Radioimmunodetection of Human Tumor Xenografts by Monoclonal Antibodies

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ABSTRACT

Mouse IgG2a monoclonal antibodies with specific binding reactivity in vitro to human tumors of the gastrointestinal tract were radioiodinated and injected into immunosuppressed mice xenografted with human colon carcinoma tumors. The antibodies preferentially localized in tumor tissue compared to normal mouse tissue, as determined by differential tissue counting of radioactivity. Preferential antibody localization in tumor tissue was greatly enhanced when F(ab')2 fragments of the antibodies were used, and the fragments localized specifically only in those tumors that bind the antibodies in vitro and not in unrelated tumors. Radiolabeled fragments of an anti-hepatitis virus monoclonal antibody of the same isotype as the specific antibody did not localize in tumors. Tumors could be located by whole-body γ-scintigraphy with radiolabeled specific antibody F(ab')2 fragments without background subtraction.

INTRODUCTION

Successful radioimmunolocalization of human tumors depends on the specificity of a radiolabeled antibody for a given tumor. Until now, reports on localization of human tumors by radioimmunodetection with antibodies produced in animals (polyclonal antibodies) were limited to a few tumor species xenografted in either nude mice (3, 19) or hamsters (8, 11). With the advent of MAbs4 that define specifically human tumor-associated antigens (20), it has become possible to simulate an experimental procedure of radiocalization of human tumors in an animal model system (21, 24, 26) with the ultimate aim of applying the technique to humans. Specific radiocalization of tumors in humans is of particular importance in cases of micrometastases or when sites are inaccessible to other means of localization.

We investigated the localization of colon carcinoma tumors in mice using radiolabeled MAbs that bind specifically to these tumors (16, 18, 22). Although the nude mouse is generally used in studies of human tumor xenografts, we used the immunosuppressed CBA mouse (12, 25) in this study, as frequent handling under non-pathogen-free conditions necessary in tumor-imaging experiments is better tolerated by these mice due to their lower susceptibility to intermittent infections. Using this mouse model, Moshakis et al. (21) have demonstrated specific localization of human colon carcinoma xenografts by monoclonal antibody.

We used MAbs of IgG2a isotype because only these mediate specific tumor destruction in nude mice (13, 14) and, in the future, radiocalization of tumors may be followed by specific immunotherapy. F(ab')2 fragments of MAbs were primarily used for the tumor localization studies, inasmuch as they showed higher binding selectivity in vivo as compared with intact antibody.

MATERIALS AND METHODS

Human Tumor Cell Lines. We included the CRC cell lines SW948, SW1116, and SW1222 and the melanoma cell line WM-9. These cell lines have been described elsewhere (15, 18).

Mouse and Xenografts. Four- to 6-week-old CBA mice (The Jackson Laboratory, Bar Harbor, Maine) were thymectomized and, 4 weeks later, given 900 rads whole-body radiation from a 137Cs source. The lethal effect of irradiation was prevented by i.p. injection of 1-β-o-arabinofuranosylcytosine (200 mg/kg; Cytosar; The Upjohn Co., Kalamazoo, Mich.) 2 days before irradiation (25). Human tumor cells (1 to 2 x 10⁷) were injected s.c. in the flank. Mice were maintained on sterile bedding with sterilized food and water. All mice received 0.1% (v/v) KI in the drinking water throughout the experiment, beginning 48 hr before the isotope injections in order to block uptake of free radiodiode by the thyroid.

Mouse MAbs. Anti-CRC MAb 17-1A, with binding specificity for tumors of the gastrointestinal tract (adenocarcinomas of the colon, rectum, stomach, and pancreas), has been described (16, 18). Anti-CRC MAb C2032 binds in radioimmunosassay to cells of gastrointestinal tract tumors and to mammary carcinomas but not to other human tumor cells or normal fibroblasts.5 Monoclonal anti-hepatitis virus antibody A5C3 was kindly provided by Centocor, Malvern, Pa. All antibodies are of IgG2a isotype.

Preparation of MAbs. MAbs were purified from ascitic fluid on Protein A-Sepharose columns as described previously (23).

