC₄₂ versus cCC49, biodistribution studies were conducted, which compared the ability of coadministered ¹³¹I-labeled cCC49ΔC₄₂ and ¹²⁵I-labeled cCC49 to localize to human colon carcinoma xenografts and clear from normal tissues in athymic mice. In comparison with ¹²⁵I-labeled cCC49, a lower %ID/g of ¹³¹I-labeled cCC49ΔC₄₂ was detected in the tumor and the normal tissues at all time points (Table 2). From 24 to 72 h, the tumor uptake of ¹³¹I-labeled cCC49ΔC₄₂ was approximately 2.6–10-fold less than that of ¹²⁵I-labeled cCC49, whereas approximately 2.5–100-fold less of the %ID/g of ¹³¹I-labeled cCC49ΔC₄₂ remained in the normal tissues than did ¹²⁵I-labeled cCC49. When the RIs were examined for differences between iodine-labeled cCC49ΔC₄₂ and cCC49 from 24 to 168 h, higher ¹³¹I-labeled cCC49ΔC₄₂ tumor:normal tissue ratios were observed in the blood (P < 0.05), liver (0.1 ≤ P ≤ 0.01), spleen (0.02 ≤ P ≤ 0.2), kidneys (P ≤ 0.05), and lungs (P ≤ 0.05) than were observed with ¹²⁵I-labeled cCC49 (Table 2).

In contrast to ¹³¹I-labeled cCC49ΔC₄₁, ¹³¹I-labeled cCC49ΔC₄₂ exhibited very different tumor:normal tissue ratios despite the similarity in size between the two domain-deleted cMAbs. With an overall lower %ID/g in not only the tumor but all the tissues, higher RIs were observed at earlier time points for cCC49ΔC₄₂. These results demonstrate that ¹³¹I-labeled cCC49ΔC₄₂ is a more efficient tumor-targeting agent than iodine-labeled cCC49 and cCC49ΔC₄₁.

### Immunoscintigraphy Studies of Iodine-labeled cCC49ΔC₄₂ and cCC49 in Tumor-bearing Athymic Mice

A gamma camera imaging study of LS-174T tumor-bearing athymic mice was initiated to visualize any differences in tumor localization and blood clearance of ¹³¹I-labeled cCC49ΔC₄₂ and ¹³¹I-labeled cCC49. At 24 h, ¹³¹I-labeled cCC49 had a disseminated image throughout the body of the mouse, with some accumulation in the tumor over background; however, at the same time point, ¹³¹I-labeled cCC49ΔC₄₂ exhibited a sharp image of the tumor with a low blood pool background (Fig. 3, A and B). At 72 h, ¹³¹I-labeled cCC49 had started to clear from the blood pool and exhibited a defined image of the tumor, and ¹³¹I-labeled cCC49ΔC₄₂ with a negligible blood pool.
PROPERTIES OF CHIMERIC DOMAIN-DELETED ANTIBODIES

Fig. 3. Gamma camera imaging of \( ^{131}I \)-labeled cCC49@CH2 and cCC49. Either \( ^{131}I \)-labeled cCC49@CH2 (A and C) or \( ^{131}I \)-labeled cCC49 (B and D) were injected into separate groups of LS-174T tumor-bearing athymic mice. Each mouse received 12–48 μCi either \( ^{131}I \)-labeled cCC49@CH2 or \( ^{131}I \)-labeled cCC49. Images of the mice were taken at 24 (A and B) and 72 h (C and D).

Biodistribution of cCC49ΔCH2, cCC49ΔCH1, and cCC49 Labeled with \( ^{177}Lu \) in Tumor-bearing Athymic Mice

