Radioimmunolocalization of Human Carcinoma Xenografts with B72.3 Second Generation Monoclonal Antibodies

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ABSTRACT

The B72.3 reactive antigen, TAG-72, has been purified and a series of second generation monoclonal antibodies (MAbs), designated CC (colon cancer), have been characterized by a range of in vitro immunological assays. Six CC MAbs (CC11, CC30, CC46, CC49, CC83, and CC92) were chosen for analyses of the in vivo binding to a human colon carcinoma xenograft. All 6 MAbs were previously shown to be distinct from B72.3 and each other by a series of reciprocal competition radioimmunoassays, and all were shown to have a $K_a$ higher than that of B72.3. In this study we demonstrate that all six CC MAbs evaluated are superior to B72.3 in an in vivo tumor targeting model, using human colon carcinoma (LS-174T) xenografts in athymic mice, in terms of both the percentage of the injected dose of radiolabeled MAb delivered per g of tumor and tumor:normal tissue ratios. Differences in the in vivo binding patterns and pharmacokinetics among the CC MAbs are also evaluated. Thus, in light of the fact that B72.3 has been shown to successfully target approximately 75% of primary and metastatic carcinoma lesions in a variety of different carcinoma types in over 300 patients, these studies serve as further evidence to support the clinical evaluation of the second generation CC MAbs, either alone or in combination with B72.3.

INTRODUCTION

Several MAbs2 have been used in the radiolocalization of tumors in carcinoma patients (1-10). Some of the variables being evaluated in these studies include: route of MAb administration; dose and specific activity of the radiolabeled MAb; radionuclide and conjugate; form of the MAb (whole IgG or fragment); species of MAb; and MAb specificity and binding properties.

MAb B72.3 murine IgG, (11-13) has now been administered to over 300 patients with a variety of carcinomas (6-10). B72.3 has been shown to react with a high molecular weight glycoprotein, with properties of a mucin, termed TAG-72 (14). Both primary and metastatic lesions in patients with colorectal cancer and ovarian cancer have been localized by B72.3 without any associated toxicity; tumors of selected patients with breast, gastric, pancreatic, and lung carcinomas have also been localized.3 Administration of $^{131}$I-B72.3 IgG i.v. in colorectal cancer patients has shown localization of the radiolabeled MAb in approximately 75% of the metastatic lesions found at various body sites (6, 7, 9). Localization of the MAb in tumor tissue as measured by RI (% injected dose/g of tumor divided by the % injected dose/g of normal tissue) showed RI values of from 3 to over 30 for over 70% of the tumor lesions that were surgically biopsied (6, 7).

An intraoperative hand held gamma detecting probe has been used in patients that have been previously given $^{131}$I-B72.3 i.v. (9). These studies have shown the localization of B72.3 in approximately 80% of carcinoma lesions. Furthermore, in approximately 20% of patients, occult tumor was detected by the gamma detecting probe at surgery.

Recent studies using i.p. administered $^{131}$I-B72.3 have shown good localization of peritoneal implants with RI values of as high as 60 (8). In 3 of the first 10 patients studied the MAb gamma scan localized tumor (confirmed at surgery) that was not detected by conventional techniques including computer-assisted tomographic scans. Dual label studies, using $^{131}$I-B72.3 administered i.p. and $^{131}$I-BT2.3 administered i.v., showed that i.v. administered MAb could localize peritoneal implants better than i.v. administered MAb, while i.v. administration of labeled MAb was better than i.p. administration for the detection of hemato-genously borne metastases (8).

In view of the potential utility of B72.3 in diagnostic and potentially therapeutic applications, we have prepared a series of second generation anti-TAG-72 MAbs (15). These second generation MAbs have been given a CC designation (for colon cancer) since the TAG-72 used as immunogen was purified from a colon cancer xenograft. They have all been shown to be distinct from each other and from B72.3 and all have $K_a$ values higher than that of B72.3. Their range of reactivity for various human carcinomas versus normal tissue has been shown to be similar or superior to B72.3 using a variety of in vitro immunological assays (15).