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Preparation of F(ab')2 Fragments. Purified antibodies (approximately 10 mg/ml) in 0.1 M sodium acetate buffer, pH 4.5, were digested with 1% (v/v) pepsin at 37°C for approximately 24 hr. The digestion was then stopped by adjusting the reaction solution to pH 8.0 with 1 M Tris buffer. The digestion solution was applied to a Protein A-Sepharose column to remove undigested IgG and heavy chain fragments. The effluent containing F(ab')2 fragments [yield, 0.5 to 1 mg of F(ab')2/10 mg of intact IgG] and peptides was chromatographed on LKB Ultrogel AcA44 equilibrated in PBS. Purity of the chromatographed F(ab')2 was demonstrated in sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Immunoelectrophoresis of the F(ab')2 fragments showed no reaction with antisera specific for the Fc region.

Radiolabeling of MAbs and F(ab')2 Fragments. MAbs and their F(ab')2 fragments were labeled with 125I or 131I using the iodogen method.
(6) Briefly, 25-μl aliquots of iodogen (1,3,4,6-tetrachloro-3,6-diphenylglycoluril; Pierce Chemical Co., Rockford, Ill.) at 400 μg/ml in chloroform were evaporated to dryness at 37°C in 10-× 75-mm glass tubes. Na[131]I or Na[125]I (for protein iodination; Radiochemical Centre, Amersham, England) and 50 μg of protein in 100 μl PBS were added to the tubes, and the tubes were incubated for 10 min at room temperature. The radiolabeled protein was then separated from free iodine on Dowex (Bio-Rad Laboratories, Richmond, Calif.) columns equilibrated with PBS. For organ distribution, antibodies and their fragments were labeled to 1.0 to 3.0 μCi of [131]I or [125]I per μg of protein. For γ-sciintigraphy, antibodies were labeled to 5.6 to 13 μCi/μg of protein. Before inoculation into mice, the iodinated antibody preparations were centrifuged at 45,000 rpm in a SW 50.1 rotor for 45 min at 4°C to remove aggregates. Filtration of labeled antibodies over Sephadex G-200 columns revealed no aggregates.

Antibody Binding Assay. Labeled antituor MAbs and the ASC3 control MAb as well as their F(ab')2 fragments were tested for their binding specificity using CRC and melanoma cells as targets. PBS containing 5% heat-aggregated horse serum and 0.02% sodium azide was used throughout. A constant number of target cells (5 × 10^6) was incubated with varying quantities of [131]I- or [125]I-labeled antibody or F(ab')2 fragments in 100 μl of buffer in wells of microtiter plates for 1 hr at room temperature. Cells were then washed 3 times in buffer and radioactivity in the pellet was determined with a gamma counter.

In Vivo Antibody Distribution. Labeled antibody or its F(ab')2 fragments were injected into immunosuppressed mice bearing 7- to 8-day-old tumors weighing between 70 and 90 mg. Histological preparations of a proportion of tumors in each experiment showed well-vascularized tumor tissue with no evidence of necrosis. Mice were inoculated i.p. with 15 μCi (5 to 15 μg) of [131]I-labeled antitumor antibody or its F(ab')2 fragments. Some mice were given simultaneous injections of 15 μCi each of [131]I-labeled specific and [125]I-labeled indifferent F(ab')2 fragments. Two to 14 days later, mice were sacrificed and dissected. Tumors, blood, visceral organs, and muscle samples were weighed and assayed for radioactivity.

The results are expressed as: (a) ratios of specific activity of antibody (or its fragments) in tumor to normal mouse tissues [(cpm/mg in tumor)/(cpm/mg tissue)]; (b) percentage of injected radioactivity per g of tissue, corrected for physical decay; and (c) localization index, i.e., the ratio of specific ([131]I) to nonspecific ([125]I) activity in tumors and organs divided by the same ratio in the blood (21).

γ-Sciintigraphy. Mice bearing 7- to 8-day-old tumors were given i.p. injections of 60 to 100 μCi (4.5 to 18 μg) of [131]I-labeled MAb fragments. Images were obtained at various times (for up to 7 days) following antibody injection. Mice were anesthetized and placed prone on the collimator face, and images were obtained using a medium-energy parallel-hole collimator and a large-field-of-view scintillation camera (Searle Photogramma LFV) interfaced to a computer (Digital Equipment Corp., Maynard, Mass.).