As a MAb radiolabel, \( ^{177}Lu \) may be advantageous in therapeutic regimens because of its unique ratio of \( \gamma \) to \( \beta \) emissions (35). A comparative biodistribution study of the tumor localization properties and metabolic patterns of \( ^{177}Lu \)-labeled cCC49ΔCH2, \( ^{177}Lu \)-labeled cCC49ΔCH1, and \( ^{177}Lu \)-labeled cCC49 was done to ascertain potential differences among the cMAbs when labeled with a heavy metal chelate as opposed to an iodine label. Overall, \( ^{177}Lu \)-labeled cCC49ΔCH2 had a lower %ID/g in the tumor (0.05 \( \leq \) P \( \leq \) 0.001), blood (0.05 \( \leq \) P \( \leq \) 0.001), and lungs (0.05 \( \leq \) P \( \leq \) 0.001) than did \( ^{177}Lu \)-labeled cCC49ΔCH1 or \( ^{177}Lu \)-labeled cCC49 (Table 3). However, higher or equivalent %ID/g values were observed for \( ^{177}Lu \)-labeled cCC49ΔCH2 at 24–72 h in the liver (0.5 \( \leq \) P \( \leq \) 0.05), spleen (0.2 \( \leq \) P \( \leq \) 0.05), and kidneys (0.2 \( \leq \) P \( \leq \) 0.001) compared with \( ^{177}Lu \)-labeled cCC49ΔCH1 and \( ^{177}Lu \)-labeled cCC49. Comparing cCC49ΔCH1 with cCC49, no apparent differences in the %ID/g were observable in either the tumor or normal tissues (Table 3).

When the RI values were examined to determine differences in localization properties of each cMAb, there were significant differences between cCC49ΔCH2 and cCC49 and also between cCC49ΔCH2 and cCC49ΔCH1. As shown in Table 3, \( ^{177}Lu \)-labeled cCC49ΔCH2 cleared rapidly out of the plasma, and, thus, the observed tumor: blood RI values were significantly greater than those of either \( ^{177}Lu \)-labeled cCC49ΔCH1 or \( ^{177}Lu \)-labeled cCC49 (P \( \leq \) 0.05). However, because of the relatively high %ID/g of \( ^{177}Lu \)-labeled cCC49ΔCH2 observed in the liver, kidneys, and spleen at all time points in comparison with the overall low %ID...
PROPERTIES OF CHIMERIC DOMAIN-DELETED ANTIBODIES

Table 3 Biodistribution of $^{177}$Lu-labeled cCC49ΔCH2, cCC49ΔCH1, and cCC49 in athymic mice bearing LS-174T tumors

<table>
<thead>
<tr>
<th>Tissue</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%ID/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>21.1</td>
<td>52.5</td>
<td>58.7</td>
<td>12.0</td>
</tr>
<tr>
<td>Blood</td>
<td>0.4</td>
<td>7.8</td>
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<td>1.0</td>
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<tr>
<td>Liver</td>
<td>12.8</td>
<td>12.4</td>
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<td>8.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>28.4</td>
<td>14.3</td>
<td>10.8</td>
<td>10.2</td>
</tr>
<tr>
<td>Kidneys</td>
<td>11.2</td>
<td>6.8</td>
<td>6.5</td>
<td>9.9</td>
</tr>
<tr>
<td>Lungs</td>
<td>1.4</td>
<td>4.2</td>
<td>4.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

RI

<table>
<thead>
<tr>
<th>Tissue</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%ID/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>60.6</td>
<td>7.3</td>
<td>6.7</td>
<td>115.8</td>
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<tr>
<td>Liver</td>
<td>1.7</td>
<td>7.8</td>
<td>7.8</td>
<td>1.5</td>
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<tr>
<td>Spleen</td>
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<td>5.8</td>
<td>7.3</td>
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<tr>
<td>Kidneys</td>
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</tr>
<tr>
<td>Lungs</td>
<td>14.4</td>
<td>12.8</td>
<td>12.8</td>
<td>11.7</td>
</tr>
</tbody>
</table>

RI values for $^{177}$Lu-labeled cCC49ΔCH2 in these tissues never exceeded a value of 2.6 and were approximately 2.5–41-fold lower than either $^{177}$Lu-labeled cCC49ΔCH1 or $^{177}$Lu-labeled cCC49 (0.05 ≥ P ≥ 0.01). When compared with each other, $^{177}$Lu-labeled cCC49ΔCH1 and $^{177}$Lu-labeled cCC49 showed no significant differences in any of their RI values at any time point.