MATERIALS AND METHODS

Generation and Source of Monoclonal Antibodies. The CC MAbs, CC83, CC49, CC46, CC92, CC11, and CC30, were developed by the immunization of mice with purified TAG-72 as described previously (15). Purified TAG-72 was used to immunize 4-week-old BALB/c mice over an extended period of time and the spleen cells were fused with NS-1 cells to generate hybridomas. Selected wells were chosen for further study and the hybridomas were cloned twice by limiting dilution. The monoclonal antibodies were extensively characterized and their reactivity to colon carcinomas with minimal reactivity to normal human adult tissues has been described (15). Monoclonal antibodies B72.3, COL-3, and COL-12 were generated using breast and colon carcinomas as immunogen as described previously (11, 16). Ascitic fluid containing the various MAbs were generated by the i.p. inoculation of approximately 1 x 106 hybridoma cells into BALB/c mice that were previously primed with pristane. The ascitic fluid was harvested and clarified at10,000 x g for 10 min before storage at −20°C.

Purification of MAb IgG. MAb IgG was purified from ascitic fluid by ammonium sulfate precipitation followed by ion-exchange chromatography using DEAE, either DE-52 (Whatman, Clifton, NJ) or DEAE-5PW (Waters, Milford, MA), or a Bakerbond AXB column (J. T. Baker Chemical Co., Philipsburg, NJ). The antibodies were eluted by a 10 to 150 mm sodium phosphate (pH 7.5) gradient, a gradient of 20 mm Tris-HCl (pH 8.5) to 300 mm NaCl in 20 mm Tris-HCl (pH 7.0), or a 25 mm 2-(N-morpholino)ethanesulfonate (pH 5.6) to 1 mm sodium acetate (pH 7.0) gradient for the 3 columns, respectively. Column fractions were analyzed by SDS-polyacrylamide gel electrophoresis and appropriate fractions, with purity of over 90% of the protein being IgG, were pooled and dialyzed against PBS. The concentration was determined by the method of Lowry et al. (17) using a BSA standard.
BIODISTRIBUTION OF ANTI-TAG-72 MONOCLONAL ANTIBODIES

Analysis of the Purified MABs. MAB purity was analyzed by electrophoresis in 5–20% gradient SDS-polyacrylamide gels with or without disruption with β-mercaptoethanol according to the method of Laemmli (18) using a stacking gel of 3% acrylamide. The proteins were stained with Coomassie Blue R-250. Radiolabeled antibodies were detected by autoradiography using Kodak (Rochester, NY) XAR X-ray film and DuPont (Wilmington, DE) Lightning-Plus intensifying screens at -70°C.

Samples were also analyzed by isoelectrofocusing on a pH 3.5–9.5 acrylamide gel (LKB, Bromma, Sweden). MABs (50 μg in 20 μl of distilled water) were applied to 5-×10-mm application pieces of filter paper and placed on 5% polyacrylamide gels (245 × 110 × 1 mm) containing 2.3% ampholines. The isoelectrofocusing was run for 2.5 h at 10 W resulting in approximately 50 mA and 1500 V at 10°C using an anode electrode solution of 1 m H3PO4 and a cathode solution of 1 m NaOH. The pH gradient was measured, and the gel was fixed for 30 min in 11.5% sulfosalicylic acid with 3.5% trichloroacetic acid and stained with Coomassie Blue R-250.

