Comparative Binding and Preclinical Localization and Therapy Studies with Radiolabeled Human Chimeric and Murine 17-1A Monoclonal Antibodies

Donald J. Buchsbaum, Pamela G. Brubaker, David E. Hanna, Arthur A. Glatfelter, Valeri H. Terry, Dianne M. Guilbault, and Zenon Steplewski


Abstract

Murine MoAb 17-1A is an IgG2a antibody reactive with a gastrointestinal cancer-associated cell surface antigen. Human-mouse chimeric 17-1A MoAbs were constructed in which the murine variable region of 17-1A was joined with human IgG1, IgG2, IgG3, and IgG4 constant regions. Human-mouse IgG1, IgG2, and IgG4 chimeric antibodies were compared with the parental murine antibody and its F(ab')2 fragments for their ability to bind to colon carcinoma cells in vitro, for their blood clearance in normal nude mice, and for their localization and tumor growth inhibition of colon carcinoma xenografts in nude mice. Indirect immunofluorescence experiments with fluorescein-conjugated goat anti-mouse or goat anti-human antibody verified that the substitution of human constant regions in the chimeric MoAbs did not significantly alter the ability of the murine variable region to bind to colon adenocarcinoma cell lines (LS174T, SW948, and C0112). The immunoreactivities of 125I-labeled murine and chimeric 17-1A MoAbs measured in a live cell-binding assay with LS174T, SW948, and C0112 cells revealed that chimeric IgG1, IgG4, and 17-1A F(ab')2 were comparable to murine 17-1A while chimeric IgG2 showed lower binding. The blood half-lives of 125I-labeled murine 17-1A, its F(ab')2 fragments, and chimeric IgG1, IgG2, and IgG4 in normal nude mice determined by serial eye bleeding were 7.5, 0.5, 5.2, 6.9, and 1.9 days, respectively. In biodistribution studies at 4 days after injection of 125I-labeled MoAbs in nude mice bearing LS174T tumors, chimeric IgG1 had the highest tumor concentration of 20.5% injected dose/g with a tumor/blood ratio of 3.2. 131I-labeled murine 17-1A administered in a single injection of 300 µCi or 3 injections of about 300 µCi each to nude mice bearing established LS174T tumors inhibited tumor growth, whereas a comparable amount of unlabeled murine 17-1A did not inhibit tumor growth. 131I-labeled chimeric IgG1 MoAb showed a similar level of tumor growth inhibition. The results of the present study indicate that 17-1A chimeric IgG1 antibody may be the best choice for clinical radioimmunodetection and radioimmunotherapy studies.

Introduction

Murine MoAb 17-1A is an IgG2a antibody reactive with a M, 30,000-41,000 gastrointestinal cancer-associated cell surface antigen expressed on colon, gastric, and pancreatic carcinoma (1–3). Murine 17-1A is active in in vitro ADCC assays with murine (2, 4–7) and human (5–8) effector cells. This MoAb has been shown to inhibit the growth of human colon carcinoma xenografts in nude mice with multiple injections starting immediately after tumor cell inoculation (6, 7, 9, 10). In patients with colon and pancreatic adenocarcinoma treated with unlabeled murine 17-1A, complete and partial remissions have been described (11–17). However, murine MoAbs administered to humans have a short blood half-life (12–18 h) and induce a HAMA response (11–14, 17–19) leading to more rapid clearance of subsequent injections of the MoAb that may reduce the therapeutic effects of MoAb and can lead to anaphylactic reactions. Antibody-dependent antibodies directed against the antigen- combining site of murine 17-1A MoAb have also been detected in cancer patients treated with this antibody (20). One approach to avoid these problems would be the use of human MoAbs. Although some human MoAbs have been produced that are reactive with gastrointestinal cancers (21), they have been of the IgM subclass and have shown limited tumor localization (22, 23). However, in a Phase I clinical trial up to 100 mg of human MoAb could be administered weekly for up to 4 weeks without elicitation of an antiallotypic or antiidiotype response (23).