Pharmacokinetic Studies

Pharmacokinetics of $^{177}$Lu-conjugated cMAbs in Athymic Mice. A plasma clearance study using non-tumor-bearing athymic mice was initiated to determine whether any differences exist in the pharmacokinetics among lutetium-labeled cMAbs. As shown in Fig. 4, $^{177}$Lu-conjugated cCC49ΔCH2 cMAb cleared significantly faster than $^{177}$Lu-conjugated cCC49, starting at 0.5 h (P < 0.002). At 6 h, approximately 75% of the $^{177}$Lu-conjugated cCC49ΔCH2 had cleared the plasma, but less than 45% of $^{177}$Lu-conjugated cCC49 was cleared. By 24 h, more than 99% of the CH2 domain-deleted cMAb had cleared from the plasma, however, even at 168 h, only 70% of cCC49 had cleared. The T1/2a and T1/2b half-lives were calculated for each cMAb and were 3.2 and 18.5 h, respectively, for cCC49ΔCH2 and 1.8 and 179.4 h, respectively, for cCC49 (Table 4).

The plasma clearance of $^{177}$Lu-conjugated cCC49ΔCH2 was similar in non-tumor-bearing (Fig. 4) and tumor-bearing athymic mice (Fig. 4, inset). Starting at 6 h and continuing to 168 h, $^{177}$Lu-conjugated cCC49ΔCH2 had a more rapid rate of clearance than either $^{177}$Lu-conjugated cCC49 or $^{177}$Lu-conjugated cCC49ΔCH1 (0.05 ≥ P ≥ 0.01) (Fig. 4, inset). When compared with each other, lutetium-labeled cCC49 and cCC49ΔCH1 showed no statistically significant difference in their clearance in the tumor bearing mice (Fig. 4, inset).

Pharmacokinetics of Iodine-labeled cCC49ΔCH2, cCC49ΔCH1, and cCC49 in Non-Tumor-bearing Athymic Mice. Pharmacokinetic studies using iodine-labeled cMAb variants were also conducted in non-tumor-bearing mice to compare pharmacokinetic differences between cCC49ΔCH2, cCC49, and cCC49ΔCH1 labeled with iodine. As shown in Fig. 5A, plasma clearance studies in which $^{131}$I-labeled cCC49ΔCH1 was coinjected i.v. with $^{125}$I-labeled cCC49 into non-tumor-bearing athymic mice showed that these cMAbs cleared at a similar rate. No statistically significant difference was observed at any time point. At 6–8 h, approximately 50% of both cMAbs had cleared from the plasma; by 24 h, approximately 80% of $^{125}$I-labeled cCC49 and 90% of the $^{131}$I-labeled cCC49ΔCH1 had cleared. Thus, the deletion of the CH1...
domain did not seem to affect plasma clearance in normal athymic mice. The serum \( T_{1/2a} \) were 4.4 and 5.6 h, and \( T_{1/2B} \) were 77.2 and 45.2 h for cCC49 and cCC49ΔCH1, respectively.

When \(^{131}I\)-labeled cCC49ΔCH2 and \(^{125}I\)-labeled cCC49 were coinjected i.v. in athymic mice, by 2 h, the clearance of \(^{131}I\)-labeled cCC49ΔCH2 was significantly faster than that of the intact cMAb (0.05 > \( P > 0.01 \); Fig. 5B). At 24 h, more than 99% of \(^{131}I\)-labeled cCC49ΔCH2 and less than 75% of \(^{125}I\)-labeled cCC49 had cleared out of the plasma. The above results observed for cCC49ΔCH2, cCC49ΔCH1, and cCC49 were similar to those observed when \(^{177}Lu\) was used as the radiolabel for these cMAbs. The \( T_{1/2a} \) values were 3.4 and 2.5 h, and the \( T_{1/2B} \) values were 140.0 and 8.9 h for cCC49 and cCC49ΔCH2, respectively (Table 4).