Radiolabeling of MABs. Purified CC MABs and B72.3 were labeled with Na125I or Na131I by the iodogen (Pierce, Rockford, IL) method (19). Forty μg of the purified IgG were adjusted to 100 mM sodium phosphate (pH 7.2) and added to a 12 × 75 mm glass test tube that had been coated previously with 20 μg of iodogen. Two min after the addition of 0.5 mCi of Na125I or Na131I (DuPont-NEC, Boston, MA) the plates were removed from the tube and the unincorporated Na125I was separated by gel filtration through a 10-ml column of Sephadex G-25 (Pharmacia, Piscataway, NJ). The labeled antibody in the void of the column was pooled and diluted with PBS for in vitro studies or PBS containing 1% BSA for in vitro studies. The iodination protocol resulted in 125I- and 131I-labeled MAB of approximately 5–10 μCi/μg with approximately 50% of the input iodine bound to protein.

Cell Lines and Tumor Growth. The LS-174T human colon carcinoma cell line (20) was obtained from Dr. P. Noguchi (Bureau of Biologics, FDA, Bethesda, MD) and was routinely grown in minimum essential medium supplemented with 1% L-glutamine, 1% nonessential amino acids, 50 μg/ml gentamicin, and 10% heat-inactivated fetal bovine serum. The A375 human melanoma cell line was obtained from Dr. S. Aaronson (National Cancer Institute, Bethesda, MD) and was grown in Dulbecco's modified Eagle’s medium with the same supplements as the LS-174T cell line. The cell lines were routinely subpassaged weekly at a 1:5 or 1:10 split ratio. Cells were removed from the culture flasks with 0.1% trypsin containing 0.5 mM EDTA and washed twice in growth medium without serum prior to inoculation into mice.

Female athymic mice (nu/nu) were obtained from Charles River, Inc. (Wilmington, MA) at approximately 4 weeks of age. One week later the mice were given s.c. injections on the back of 1-4 × 106 LS-174T or A375 cells (0.1 ml). Approximately 2 weeks later, when the tumor were 400–700 mg (approximately 0.5 cm in diameter), the mice were used for the biodistribution studies. Approximately 5 μCi of iodinated MAB IgG were injected i.p. and the mice were sacrificed at various times. Tumors and selected normal tissues were removed and weighed. The percentage of the injected dose per g was determined and RIs were calculated (%ID/g in the tumor divided by the %ID/g in the tissue).

Solid-Phase Radioimmunoassays. Radiolabeled CC and B72.3 MABs were tested for the retention of their immunoreactivity using extracts that contain the TAG-72 antigen that they were generated against. The assays were run as previously described (21) with minor modifications. Extracts from human colon carcinoma xenografts (LS-174T, TAG-72 positive) and human melanoma xenografts (A375, TAG-72 negative) were generated as described previously (14). Twenty μg (in 50 μl of PBS) of the tumor extracts were added to each well of 96-well polystyrene microtiter plates and allowed to dry overnight at 37°C. The plates were then treated with 100 μl of 5% BSA in PBS for 1 h at 37°C to minimize nonspecific absorption and the plates were then stored at -20°C until used. The plates were washed with 1% BSA in PBS and varying amounts of radiolabeled antibody were added in 50 μl of 1% BSA in PBS. After overnight incubation at 4°C, the unbound antibody was removed by washing with 1% BSA in PBS and the bound radioactivity was detected by counting the individual wells in a gamma counter.

All the radiolabeled MABs bound to the LS-174T extracts (191-
MABs: B72.3, 44.5%; CC83, 64.0%; CC49, 64.0%; CC46, 60.5%; CC92, 52.5%; CC11, 43.4%; CC30, 47.9%) with less than 2% binding to the TAG-72 negative A375 tumor extract. While the assays were routinely performed at 4°C overnight several of the CC MABs, e.g., CC49, demonstrated significantly better binding after limited incubations than did B72.3. 125I-CC49 IgG was studied in the solid-phase RIA for 1 h at 37°C in addition to the overnight incubation at 4°C. Similar extents of binding to the TAG-72 positive LS-174T tumor xenograft extract were observed with the CC49 MAB; B72.3 IgG, however, binds after 1 h at 37°C only one-fourth to one-half the extent that can be seen after overnight incubation at 4°C. The high extent of binding of some of the CC MABs after limited incubations may be a function of the high affinity constants of these MABs (15).