Another approach that has been taken to avoid the HAMA response has been to produce human-mouse chimeric antibodies by recombinant DNA techniques composed of the antigen-binding variable region from a murine MoAb and the heavy and light chain constant regions of human immunoglobulins (24–26). Chimeric 17-1A MoAbs of the IgG1, IgG2, IgG3, and IgG4 subclasses have been constructed (7, 27, 28), and their characteristics and activity in ADCC assays (7, 28–31), xenogeneic tumor growth inhibition (7), and clearance from patients (32) have been described.

Murine 17-1A and its F(ab')2 fragments have been radiola- beled with 125I, 125I, 121I, and 111In and have been shown to localize human colon carcinoma xenografts in nude mice (33–38). Radioimmunotherapy studies in nude mice bearing human colon carcinoma xenografts have been conducted with 131I-labeled murine MoAbs (39–43). 131I-labeled 17-1A has been reported to successfully localize primary and metastatic colorectal cancer in patients with carcinoma of the colon and rectum (44–49).

The goals of the present study were to demonstrate that 131I-labeled murine 17-1A MoAb has therapeutic utility in a nude mouse human colon cancer xenograft model and to determine which of the radiolabeled chimeric 17-1A MoAbs might be most useful in future clinical radioimmunodetection and radioimmunotherapy studies based on the results of in vitro tumor binding, animal localization, and tumor growth inhibition studies.

Materials and Methods

Monoclonal Antibodies. Intact murine MoAb 17-1A and F(ab')2 fragments of this antibody were provided in purified form at concentrations of 9.3 and 10.6 mg/ml, respectively, by Centocor (Malvern, PA). Chimeric IgG1, IgG2a, and IgG4s antibodies were also provided in purified form by Centocor at concentrations of 1.5, 1.7, and 0.9 mg/ml, respectively. Murine MoAb H24B5, an IgG2a antibody that recognizes influenza virus, and T101, a murine IgG2a MoAb that binds to human T-cells, were used as control antibodies.

Radiolabeling and Characterization of Labeled Antibodies. Iodination
and characterization of radiolabeled MoAbs were performed by standard techniques. Purified murine and chimeric 17-1A antibody preparations were labeled with $^{125}$I (Amersham Corp., Arlington Heights, IL) or $^{131}$I (ICN Biomedical, Inc., Irvine, CA) by the ICI method of Buchsbaum et al. (50). Free iodine was removed by passage of the labeled preparations over a PD-10 column (Pharmacia Fine Chemicals, Piscataway, NJ). Labeling efficacy was determined as the amount of radioactive iodine incorporated into the recovered product as compared to the amount of radioactive iodine added to the reaction mixture. Specific activity is expressed as a ratio of mCi of radionuclide attached per mg of antibody in the final product. High pressure liquid chromatography and gel electrophoresis were used to analyze the radiolabeled MoAbs as described elsewhere (38).

Cell Lines. The LS174T human colon adenocarcinoma cell line was obtained from the American Type Culture Collection (Rockville, MD). The SW948 human adenocarcinoma cell line was obtained from Dr. Kenneth Foon (Roswell Park Memorial Institute, Buffalo, NY). The C0112 human colon carcinoma cell line (S1) was provided by Dr. Jean-Pierre Mach (University of Lausanne, Epalinges, Switzerland). The adherent human melanoma cell line UMCC-MEL-1, obtained from Dr. Thomas Carey at the University of Michigan (Ann Arbor, MI), was used as a control cell line. The colon carcinoma and melanoma cell lines were grown as monolayers in T75 flasks (Corning, Corning, NY) with Eagle’s minimal essential medium with Earle’s salts, 2 mM l-glutamine, and nonessential amino acids (Whittaker M. A. Bioproducts, Walkersville, MD) supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT). Serial passaging and harvesting for injection into nude mice were accomplished with trypsin-Versene (Whittaker M. A. Bioproducts) for C0112 and LS174T cells or EDTA-PBS for SW948 and MEL-1 cells. Adherent cell lines were detached with EDTA/PBS prior to binding assays. The human T-cell acute lymphoblastic leukemia cell line CEM was obtained from the American Type Culture Collection. The cells were maintained as a suspension culture in T75-flasks in RPMI 1640 (Whittaker M. A. Bioproducts) supplemented with 2 mM l-glutamine and 10% fetal bovine serum.