**Pharmacokinetics of Iodine-labeled cCC49ΔCH2 and cCC49 in Nonhuman Primates.** Previous studies of MAb pharmacokinetics have shown that nonhuman primates predict the plasma clearance of MAbs more accurately in humans than do mice (36). Because the rapid clearance of cCC49ΔCH2 may be important in both in development and therapy of human carcinomas using a iodinated MAb, the plasma clearance of \(^{131}I\)-labeled cCC49ΔCH2 and \(^{125}I\)-labeled cCC49 was compared in juvenile rhesus monkeys.

As shown in Fig. 6, approximately 50% of \(^{131}I\)-labeled cCC49ΔCH2 cleared from the rhesus plasma at 6 h, and by 30 h, approximately 92% cleared. In comparison, at 6 h, only 1% of \(^{125}I\)-labeled cCC49 cleared, whereas 50% cleared the plasma at 30 h. Between 96 and 168 h, more than 90% of cCC49 was removed from the monkey plasma. The \( T_{1/2a} \) and \( T_{1/2B} \) shown in Table 4 are 6.1 and 50.2 h, respectively, for cCC49ΔCH2 and 16.5 and 65.0 h, respectively, for cCC49. Thus, \(^{131}I\)-labeled cCC49ΔCH2 cleared from the plasma of both athymic mice and rhesus monkeys at a significantly faster rate than \(^{125}I\)-labeled cCC49.

**Discussion**

Currently, MAbs are being evaluated for both the detection and treatment of a variety of carcinomas (6). The generation of recombinant chimeric or complementarity determining region-grafted MAbs may circumvent one of the potential disadvantages inherent in using murine MAbs, i.e., HAMA. CC49 is a second-generation MAb that has high affinity for TAG-72. In mice bearing human colon carcinoma xenografts, CC49 has demonstrated efficient tumor localization (27). In patients, approximately 90% of tumor lesions can be detected with iodine-labeled CC49 by radioimmunoguided surgery (37). Furthermore, in a subgroup of colorectal carcinoma patients expressing high levels of TAG-72, radiolabeled CC49 localizes to more than 94% of evaluable TAG-72-positive lesions (28), and approximately 95% of patients' breast carcinomas were detected using iodine-labeled CC49. Currently, Phase II clinical trials using radiolabeled CC49 are ongoing.

Because of the efficiency of tumor localization in preclinical and clinical studies, CC49 was chimerized. The first goal of this study was to produce and characterize a C\(\text{H}_2\) domain-deleted chimeric variant of CC49. Our previous studies with cB72.3 and those of others suggested that an immunoglobulin of this type might be useful in clinical settings that require a MAb designed to clear at a rapid rate from the blood pool and yet maintain a high relative affinity for the antigen. The second goal was to evaluate the clinical potential of a previously generated, domain-deleted cCC49 variant (cCC49Δ\(\text{CH}_1\); Ref. 29).

Overall, the removal of the C\(\text{H}_2\) region did not seem to affect the ability of the MAb to bind to antigen: the relative affinity of cCC49Δ\(\text{CH}_2\) was similar to that of cCC49. However, the deletion of the C\(\text{H}_2\) domain did change the patterns of tumor localization observed in athymic mice bearing human colon carcinoma xenografts. When cCC49Δ\(\text{CH}_2\), cCC49Δ\(\text{CH}_1\), and cCC49 were radiolabeled with iodine, optimal localization of cCC49Δ\(\text{CH}_2\) to LS-174T tumor xenografts occurred at earlier time points than those observed for either intact cCC49 or the cCC49Δ\(\text{CH}_1\). The rapidity of localization of cCC49Δ\(\text{CH}_2\) is similar to rates of localization reported for ch14.18Δ\(\text{CH}_2\) (21) and cB72.3Δ\(\text{CH}_2\) (22). The fact that iodine-labeled cCC49Δ\(\text{CH}_2\) localized to the tumor and cleared at a rate similar to cCC49 was surprising, given that cCC49Δ\(\text{CH}_1\) is similar in size to cCC49Δ\(\text{CH}_2\); cCC49Δ\(\text{CH}_2\) had a lower overall %ID/g remaining in