Competition Radioimmunoassay. Antibody samples were serially diluted in 1% BSA in PBS and 25 μl were added to plates, containing 1 μg/well of the LS-174T xenograft extract, that were prepared as described above. Following a 6-h incubation at 4°C, 25 μl of 125I-CC49 IgG (50,000 cpm) were added per well and the assay plate incubated at 4°C for 18 h. The plates were then washed and counted as described above and the percentage of inhibition, compared to a buffer control, was determined.

Fragmentation of CC MABs. F(ab’2) fragments of selected CC MABs were generated by the digestion of the purified IgG using pepsin (22). Ten μg of IgG were adj usted to 100 mM sodium acetate (pH 4.5) and 100 μg of pepsin were then added. After incubation at 37°C for 16 h, the sample was adjusted to pH 8.0 with Tris and concentrated to 1.5 ml. The F(ab’2) fragments were purified by size exclusion chromatography using Sephacyrl S-200 (Pharmacia, Piscataway, NJ) run in 200 mM NaCl and 3 mM EDTA in 10 mM Tris-HCl (pH 8.0).

RESULTS

Purification, Characterization, and Iodination of CC MABs. The CC MABs and B72.3 were purified as described in “Materials and Methods” using a combination of differential salt precipitation and ion-exchange chromatography. The purified IgGs were subjected to electrophoresis on 5–20% SDS-polyacrylamide gradient gels (Fig. 1A). Analysis of the gels demonstrated that over 90% of the stained protein on the gel was associated with the heavy and light chains of the IgG molecule. Differences can be seen in the migration of both the heavy and light chains of the CC MABs with the CC30 heavy chain and the CC83 light chain migrating with a notably lower mobility than the other CC MABs and light chains. The purified IgGs were also subjected to isoelectrofocusing on a pH 3.5–9.5 acrylamide gel (Fig. 1B). All the CC MABs exhibited limited heterogeneity with 2 or 3 major bands with plS of 6.5–6.7. The limited distribution of the plS of the CC MABs may be a function of the TAG-72 antigen that they all recognize; the pl of B72.3 IgG, the MAB that first identified the TAG-72 antigen, is also approximately 6.5 (21). This is in contrast to the plS observed with several of the control MABs we have generated against the carcinoembryonic antigen molecule (Fig. 1B).

The CC MABs were iodinated using the iodogen method (19) as described in “Materials and Methods.” Forty μg of IgG were reacted with 0.5 mCi of Na125I in the presence of 20 μg of iodogen for 2 min and the IgG was purified by size exclusion chromatography. The iodinated MABs were subjected to SDS-polyacrylamide electrophoresis both with and without disruption with β-mercaptoethanol. As can be seen in Fig. 2, virtually all the radioactivity is associated with the IgG molecule distributed on both the heavy and light chains. The iodinated CC MABs were also tested in solid-phase RIAs against an extract of a tumor xenograft of a human colon carcinoma cell line (LS-174T) that contains the TAG-72 antigen and an extract of a human melanoma xenograft (A375) that lacks the reactive antigen. The CC MABs were shown to maintain their immunoreactivity after iodination.
Biodistribution of Anti-TAG-72 Monoclonal Antibodies

Fig. 1. SDS-polyacrylamide gel electrophoresis and isoelectrofocusing of CC MAbs. CC MAbs were subjected to SDS-polyacrylamide gel electrophoresis analysis on a 5–20% gradient gel and stained using Coomassie Blue R-250 (A). Samples were run in the presence of β-mercaptoethanol, markers (M), conalbumin, M, 87,000; BSA, M, 68,000; ovalbumin, M, 47,000; lactate dehydrogenase, M, 36,000; carbonic anhydrase, M, 28,000; myoglobin, M, 17,000; and cytochrome c, M, 11,700; CC30 (Lane 1), CC46 (Lane 2), CC49 (Lane 3), CC83 (Lane 4), and CC92 (Lane 5). CC MAbs were also analyzed by isoelectrofocusing (B) using a pH 3.5–9.5 gradient acrylamide gel; CC30 (Lane 1), CC46 (Lane 2), CC49 (Lane 3), CC83 (Lane 4), CC11 (Lane 2), CC30 (Lane 3), CC46 (Lane 4), CC49 (Lane 5), CC83 (Lane 6), and CC92 (Lane 7).