Indirect Immunofluorescence. Reactivity of the intact murine 17-1A MoAb and F(ab')2 fragments and the intact chimeric MoAbs with LS174T, SW948, and C0112 human colon carcinoma cell lines and the MEL-1 human melanoma cell line was determined by flow cytometry analysis. Cells were harvested, counted, and adjusted to $10^5$ viable cells/ml in DPBS containing 2% (v/v) bovine serum albumin (Sigma Chemical Co., St. Louis, MO) and 0.02% sodium azide (Aldrich, Milwaukee, WI). One hundred $\mu$l of cell suspension were added per well of a V-bottomed 96-well plate (Costar, Cambridge, MA). One hundred $\mu$l of antibody (5 $\mu$g/ml final concentration) were added to cells and they were incubated at 4°C for 1 h. Cells were washed once with 0.5 ml RPMI 1640 containing 10% fetal bovine serum, the tubes were recounted to determine the percentage of radiolabeled antibody bound to the cells. The percentage binding was calculated by dividing the amount of radioactivity above background bound after washing by the amount added to the tubes less background bound x 100.

Mice. Athymic nude female CD-1 or NIH Swiss mice, 5-8 weeks old, were obtained from Charles River, Inc. (Wilmington, MA), or Taconic Farms, Inc. (Germantown, NY). Mice were kept under sterile conditions in a laminar flow room in cages with filter bonnets and were fed sterile mouse diet and sterilized tap water.

Establishing Colon and Melanoma Tumors in Nude Mice. The LS174T, SW948, and MEL-1 tumor cells were harvested and suspended in DPBS at a concentration of $25 \times 10^6$ cells/ml. Cell viability was determined by trypan blue dye exclusion. Cells ($5 \times 10^5$ viable) in sterile PBS were injected s.c. into the flank of nude mice. When the tumors were approximately 3-11 mm in diameter, the animals were given injections of radiolabeled antibodies and the biodistribution and tumor growth were determined.

Biodistribution. Serial eye bleeding was done using 10-50 $\mu$l capillary tubes in groups of 4-8 normal nude mice at 4 and 12 h and 1, 2, 3, 4, 5, 6, 8, and 10 days after i.p. injection of 0.7-1.1 $\mu$Ci $^{131}$I-labeled 17-1A MoAbs and 1.8-3.7 $\mu$Ci $^{125}$I-labeled murine 17-1A, chimeric 17-1A MoAbs, or H4-485 and 0.4 and 4 $\mu$Ci of $^{125}$I-labeled murine 17-1A F(ab')2 fragments. The blood samples were counted in a well-type gamma counter to determine the rate of clearance from the slope of the elimination curves. In vivo tissue distribution was conducted in nude mice bearing LS174T, SW948, or MEL-1 tumors following i.p. administration of 0.5 $\mu$Ci $^{125}$I-labeled murine 17-1A, chimeric 17-1A MoAbs, or H4-485 and 0.4 or 4 $\mu$Ci of $^{125}$I-labeled murine 17-1A F(ab')2 fragments. Groups of 4-8 animals were bled and sacrificed 4 days after i.p. injection. Tissues and organs were immediately dissected, rinsed with saline, blotted dry, and placed in 12 x 75-mm polystyrene tubes and weighed. The samples of blood, tumor, spleen, liver, heart, lung, kidney, muscle, skin, small intestine, stomach, and femur were counted in a well-type gamma counter. All animals received potassium iodide in their drinking water starting 1 day prior to administration of radiolabeled antibodies.

Results of labeled antibody biodistribution are expressed as % ID/g and as tissue/blood ratios of the concentration (cpm/g) in the tissue relative to the blood.