---

**Table 4**

<table>
<thead>
<tr>
<th>cMAb</th>
<th>Radiolabel</th>
<th>Species</th>
<th>( T_{1/2a} ) (h)</th>
<th>( T_{1/2B} ) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cCC49ΔCH2</td>
<td>(^{131}I)</td>
<td>Murine</td>
<td>2.5</td>
<td>8.9</td>
</tr>
<tr>
<td>cCC49</td>
<td>(^{125}I)</td>
<td>Murine</td>
<td>3.4</td>
<td>140.0</td>
</tr>
<tr>
<td>cCC49ΔCH1</td>
<td>(^{131}I)</td>
<td>Murine</td>
<td>4.4</td>
<td>45.2</td>
</tr>
<tr>
<td>cCC49</td>
<td>(^{125}I)</td>
<td>Murine</td>
<td>5.6</td>
<td>77.2</td>
</tr>
<tr>
<td>cCC49ΔCH2</td>
<td>(^{177}Lu)</td>
<td>Murine</td>
<td>3.2</td>
<td>18.5</td>
</tr>
<tr>
<td>cCC49</td>
<td>(^{177}Lu)</td>
<td>Murine</td>
<td>1.8</td>
<td>179.4</td>
</tr>
<tr>
<td>cCC49ΔCH2</td>
<td>(^{131}I)</td>
<td>Primate</td>
<td>6.1</td>
<td>50.2</td>
</tr>
<tr>
<td>cCC49</td>
<td>(^{125}I)</td>
<td>Primate</td>
<td>16.4</td>
<td>65.0</td>
</tr>
</tbody>
</table>

*The serum \( T_{1/2} \) of radiolabeled cCC49, cCC49ΔCH2, and cCC49ΔCH1 were determined as described in “Materials and Methods” in non-tumor-bearing athymic mice and nonhuman primates as indicated above.

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the tumor compared with both cCC49ΔC\textsubscript{H}\textsubscript{1} and cCC49. Because cCC49ΔC\textsubscript{H}\textsubscript{2} has a similar relative affinity to that of intact cCC49, the lower %ID/g observed in the tumor is probably due to its rapid clearance out of the body, thus limiting its exposure to antigen on the tumor. Also, in comparison with either iodine-labeled cCC49ΔC\textsubscript{H}\textsubscript{1} or cCC49, cCC49ΔC\textsubscript{H}\textsubscript{2} had a lower %ID/g in the normal tissues, especially the kidneys. The low %ID/g of 131I-labeled cCC49ΔC\textsubscript{H}\textsubscript{1} in the kidneys is in contrast to previously reported high %ID/g in the kidneys of iodine-labeled Fab' and F(ab')\textsubscript{2} fragments (14).

Lutetium has lower-energy γ emissions than iodine. The low-energy γ emissions may lower marrow toxicity and decrease problems of handling radionuclides for health care workers (35). Furthermore, the β-emissions of 177Lu can cause cell killing effectively between 12 and 50 cell diameters (35). Previous studies have shown that 177Lu has an antitumor effect on established human colon carcinoma xenografts in mice (35). In this study, we report that 177Lu-conjugated cCC49ΔC\textsubscript{H}\textsubscript{2} had a significantly faster plasma clearance and targeted tumor xenografts at a lower efficiency than cCC49ΔC\textsubscript{H}\textsubscript{1} and cCC49. However, unlike iodine-labeled cCC49ΔC\textsubscript{H}\textsubscript{2}, 177Lu-conjugated cCC49ΔC\textsubscript{H}\textsubscript{2} seemed to remain in normal tissues, especially the kidneys, liver, and spleen. Coupled with the lower %ID/g of cCC49ΔC\textsubscript{H}\textsubscript{2} observed in the tumor, the RI values for these tissues were relatively low at all time points when compared with those of cCC49 and cCC49ΔC\textsubscript{H}\textsubscript{1}. Interestingly, when conjugated with lutetium, cCC49 and cCC49ΔC\textsubscript{H}\textsubscript{1} exhibited similar patterns of distribution in all normal tissues and the tumor.