In Vivo Comparison of CC MAbs to B72.3. Dual label studies were performed to investigate the relative efficiency of the CC MAbs to localize human tumor xenografts in comparison to B72.3 IgG. B72.3 IgG has been used extensively in both model systems (21, 23–25) and clinical studies (6–10) and has been shown to be highly effective in localizing carcinoma lesions. Previous studies have shown a wide variation in the biodistribution of radiolabeled B72.3 IgG. Localization of iodinated B72.3 in LS-174T tumor xenografts ranged from 4.5% ID/g (21) to 26.8% ID/g (23); with other radionuclides even higher %ID/g in the LS-174T xenografts were observed. Similar differences in tumor uptake of the radiolabeled MAbs were observed in another model system using MAb B6.2 and a human mammary carcinoma xenograft (Clouser) where tumor:blood ratios varied from 1.2:1 to over 14:1 (26). Differences were also observed in the pharmacokinetics of radioiodinated B72.3 IgG where blood levels at 24 h of the iodinated MAb ranged from 9.9%ID/g (21) to 20.0%ID/g (27). In order to minimize the variability in the tumor uptake and the pharmacokinetics of the radiolabeled MAbs dual label studies, using B72.3 and the CC MAbs, were performed. MAbs CC83, CC49, CC46, CC92, CC11, and CC30 IgGs were radiolabeled with 125I and individually mixed with 131I-labeled B72.3 IgG (at the same dose and specific activity) and injected into mice bearing s.c. LS-174T xenografts. After 5 days the mice were sacrificed and the %ID/g in tumor and normal tissues of the two radionuclides was determined. As can be seen in Table 1, the %ID/g of the CC MAbs in the human colon carcinoma xenograft (3 mice/group) was 1.4 to 5.4 times greater than that observed with the co-injected B72.3 IgG. With CC83, CC49, and CC92, moreover, significantly lower levels of activity were found in the normal organs when compared to that found with the B72.3 IgG. RIs were determined. As can be seen in Table 2, the higher %ID/g in the tumor of the CC MAbs coupled with the lower levels of activity found in the normal organs with CC83, CC49, and CC92 resulted in much higher RIs with the CC MAbs as compared to B72.3. RIs for tumor versus many normal organs of over 15 were observed with all of the CC MAbs, while RIs of greater than 30 were obtained with CC83, CC49, and CC46. CC49 gave the highest tumor:blood ratio, with an RI of 18, while CC83 and CC46 exhibited the highest tumor:liver, tumor:spleen, and tumor:kidney ratios.

Specificity of the in Vivo Radioimmunolocalization. The CC MAbs tested have exhibited an increased binding to the I.S174T human colon carcinoma xenograft as compared to B72.3.

Table 1 Coadministration of 131I-CC MAbs and 125I-B72.3 in mice bearing human colon carcinoma xenografts: comparison of %ID/g