Statistical Analysis. The statistical significance of differences between experimental and control values was determined with Student's t test.

RESULTS

In Vitro Binding Specificity of Radiolabeled 17-1A Antibody and Its F(ab')2 Fragments. Iodinated 17-1A antibody and its F(ab')2 fragments both bound equally well to CRC cells, but they did not bind to melanoma cells. A linear relationship existed between the amount of labeled antibody or fragments added (0.025 to 0.1 μg/5 × 10⁶ cells) and the amount bound to CRC cells. Between 35 and 40% of the labeled protein added was bound to the target cells. Monoclonal anti-hepatitis virus control antibody fragments, on the other hand, did not bind to CRC cells (results not shown).

In Vivo Distribution of [131]I-Labeled MAb 17-1A and Its F(ab')2 Fragments in Various Tissues of Mice Grafted with SW948 Human CRC Cells. Immunosuppressed mice bearing human CRC tumors were given injections of [131]I-labeled anti-CRC MAb 17-1A or its fragments, and the specific activity of tumor tissue was compared with the activity in normal mouse tissues (Table 1). As compared with intact antibody, the use of F(ab')2 fragments resulted in a significant increase in tumor/tissue ratios of radioactivity (Table 1). For example, tumor/blood ratios increased 13-fold, and the tumor/tissue ratios in spleen, lung, heart, and muscle increased over 4-, 3-, 6-, and 5-fold, respectively, when compared 5 days following antibody injection.

Comparative Clearance Studies with 17-1A Antibody and Its F(ab')2 Fragments. Of the many factors that could account for the increased binding selectivity to tumor tissue of MAb fragments as compared to intact MAb, the faster clearance of the fragments from body fluids was considered here. To test this possibility, mice were given i.p. inoculations of radiolabeled F(ab')2 fragments, and the clearance of the fragments from blood and tumor tissue was compared to the clearance obtained with intact immunoglobulin. The 17-1A F(ab')2 was cleared more rapidly from the blood of human CRC tumor-bearing mice than was the intact antibody (Chart 1). By Day 5, only 0.03% of the administered dose of 17-1A F(ab')2 as compared with more than 6% of the intact antibody was present per ml of blood. The biological half-lives of the fragments and intact antibody molecules determined from the clearance of the antibody preparations from the blood of tumor-bearing animals from Day 2 onwards were 14.4 and 72 hr, respectively. Although the fragments disappeared from the tumors at a faster rate than did intact antibody, the tumor/blood ratios of radioactivity were significantly higher with the fragments. F(ab')2 fragments were therefore used in all further tumor localization and imaging studies.

Specificity of Tumor Localization of 17-1A F(ab')2 Fragments. Preferential localization of 17-1A F(ab')2 in CRC tumors as opposed to normal mouse tissues is shown in Chart 2. Selectivity of this process increased with time, with the highest tumor/tissue ratios found between Days 4 and 5. Among normal tissues, the highest concentration of radioactivity was in kidney and the lowest concentration was in muscle.

The specificity of MAb 17-1A binding in vivo for CRC cells was demonstrated in mice xenografted with melanomas (Chart 2). Although specific activity ratios in melanoma tissue compared to normal mouse tissue increased slightly with time, these ratios were significantly lower (at p < 0.05, by t test) on Days 4 and 5 in melanoma tumors than in CRC tumors (Chart 2).

### Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>17-1A</th>
<th>17-1A (F(ab')2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1.4 ± 0.26</td>
<td>18.7 ± 6.8</td>
</tr>
<tr>
<td>Liver</td>
<td>6.6 ± 1.9</td>
<td>6.7 ± 1.9</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.9 ± 1.3</td>
<td>20.4 ± 9.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.1 ± 0.7</td>
<td>3.6 ± 1.7</td>
</tr>
<tr>
<td>Lung</td>
<td>2.4 ± 0.5</td>
<td>7.7 ± 4.3</td>
</tr>
<tr>
<td>Heart</td>
<td>4.0 ± 0.5</td>
<td>26.0 ± 5.7</td>
</tr>
<tr>
<td>Muscle</td>
<td>11.3 ± 3.4</td>
<td>66.8 ± 26.7</td>
</tr>
</tbody>
</table>

At 5 days following injection of 20 μCi (8 μg) of [131]I-labeled 17-1A and 15 μCi (8.5 μg) of [125]I-labeled 17-1A F(ab')2.