The dehalogenation of radiolabeled MAbs in the tissues (38) and the subsequent excretion of metabolic forms of iodine from the cells may be an explanation for the observed low %ID/g of 131I-labeled cCC49ΔC\textsubscript{H}\textsubscript{2} in normal tissues, whereas a lutetium chelate, because of its large size and charge, unlike iodine metabolites, may not be cleared from normal organs readily (19). Thus, the low RIs observed with 177Lu-labeled cCC49ΔC\textsubscript{H}\textsubscript{2} compared with either 177Lu-labeled cCC49ΔC\textsubscript{H}\textsubscript{1} and 177Lu-labeled cCC49 or 131I-labeled cCC49ΔC\textsubscript{H}\textsubscript{2} may be due to the rapid clearance of cCC49ΔC\textsubscript{H}\textsubscript{2} from the blood, which may lead to a lower distribution of cCC49ΔC\textsubscript{H}\textsubscript{2} in the tumor, whereas normal organs may retain the 177Lu-PA-DOTA chelate. Alternatively, there may be differences in how cCC49ΔC\textsubscript{H}\textsubscript{2} is metabolized compared with cCC49ΔC\textsubscript{H}\textsubscript{1} and cCC49. The %ID/g values in normal tissues of iodinated cCC49ΔC\textsubscript{H}\textsubscript{1} and cCC49 were similar to those %ID/g values obtained when cCC49ΔC\textsubscript{H}\textsubscript{1} and cCC49 were 177Lu labeled. As stated above, when iodinated, cCC49ΔC\textsubscript{H}\textsubscript{2} had very low %ID/g values in normal tissues, but when labeled with the lutetium chelate, cCC49ΔC\textsubscript{H}\textsubscript{2} had fairly high %ID/g values in normal tissues. This change in the %ID/g values of 177Lu-labeled cCC49ΔC\textsubscript{H}\textsubscript{2} versus 131I-labeled cCC49ΔC\textsubscript{H}\textsubscript{2} may suggest a different metabolic pathway for cCC49ΔC\textsubscript{H}\textsubscript{2} than either cCC49ΔC\textsubscript{H}\textsubscript{1} or cCC49. Possibly, Fc receptors are able to clear cCC49ΔC\textsubscript{H}\textsubscript{1} and cCC49 via their Fc regions. However, because cCC49ΔC\textsubscript{H}\textsubscript{2} lacks a portion of its Fc region, it may have to be cleared by an alternative pathway in which glycosylation may be a factor also. It is important to point out that the studies described here with 177Lu-labeled cMAb were conducted in tumor-bearing mice in which the TAG-72 antigen is shed into the serum. We have shown previously (27) that the presence of a circulating antigen in mice will alter the biodistribution and metabolic patterns of radiolabeled MAb to increase liver and spleen uptake. Future studies also should be conducted in mice free of tumors and bearing small tumors using both iodine-labeled and heavy metal-chelated MAb.

Size does not seem to be the sole determinant for the more rapid clearance of the cCC49ΔC\textsubscript{H}\textsubscript{2}. In a report published by Milenic et al. (14), 99% of the ID/g F(ab')\textsubscript{2} of murine CC49 had cleared the mouse plasma by 24 h, and more than 99.9% had been cleared by 72 h. Although cCC49ΔC\textsubscript{H}\textsubscript{2} has a larger apparent molecular weight than a F(ab')\textsubscript{2}, the work presented here shows that cCC49ΔC\textsubscript{H}\textsubscript{2} has a murine plasma clearance pattern similar to that of previously reported CC49 F(ab')\textsubscript{2}. And cCC49ΔC\textsubscript{H}\textsubscript{1}, which is similar in weight to cCC49ΔC\textsubscript{H}\textsubscript{2}, had a slower overall rate of clearance than cCC49ΔC\textsubscript{H}\textsubscript{2}. In fact, although cCC49ΔC\textsubscript{H}\textsubscript{1} has the C\textsubscript{H}\textsubscript{1} and C\textsubscript{L} domains deleted, cCC49ΔC\textsubscript{H}\textsubscript{1} showed no significant difference in its clearance pattern from that of cCC49. The plasma clearance studies were repeated, and although some variation was observed between each study, possibly due to normal variations in the murine model, similar results were obtained with all three cMAbs. Using domain mutant MAbs,
of greater benefit in therapeutic protocols using radiolabeled MAbs. cCC49@CH2 has a shorter plasma half-life, a lower %ID/g in normal tissues, and earlier and greater radiolocalization to tumors, as manifested by greater tumor:normal tissue ratios. cCC49@CH2 thus may be observed.