Inhibition of Tumor Growth. Nude mice bearing LS174T tumors approximately 8-11 mm in maximal dimension were given i.p. injections of 300 $\mu$Ci (26.3 $\mu$g) of $^{131}$I-labeled murine 17-1A or 300 $\mu$Ci (65.2 $\mu$g) of 17-1A chimeric IgG1. The bedding in cages was changed at 1 and 4 days after radiolabeled antibody injection. The tumor size was measured in 2 dimensions 3 times weekly using a vernier caliper until the mice were sacrificed when tumors exceeded 700 mm$^2$. In a fractionation experiment, nude mice bearing LS174T tumors approximately 7-10 mm in maximal dimension were given i.p. injections of 300 $\mu$Ci (26.3 $\mu$g) of $^{131}$I-labeled murine 17-1A, 320 (15.5 $\mu$g) of $^{131}$I-labeled murine 17-1A at days 9, 16, and 28 after tumor cell injection. Mice given injections of unlabeled murine or chimeric IgG1 17-1A MoAb and mice not given antibody were used as controls. The animals received potassium iodide to block uptake of $^{131}$I in the thyroid. The change in average tumor size for a group of 4-15 animals expressed as a product of length x width in mm$^2$ was determined.

Results

Flow Cytometric Analysis of Murine and Chimeric 17-1A MoAb Reactivity with Human Colon Cancer Cells. Reactivity of murine 17-1A MoAb, its F(ab')2 fragments, and chimeric IgG1, IgG2, and IgG4 17-1A MoAbs with LS174T, SW948, C0112, and control MEL-1 cell lines was determined by indirect immunoassay.
mumonofluorescence with fluorescein-conjugated goat anti-mouse IgG or goat anti-mouse (GAM) IgG plus IgM.

The results of consecutive 17-1A MoAb incubations appear in Table 2. Two incubations of colon cancer cells with chimeric IgG1 and staining with FITC-conjugated goat anti-human (GAH) IgG or FITC-goat anti-human IgG produced a comparable percentage of positive staining cells and mean fluorescence intensity as two incubations with murine 17-1A and staining with FITC-goat anti-mouse IgG plus IgM. Immunoassay of unlabeled and radiolabeled products. The I25I- and I31I-labeled products showed minimal (<2%) free iodine in the preparations.

Table 1 Reactivity of intact murine 17-1A (M-17-IA), its F(ab')2 fragments, and chimeric 17-1A (Ch-17-IA) MoAbs with human colon and melanoma cancer cell lines determined by flow cytomtery

<table>
<thead>
<tr>
<th>Antibody type</th>
<th>Cell type</th>
<th>M-17-1A</th>
<th>Ch-G1</th>
<th>Ch-G2</th>
<th>Ch-G4</th>
<th>H4B5</th>
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<tr>
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<td>M-17-1A</td>
<td>M-17-1A</td>
<td>Ch-G1</td>
<td>Ch-G2</td>
<td>Ch-G4</td>
<td>H4B5</td>
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<td>LS174T</td>
<td>19 (90)</td>
<td>94 (19)</td>
<td>32 (1)</td>
<td>43 (2)</td>
<td>35 (2)</td>
<td>11 (0.5)</td>
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<tr>
<td>SW948</td>
<td>99 (54)</td>
<td>99 (12)</td>
<td>46 (1)</td>
<td>81 (2)</td>
<td>55 (1)</td>
<td>10 (0.4)</td>
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<tr>
<td>C0112</td>
<td>91 (103)</td>
<td>57 (29)</td>
<td>19 (8)</td>
<td>34 (9)</td>
<td>30 (9)</td>
<td>11 (3)</td>
</tr>
<tr>
<td>MEL-1</td>
<td>7 (0.3)</td>
<td>2 (0.3)</td>
<td>2 (0.3)</td>
<td>2 (0.3)</td>
<td>2 (0.3)</td>
<td>5 (0.3)</td>
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* Mean percentage of positive cells (mean linear fluorescence) from 2-12 experiments using fluorescein-conjugated goat anti-mouse antibody.