Mean ± S.D. of 5 animals.
Tumor Localization with MAb

Chart 1. Clearance of 131I-labeled MAb 17-1A and its F(ab')2 fragments from tumor and blood of mice. Mice bearing SW948 human CRC tumors were given i.p. injections of 20 μCi (8 μg) of 131I-labeled 17-1A (•) or 15 μCi (8.5 μg) of 131I-labeled 17-1A F(ab')2 (○), and the amount of antibody present in tumor (---) and blood (----) was determined at different time intervals and expressed as the percentage of antibody dose injected per g of tissue. The radioactivity in tumor and blood differed significantly (p < 0.05) for F(ab')2 on Days 3, 4, and 5. Results are means of 3 to 7 mice. Bars, S.D.

Further evidence of specific localization of MAb 17-1A in CRC tumors was established by comparing the tumor and the organ distribution of 131I-labeled 17-1A F(ab')2 and 125I-labeled F(ab')2 fragments of the A5C3 anti-hepatitis virus MAb injected simultaneously into CRC-bearing mice (Chart 3). The localization indices derived from specific antibody/indifferent antibody ratios in tumor tissue relative to blood were between 3.8 and 11.3 on various days. Thus, accumulation of antibody 17-1A in tumor tissue was significantly greater compared to accumulation of control antibody. These differences were most pronounced 4 days following antibody injection. In comparison, the localization indices in all normal mouse tissues tested were between 1.0 and 2.2, indicating similar distribution of both antibodies in these tissues on all days tested.

In Vivo Distribution of 131I-labeled F(ab')2 Fragments of Anti-CRC MAb C2032. 131I-Labeled F(ab')2 fragments of MAb C2032 were prepared as described for MAB 17-1A and showed similar binding reactivity and specificity in vitro (not shown). Preferential localization of antibody fragments C2032 in SW1222 CRC tumor tissue as compared to normal mouse tissue could be demonstrated in xenografted mice (Table 2). The specificity of 131I-labeled C2032 F(ab')2 binding in vivo for CRC cells was demonstrated in mice xenografted with melanoma (Table 2). Tumor/tissue ratios were significantly lower (at p < 0.05, by t test) in melanoma tumors than in CRC tumors. Furthermore, accumulation of MAb C2032 fragments in CRC tumor tissue was significantly higher than was the concentration of anti-hepatitis virus control antibody fragments, as indicated by the high localization index (11.5; average of 4 animals) obtained in tumor at 5 days after antibody administration (results not shown).

Tumor Localization of 131I-labeled F(ab')2 Fragments of Antibodies 17-1A and C2032 by γ-Scintigraphy. γ-Scintigraphy was performed in mice grafted either with one of 2 different CRC tumors (SW948 and SW1116) or with one melanoma (WM-9) and given injections of 60 to 100 μCi (4.6 to 18 μg) of 131I-labeled 17-1A F(ab')2. Of 21 mice with xenografted CRC tumors, activities were 5- to 10-fold higher in CRC tumors than in melanomas on various days tested (not shown).

Chart 2. Distribution of 131I-labeled 17-1A F(ab')2 in mice grafted with SW948 human CRC (•) or WM-9 melanoma (○) cells. Tumor-bearing mice were given i.p. injections of 15 μCi (8.5 μg) of 131I-labeled 17-1A F(ab')2, and tumor (T)/tissue ratios of radioactivity were determined at different time intervals as described in "Materials and Methods." Values represent the means of 2 to 7 mice. CRC tumors bound significantly (p < 0.05) more 17-1A F(ab')2 than did melanomas on Days 4 and 5. Mean CRC tumor weight increased from 83 mg on Day 2 to 143 mg on Day 6; melanoma tumor weight increased from 68 to 110 mg.