| Tissue | CC83 | B72.3 | CC49 | B72.3 | CC46 | B72.3 | CC92 | B72.3 | CC11 | B72.3 | CC30 | B72.3 | CC46 | B72.3 | CC92 | B72.3 | CC83 | B72.3 | CC49 | B72.3 | CC46 | B72.3 | CC92 | B72.3 | CC11 | B72.3 | CC30 | B72.3 |
|--------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|
| Tumor  | 9.89 | 5.38  | 1.84 | 9.94  | 5.18 | 1.92  | 17.57 | 3.28  | 5.35  | 6.66  | 4.92  | 1.35  | 20.08 | 5.10  | 3.94  | 16.17 | 4.86  | 3.33  |
| Blood  | 1.21 | 5.01  | 0.24 | 5.29  | 0.12 | 2.72  | 2.75  | 0.99  | 2.57  | 1.40  | 3.98  | 0.35  | 6.41  | 4.74  | 1.35  | 4.27  | 4.90  | 0.87  |
| Liver  | 0.41 | 1.53  | 0.26 | 4.36  | 1.40 | 3.12  | 0.66  | 0.69  | 0.95  | 0.46  | 1.11  | 0.41  | 1.72  | 1.28  | 1.34  | 1.01  | 1.14  | 0.88  |
| Spleen | 0.38 | 1.14  | 0.34 | 1.10  | 0.94 | 0.61  | 0.54  | 1.14  | 1.29  | 0.90  | 1.44  | 1.36  | 1.02  | 1.34  | 0.93  | 1.05  | 0.89  |
| Kidney | 0.29 | 1.01  | 0.29 | 0.34  | 1.05 | 0.32  | 0.49  | 0.55  | 0.88  | 0.49  | 0.12  | 0.48  | 1.23  | 0.92  | 1.33  | 0.81  | 0.93  | 0.87  |
| Lung   | 0.77 | 2.70  | 0.29 | 0.42  | 2.41 | 0.17  | 1.33  | 1.23  | 1.08  | 0.88  | 1.99  | 0.44  | 3.67  | 2.57  | 1.43  | 2.32  | 2.47  | 0.94  |
The binding of the B72.3 IgG to the LS-174T tumor has been shown to be specific in that there was no localization in the tumors using a control myeloma IgG of the same isotype (21). The LS-174T tumor therefore does not act as a "sink" for murine IgG1. It was still necessary, however, to demonstrate that the CC MAbs are binding specifically to tumors that contain the TAG-72 antigen such as the LS-174T colon carcinoma. Radiolabeled CC83, CC49, CC46, CC92, CC11, and CC30 IgGs were injected into mice bearing either the LS-174T tumor or a human melanoma xenograft (A375) that does not contain TAG-72. After 7 days the mice were sacrificed and the RIs of various tissues were determined (Table 3). All the CC MAbs exhibited RIs of greater than 10 for the major organs of mice bearing the LS-174T xenograft while CC83, CC49, CC46, and CC30 had RIs of over 30 for several of the tissues examined. RIs of the major organs of mice bearing the A375 melanoma xenograft were less than 3.0 (and in many cases <1.0) in all the tissues tested. This demonstrates that the localization of the CC MAbs in the LS-174T xenografts were specific for the tumors that contain the TAG-72 antigen and that the localization in the tumors was mediated by the binding sites of the CC MAbs.

Pharmacokinetics in Mice Bearing LS-174T Xenografts. Mice bearing the LS-174T xenograft were given i.p. injections of 125I-labeled CC MAbs and sacrificed at daily intervals for 7 days to examine the pharmacokinetics of the injected antibodies. Various tissues were removed and the %ID/g was determined; selected time points from these experiments are given in Table 4. The highest %ID/g in tumors was found with CC83 and CC49 with over 20 %ID/g at all the time points from 24 to 168 h. The amount of the CC MAbs found in the tumors rose over the first 48–72 h and then remained relatively constant over the 7-day period studied. Blood levels of the CC MAbs were approximately 10–18 %ID/g at 24 h and dropped to approximately 1–6 %ID/g at 7 days. The antibody levels found in the major organs also dropped at a similar rate with generally less than 1 %ID/g remaining in the organs at 7 days. Whole body clearance studies were performed with CC49 and CC92; these MAbs demonstrated a t½ of 4 to 5 days, which is similar to that obtained with B72.3 IgG (21, 23).