Table 2 Reactivity of 17-1A chimeric IgG1 (Ch-G1) and murine 17-1A (M-17-IA) MoAbs with human colon cancer cell lines determined by flow cytomtery

<table>
<thead>
<tr>
<th>Antibody type</th>
<th>Mean % of positive cells (mean linear fluorescence)</th>
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* Cells were reacted in duplicate with the first MoAb followed by one wash with PBS-bovine serum albumin solution and then a second MoAb incubation. One set of each of the doubly labeled cells was stained with FITC-conjugated goat anti-human IgG or two incubations of murine 17-1A stained with FITC-goat anti-human IgG plus IgM. The average cpm bound/cpm added for 2-6 assays each with duplicate samples was determined.

Table 3 Radiolabeling of intact murine 17-1A (M-17-IA), its F(ab')2 fragments, and chimeric 17-1A (Ch-17-IA) MoAbs with 125I and 131I

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Radionuclide</th>
<th>Amount labeled (mg)</th>
<th>Efficiency (%)</th>
<th>Specific activity (mCi/mg)</th>
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* Six x 10^6 tumor cells were incubated with 6-230 nCi of radiolabeled antibody, and the average cpm bound/cpm added for 2-6 assays each with duplicate samples was determined.

Table 4 Reactivity of 125I-labeled intact murine 17-1A (M-17-IA), its F(ab')2 fragments, and chimeric 17-1A (Ch-17-IA) MoAbs with human colon and melanoma cancer cell lines determined by live cell radioimmunoassay

<table>
<thead>
<tr>
<th>Binding (%)</th>
<th>Cell type</th>
<th>M-17-1A</th>
<th>Ch-G1</th>
<th>Ch-G2</th>
<th>Ch-G4</th>
<th>T101</th>
<th>H4B5</th>
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* The specific activities of the 131I-labeled murine 17-1A MoAb preparations ranged from 8.6 to 25.0 mCi/mg. When 200- to 300-μg aliquots of chimeric IgG1, IgG2, and IgG4 MoAbs were labeled with 1.1-1.4 mCi of 125I and 5 equivalents of IC1, the efficiency of incorporation of 125I ranged from 9.5 to 21.8%, and the radiolabeled chimeric antibodies had specific activities ranging from 0.3 to 1.4 mCi/mg. When 500 μg of chimeric IgG1 were labeled with 4.3 mCi of 131I, the efficiency of incorporation was 53.5% and the specific activity was 4.6 mCi/mg.

Chromatograms obtained by high pressure liquid chromatography analysis of unlabeled and radiolabeled murine and chimeric 17-1A MoAbs and gel electrophoresis results indicated homogeneous preparations of unlabeled and radiolabeled products. The 125I- and 131I-labeled products showed minimal (<2%) free iodine in the preparations.

Immunoreactivity of Radiolabeled MoAbs by Live Cell Radioimmunoassay. The average percentage binding of 125I-labeled murine 17-1A MoAb to 6 x 10^6 LS174T, SW948, C0112, and CEM cells was 17.9, 19.3, 35.8, and 1.0%, respectively, while the binding of 125I-labeled 17-1A F(ab')2 fragments and chimeric IgG1 and IgG4 to the same cell lines were similar, as shown in Table 4. The binding of 125I-labeled chimeric IgG2 to LS174T, SW948, and C0112 cells was 11.7, 9.7, and 22.2%, respectively. The binding of control 125I-labeled irrelevant control MoAb H4B5 to colon cancer cell lines was consistently less than 1%.

Biodistribution of Radiolabeled MoAbs. The elimination curves of the 125I-labeled murine and chimeric 17-1A MoAbs from the blood of normal nude mice are shown in Fig. 1. The blood half-lives for intact murine 17-1A, its F(ab')2 fragments, and chimeric IgG1, IgG2, and IgG4 MoAbs determined from 12 h to 8 days by linear regression were 7.5, 0.5, 5.2, 6.9, and 1.9 days, respectively. The concentrations of the various MoAbs in tumors and blood at 4 days after injection are shown in Table 4.
corresponding concentrations of chimeric IgG2 and IgG4 in normal nude mice at varying times after i.p. injection of 0.7–3.7 Ci of radioactivity.