Chart 3. Specific localization indices in human tumor xenografts and mouse tissues. Mice were given injections of radiolabeled F(ab')2 fragments of both 17-1A (15 μCi; 8.5 μg) and the A5C3 (15 μCi; 7.2 μg) control MAb, and localization indices in human CRC tissue (•) and mouse liver (○) or lung tissue (○) were calculated as described in "Materials and Methods." Values represent the means of 2 to 7 mice. Bars, S.D.
19 mice (16 mice with SW948 tumors and 3 with SW1116 tumors) showed clear tumor localization 3 and 4 days following antibody administration (Fig. 1, a and b). Tumor localization was possible without background subtraction. In only 2 mice were tumors (SW948) not localized. The failure to localize those tumors could not be attributed to small tumor size, as localization was possible with tumors as small as 80 mg (results not shown). The ratios of counts within the tumor area and the contralateral site ranged from 1.6 (average of group of mice with good localization) to 3.0 (average of group of mice with best localization) in the mice with positive tumor images, compared with an average value of 1.1 in the 2 mice in which CRC tumors could not be localized. Localization of 17-1A antibody fragments in CRC tumors was specific for this tumor type, inasmuch as melanoma tumors could not be localized with the antibody in any of the 10 mice tested (Fig. 1c). In these animals, the ratios of counts within the tumor area to counts in the contralateral site ranged from 0.7 to 1.0.

All 4 mice xenografted with SW1222 CRC cells and given injections of 100 μCi (11.7 μg) of 131I-labeled C42032 F(ab’)2 showed clear tumor localization 4 days following antibody administration (Fig. 1d). In these animals, the ratios of counts within the tumor area and contralateral site ranged from 2.5 to 4.3. Melanoma tumors could not be localized with antibody fragments C42032 (Fig. 1e).

**DISCUSSION**

We have demonstrated here the specific radiolocalization of xenografts of colon carcinomas in mice by 131I-labeled MAbs. The F(ab’)2 fragments of monoclonal anti-CRC antibodies showed greater selectivity and specificity in binding to CRC tumors in vivo as compared to intact antibody. Similarly, F(ab’)2 fragments of mouse MAbs directed against Thy 1.1 antigen showed higher binding selectivity in vivo as compared with intact antibody (17). On the other hand, radiolabeled intact polyclonal goat antibody directed against carcinoembryonic antigen was superior to the F(ab’)2 fragments in localizing colon carcinoma tumors in hamsters (7). Thus, it might be necessary to base the choice of using intact antibody or its fragments on experimental evidence in each system.

In the present study, the greater in vivo binding selectivity of antibody fragments as compared to intact antibody might be explained by the significantly faster rate of clearance of the fragments from the blood (Chart 1). Of several explanations for the inferior binding selectivity of the intact MAb, one must consider the affinity of the intact MAb for the Fc receptors of mouse lymphocytes and macrophages (13), as evidenced by the accumulation of radioactivity in blood, lung, and spleen (Table 1), tissues that contain large numbers of these Fc receptor-bearing cells.

Although the 17-1A and C42032 MAbs bind to different antigenic moieties on CRC cells, their specificity and high selectivity in radiolocalization in the tumor xenografts were similar.

The selectivity and specificity of binding of MAb fragments to human tumor cells, as demonstrated by differential tissue counting, suggested the feasibility of nuclear imaging of the targeted tumors. Polyclonal antitumor antibodies derived from animal serum that showed much lower uptake ratios have been used to scan solid tumors (2, 5, 9, 10). Our study confirms a previous
report of tumor imaging by MAbs (1) and shows that clear tumor visualization is possible with radiolabeled MAbs without using subtraction methods. Chatal et al. (4), using $^{131}$I-labeled intact 17-1A MAb for localization of CRC in humans, obtained positive imaging in 81% of the tumor sites, often without the use of subtraction methods.

Although, in the present study, mice received relatively large doses (in terms of body weight) of antibody fragments and of radioactivity (4.6 to 18 μg or 60 to 100 μCi) in order to facilitate imaging, recent studies in patients have shown satisfactory imaging of CRC tumors following administration of only 200 to 500 μg (1 to 2 μCi) of F(ab')₂ fragments of 17-1A MAb. Since the IgG2a MAbs, which in the present study were found to localize specifically and selectively in human tumor xenografts, are also those that destroy specifically the same tumor xenografts in mice (13, 14), imaging of tumors with MAbs may precede their therapeutic use.

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REFERENCES


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