Radio localization indices of the major organs were determined for the CC MAbs (Fig. 3). The RIs of all the CC MAbs rose over the 7-day period studied. CC83 and CC49 exhibited the highest RIs, with approximately 60 times more radioactivity found in the tumor as compared to normal kidney at 7 days postinjection of the MAbs. CC46 and CC30 had RIs of greater than 25 for tumor: liver, tumor: spleen, and tumor: kidney at 7 days, while CC11 and CC92 had RIs of greater than 12 for the major organs. CC49 exhibited the highest RIs values for tumor: blood which rose over the 7-day period, reaching a level of 20 times more radioactivity in the tumor as compared to blood. The low levels of circulating CC49 IgG resulted in high RIs for the kidney and liver, while the tumor to liver and spleen ratios were still approximately 20:1.

Table 2 Comparison of radiolocalization indices in mice bearing human colon carcinoma xenografts: coadministration of 125I-CC MAb and 131I-B72.3

<table>
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<tr>
<th>Tissue</th>
<th>CC83</th>
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<th>CC46</th>
<th>CC92</th>
<th>CC11</th>
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<td>7.94</td>
<td>18.01</td>
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<td>Lung</td>
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Table 3 Specificity of localization of CC MAbs to TAG-72 positive LS-174T colon carcinoma versus TAG-72 negative A375 melanoma: radiolocalization indices

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Table 4 Pharmacokinetics of biodistribution of CC MAbs in mice bearing human colon carcinoma xenografts

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<td>6.79</td>
<td>1.40</td>
<td>0.59</td>
</tr>
</tbody>
</table>

% injected dose/g

Fig. 3. Pharmacokinetics of 125I-labeled CC MAbs. Athymic mice bearing LS-174T tumors were given i.p. injections of approximately 5 μCi of 125I-labeled CC MAbs. Mice (3/group) were sacrificed at 24 h (A), 48 h (B), 72 h (C), 96 h (D), and 168 h (E). Radiolocalization indices were determined.

Fig. 4. Radioimmunoimaging of athymic mice bearing human xenografts. Mice bearing LS-174T colon carcinoma xenografts (A to C), TAG-72 positive, or A375 melanoma xenografts (D to F), TAG-72 negative, were given injections of approximately 10 μCi of 125I-labeled MAb CC49 IgG. Scans were performed at Day 1 (A, D), Day 4 (B, E), and Day 6 (C, F) post-i.p. administration of the MAb.

Fig. 5. Effect of tumor size and MAb specific activity. It has been suggested that monoclonal antibodies may localize in small lesions better than larger ones. CC49 IgG was administered to mice bearing LS-174T colon carcinoma xenografts of 40–100 mg (approximately 0.2 cm in diameter), and the mice were sacrificed 48 h later (Table 6). Approximately 95%ID/g of the 125I-CC49 IgG was found in the tumors as compared to 35%ID/g in the 400–700 mg tumors (approximately 0.5 cm in diameter) usually used in our studies. The antibody levels in the blood, spleen, kidney, and lung were independent of tumor size. Lower levels of 125I-CC49 were found in the liver of mice bearing the small LS-174T tumors as compared to larger (>400 mg) tumors. Whether this is due to an impairment of liver function or the release of antigen into the circulation, with deposition in the liver, from the larger tumors is currently under investigation.

Studies were performed to determine at which point the binding of the radiolabeled CC49 to the LS-174T colon carcinomas could be affected by the addition of unlabeled antibody. 125I-CC49 IgG was diluted with unlabeled antibody and injected into mice bearing small (40–100 mg) LS-174T tumors. Approximately 250–500 μg of 125I-CC49 was needed to reduce the amount of radiolabeled antibody to one-half the level obtained without the carrier IgG (Table 6). Even when 1000 μg of unlabeled CC49 IgG were added, however, 24%ID/g was still found in the tumor. Thus, LS-174T tumors of 100 mg in weight could bind 24% of the injected dose; since 1000 μg were
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Fig. 5. SDS-polyacrylamide gel electrophoresis analysis of CC MAb F(ab')2 fragments. Selected CC MAb antibodies were subjected to pepsin digestion and analyzed by SDS-polyacrylamide gel electrophoresis in the presence (A) and absence (B) of β-mercaptoethanol on a 5–20% gradient gel. Markers (M) as in Fig. 1; CC46 (Lane 1), CC49 (Lane 2), and CC92 (Lane 3).