Table 5. Biodistribution results with I25l-labeled murine 17-1A in LS174T tumor-bearing nude mice. The respective tumor/blood ratios were 1.4 ± 0.3 and 0.7 ± 0.2. Tissue biodistribution results with I25l-labeled murine 17-1A F(ab')2 fragments in LS174T tumor-bearing animals appear in Table 5. The concentrations of 17-1A F(ab')2 fragments in tumor and blood were 0.33 and 0.03% ID/g, respectively. The tumor/blood ratio at 4 days after injection in 0.07–0.34-g LS174T tumors was 12.2 ± 6.7. The tissue biodistribution of nonspecific control I25l-labeled H24B5 MoAb directed against influenza virus was studied in LS174T tumor-bearing nude mice. The concentration in tumor at 4 days after administration was 1.0% ID/g, which was lower than the concentrations obtained with the intact murine and chimeric 17-1A MoAbs. The corresponding concentration in blood was 1.1% ID/g. The tumor/blood ratio was 1.0 ± 0.5, which was lower than those obtained with the 17-1A MoAbs.

Effect of 125l-labeled MoAbs on Tumor Growth. In the first radioimmunotherapy experiment, a group of 27 athymic nude mice were given s.c. injections of the LS174T human colon carcinoma cell line. At 9 days after the tumor injection, when the tumors measured approximately 10 mm in maximal dimension, the mice were divided into 3 groups. The first group of 15 mice were given i.p. injections of 300 μCi of 125l-labeled intact murine 17-1A MoAb. The second group of 6 mice were given i.p. injections of 26.3 μCi of unlabeled intact murine 17-1A MoAb. The third group of 6 mice did not receive any antibody. The tumors were measured 3 times weekly until the mice were sacrificed when tumors exceeded 500–600 mm2. The average tumor size expressed as a product of length × width in mm2 for the 3 groups of animals is shown in Fig. 3. The growth rate of the tumors in mice given injections of 300 μCi was minimal during the first 12 days with tumors increasing in average size from 80 mm2 to 172 mm2. Tumors from this group showed some growth from 12 to 19 days after 125l-labeled
CHIMERIC 17-IA MoAb TUMOR LOCALIZATION AND THERAPY

17-IA administration, followed by an increased rate of growth thereafter. Tumors from the control mice given injections of unlabeled murine 17-1A or given no antibody at all, experienced rapid growth and were approximately twice as large as the tumors from the mice given injections of 300 μCi 131I-labeled 17-1A MoAb at 12–26 days after antibody injection.

In the second tumor growth inhibition experiment, a group of 22 nude mice were given s.c. injections of LS174T cells. At 9 days after tumor cell injection, when the tumors measured approximately 8–11 mm in maximal dimension, the mice were divided into 3 groups. The first group of 12 mice were given i.p. injections of 260 μCi (30.2 μg) of 131I-labeled intact murine 17-1A MoAb. Seven days later, these mice received a second i.p. injection of 320 μCi (15.5 μg) of 131I-labeled intact murine 17-1A MoAb. Twelve days later, these mice received a third i.p. injection of 300 μCi (12.0 μg) of 131I-labeled murine 17-1A MoAb. The second group of 5 mice were given i.p. injections of 30.2 and 15.5 μg of unlabeled intact murine 17-1A MoAb on days 9 and 16 after tumor cell injection. The third group of 5 mice did not receive any antibody. The change in average tumor size for the 3 groups of animals is shown in Fig. 5. At 20 days after 131I-labeled chimeric IgG1 17-1A MoAb administration, the average tumor size was 133 mm². By contrast, the average tumor size in the mice receiving unlabeled chimeric IgG1 17-1A MoAb was 531 mm² at 20 days after antibody injection, and the average tumor size in mice receiving no antibody was 909 mm².

Discussion

The 17-1A chimeric IgG1, IgG2, and IgG4 MoAbs described in this study retained the antibody-binding specificity of murine 17-1A MoAb, as determined by indirect immunofluorescence and a live cell radioimmunoassay. Findings similar to these
were obtained by other investigators (28-31) using competitive binding inhibition assays evaluating the binding of \(^{125}\text{I}\)-labeled murine 17-1A to colon tumor cells in the presence of varying concentrations of unlabeled murine 17-1A or chimeric 17-1A MoAbs. The results of the consecutive incubation immunofluorescence study indicate that 17-1A chimeric IgGl bound colon cancer cells to an extent equal to that of murine 17-1A.