Cleared significantly faster from the plasma than did 125I-labeled I gG demonstrated similar rates of iv. plasma clearance in mice and in nonhuman primates (30). Thus, although altering the glycosylation pattern of a chimeric immunoglobulin may affect clearance, the complete ablation of an IgG glycosylation site may have a minimal effect on clearance. A hypothesis extended by Brambell et al. (41) and later modified by Waldmann and Strober (42) suggests that immunoglobulins are cleared by receptors to the Fc region, and the more rapid clearance of the Fab' and F(ab')2 fragments is related to the fact that the cellular receptors are bypassed, and the Abs are cleared possibly through cellular diffusion. In concordance with this theory, when small amounts of radiolabeled Ab were injected into mice, followed by a large amount of cold antibody, the amount of circulating radiolabeled Ab found in the plasma was reduced by approximately 40% in mice receiving both labeled and unlabeled versus mice receiving only radiolabeled MAb (43). Possibly, with the large amount of unlabeled MAb saturating cellular Fc receptors, the radiolabeled MAb is cleared quickly through an alternative mechanism. Possibly, when the CH2 region is removed from a MAb, the Fc receptors are bypassed altogether. However, the removal of the CH2 carbohydrate moiety or its alteration may serve only to shift the Fc receptors used to clear the MAb, as observed by Wright and Morrison (25).

In studies using either murine MAbs (36) or chimeric MAbs (30, 44), the murine plasma clearance was consistently faster than the monkey plasma clearance. Because the plasma clearance of MAbs in monkeys is better reflected by the plasma clearance of MAbs in mice than in plasma clearance studies in a human immunoglobulin in nonhuman primates may prove to be a clearer indicator of how an antibody will behave in patients (36, 45). Also, these cMAbs contain human constant regions, which may lead to greater immunogenicity in mice than in monkeys or humans, and an immune response against a cMAb may affect the plasma clearance. Our studies in nonhuman primates demonstrated that 131I-labeled cCC49ΔCH2 cleared significantly faster from the plasma than did 131I-labeled cCC49. In comparison with the significantly rapid rate of clearance for cCC49ΔCH2 in monkeys and mice, cCC49ΔCH1 had similar clearance rates to cCC49 in mice, with no significant difference observed.

The studies reported here demonstrate that cCC49ΔCH1 has a longer half-life than cCC49ΔCH2. In fact, cCC49ΔCH1 is similar in pharmacokinetic and tumor localization properties to intact cCC49. In contrast to cCC49ΔCH1 and cCC49, when labeled with iodine, cCC49ΔCH2 has a shorter plasma half-life, a lower %ID/g in normal tissues, and earlier and greater radiolocalization to tumors, as manifested by greater tumor:normal tissue ratios. cCC49ΔCH2 thus may be of greater benefit in therapeutic protocols using radiolabeled MAbs and in immunodiagnostic protocols using an iodine label in which the shorter half-life of cCC49ΔCH2 would lead to earlier and more efficient use of gamma scanning techniques or of the intraoperative gamma detecting probe for the detection of a patient’s tumor after cMAb infusion. Therefore, because of the unique properties observed for cCC49ΔCH2 versus the similar properties of cCC49ΔCH1 and cCC49, cCC49ΔCH2 may have potential in clinical protocols, which necessitates a cMAb with both rapid clearance and tumor targeting.

Acknowledgments

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Biological Properties of Chimeric Domain-deleted Anticarcinoma Immunoglobulins

Dale C. Slavin-Chiorini, Syed V. S. Kashmiri, Jeffrey Schlom, et al.


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