Fig. 6. Immunoreactivity of CC49 F(ab')2 fragment. CC 49 F(ab')2 fragments were tested in a competition radioimmunoassay against 125I-CC49 IgG. The CC49 F(ab')2 fragment (O) was compared on a molar basis to the intact CC49 IgG standard (•) as described in “Materials and Methods.”

DISCUSSION

In a previous study, we have shown that all the CC MAbs described here were distinct from B72.3 in the epitope on the TAG-72 molecule that they bound. These differences were shown by a series of reciprocal competition RIAs. Moreover, the CC MAbs all had higher Ks values than B72.3 did (15). The fact that the CC MAbs all localized human tumor xenografts better than B72.3 did may be due to one or a combination of the following: (a) higher Ks; (b) quantity of epitope recognized on the tumor; (c) accessibility of the epitope on the tumor cell surface (which may be different from accessibility in a tumor extract or the purified antigen).

Another factor that may affect the biodistribution of a MAb resulting in differences in RI or its %ID/g is the rate of clearance of the MAb from the plasma. The CC MAbs demonstrated several different clearance patterns (Table 4). One fairly unique pattern was that of MAb CC49. The pharmacokinetics of B72.3 plasma clearance and tumor binding has been detailed previously (21, 23) and 5-day postinjection values are shown in Table 1 for comparative purposes. As seen in Table 4, however, the plasma clearance of CC49 is much faster than that of B72.3 and the five other CC MAbs analyzed. This resulted in much higher tumor: blood ratios for CC49 than for the other MAbs. Another potential consequence of this phenomenon is that the tumor: normal tissue ratios (RIs) of CC49 were different from that of B72.3 and the other CC MAbs analyzed. This resulted in the following ranking of RIs: tumor: liver, tumor: spleen and tumor: kidney showed the highest RIs, then tumor: lung and lastly tumor: blood. For CC49, however, the order of the RIs (from high to low) was tumor: kidney, tumor: lung, tumor: blood, tumor: spleen and tumor: liver. One possible explanation for this phenomenon is that the CC49 recognized an epitope of TAG-72 which is highly accessible when the TAG-72 is found in circulation, and the resulting MAb-TAG-72 complexes deposit in the liver and spleen, thus lowering the RIs for these organs and the MAb, therefore clearing the plasma rapidly. Further evidence for this is found in the observation that when CC49 is administered to mice with small tumors the ranking of the RI values, notably tumor: spleen and tumor: liver, of the tissues is similar to that observed for B72.3 and the other CC MAbs. Note, however, that for the small tumors (Table 6) one can observe a 95%ID/g with CC49. Regardless of this phenomenon and the resulting ranking of RIs for tumor to various normal organs, the rapid plasma clearance of the CC49 resulted in CC49 demonstrating among the highest RI values for the CC MAbs (Fig. 3).
The potential clinical utility of the CC MAbs for in vivo tumor targeting for either diagnostic or therapeutic applications is supported by the studies reported here, in that both the %ID/g of tumor and the tumor:normal tissue RI values of the second generation CC MAbs are superior to that of B72.3. It is important to point out that this is not a matter of chance occurrence, but the result of the evaluation of thousands of hybridomas derived from immunizations with purified TAG-72 antigen (15). In view of the favorable tumor targeting seen with B72.3 in several hundred patients (6–10), one is now in a position to evaluate the use of one or several of the CC MAbs in conjunction with B72.3, or as a substitute for B72.3, in a range of tumor targeting clinical applications.

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Radioimmunolocalization of Human Carcinoma Xenografts with B72.3 Second Generation Monoclonal Antibodies

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