The results of radiolabeling studies with \(^{125}\text{I}\) and \(^{131}\text{I}\) indicate that the murine and chimeric 17-1A MoAbs could be labeled to specific activities adequate for animal biodistribution, imaging, and therapy studies. Similar levels of binding to colon tumor cells of \(^{125}\text{I}\)-labeled murine 17-1A, its F(ab')\(^2\) fragments, and chimeric IgGl and IgG4 were found (Table 4). Chimeric IgG2 showed lower binding to colon tumor cells than the other chimeric MoAbs, which may have been the result of a less pure antibody preparation.

When the blood clearances of the \(^{125}\text{I}\)-labeled 17-1A MoAbs were determined in normal nude mice, the F(ab')\(^2\) fragments had the shortest half-life followed by chimeric IgG4 (Fig. 1). Murine 17-1A and chimeric IgGl and IgG2 had similar rates of blood clearance from normal nude mice. In biodistribution studies with nude mice bearing human colon cancer xenografts growing s.c., the chimeric IgGl showed the highest level of uptake in LS174T and SW948 tumors at 4 days, as compared to the other chimeric 17-1A MoAbs. Blood levels were lower for the chimeric IgGl than the other chimeric antibodies and intact murine 17-1A, resulting from the more rapid blood clearance and the higher degree of tumor uptake. Chimeric IgGl produced higher levels of tumor uptake than did murine 17-1A MoAb in this and a previous study (38). These results suggested that chimeric IgGl might be the most useful chimeric 17-1A MoAb for radioimmunotherapy studies.

In the preliminary radioimmunotherapy study in nude mice bearing LS174T colon cancer xenografts, a single injection of 300 \(\mu\text{Ci}\) \(^{131}\text{I}\)-labeled murine 17-1A MoAb slowed the growth of established tumor xenografts as compared to control animals given injections of unlabeled murine 17-1A MoAb or no antibody. Similar tumor growth inhibition results were obtained by Esteban et al. (40) using 300 \(\mu\text{Ci}\) \(^{131}\text{I}\)-labeled B72.3 administered to nude mice bearing LS174T tumor xenografts. They found no visible toxic effect in the mice with 300 \(\mu\text{Ci}\) of \(^{131}\text{I}\)-labeled B72.3, although 500 \(\mu\text{Ci}\) of \(^{131}\text{I}\)-labeled B72.3 showed increased inhibition of tumor growth and produced toxic effects in the mice including early death. Zalberg et al. (39) found that 1 mCi of \(^{131}\text{I}\)-labeled 250-30.6 MoAb directed against an antigen present on human colon secretory epithelium inhibited the growth of COLO 205 colon carcinoma xenografts in nude mice, whereas a similar quantity of \(^{131}\text{I}\)-labeled control MoAb or unlabeled specific antibody did not. Buchegger et al. (42) used a mixture of \(^{131}\text{I}\)-labeled anti-CEA MoAbs and F(ab')\(^2\) fragments for radioimmunotherapy of colon cancer xenografts in nude mice. After injection of a mixture of 200 \(\mu\text{Ci}\) of intact MoAb and 400 \(\mu\text{Ci}\) of F(ab')\(^2\) fragments, the T380 human colon carcinoma xenografts increased in size up to 6-10 days and then regressed for 4-12 weeks. A control group of mice given injections of the same amount of \(^{131}\text{I}\)-labeled normal mouse IgGl and its F(ab')\(^2\) fragments showed retarded tumor progression for 1-3 weeks as compared to untreated controls, but no tumor regression was observed. Previous reports exist concerning therapy of SW948 tumor xenografts with multiple injections of unlabeled murine 17-1A and chimeric IgGl beginning the day after tumor cell inoculation (7, 9).

In a preliminary radioimmunotherapy study with 3 injections of about 300 \(\mu\text{Ci}\) of \(^{131}\text{I}\)-labeled murine 17-1A MoAb adminis-

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References


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