Murine Monoclonal Antibodies Raised against Human Non-Small Cell Carcinoma of the Lung: Specificity and Tumor Targeting

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ABSTRACT

The tumor targeting properties of murine monoclonal antibodies (MAbs) generated in our laboratory against non-small cell carcinoma of the lung have been investigated in nude mouse xenograft models. The MAbs selected for evaluation, RS5-4H6, RS7-3G11, and RS11-51, have pancarcinoma reactivity, as shown by immunoperoxidase staining of the majority of tumors from the lung as well as breast, colon, kidney, and ovary. The localization of the three MAbs which bind to distinct antigens, and exhibit different levels of cross-reactivity with normal human epithelial tissues, are compared. The MAbs are of the IgG1 isotype. Since these MAbs were reactive with Calu-3, a human adenocarcinoma of the lung cell line grown as xenografts in nude mice, this system was selected as our initial tumor target. The MAbs were found to localize preferentially to the heterotransplanted tumors, with from 6.6 to 8.6% of the injected dose per gram accreting in the tumor at 7 days. Tumor/nontumor ratios of up to 9.7 were seen with one MAb at day 14. The targeting of MAb RS11-51 and F(ab')2, fragments of RS11-51 in GW-39, a human colon cancer grown in nude mice, was also studied. Accretion of intact RS11-51 and F(ab')2, fragments into GW-39 was greatly increased compared to Calu-3. In view of the high frequency of antigen expression on a wide variety of tumors, and the ability to target in vivo, these new MAbs may have potential use in the imaging and therapy of cancer.

INTRODUCTION

While diverse cancers have been targeted with radiolabeled monoclonal antibodies (1-4), tumors of the lung have not been studied extensively by this approach. Lung cancer is now considered to be the leading cause of cancer death in both men and women, with 155,000 new cases and 142,000 deaths estimated in the U.S. for 1989 (5). The application of hybridoma technology to the detection and treatment of lung cancer therefore warrants consideration. Our laboratory, as well as others, has been developing a panel of monoclonal antibodies against human lung cancer using a variety of immunization strategies, and the ability to target in vivo, these new MAbs may have potential use in the imaging and therapy of cancer.

MATERIALS AND METHODS

Monoclonal Antibodies and Fragments. The production and initial characterization of RS5-4H6 has been described previously (6, 9). Briefly, hybridization was performed according to the method of Galfre et al. (10) using the nonsecreting mouse myeloma cell line SP2/0-Ag14 as a fusion partner. RS7-3G11 and RS11-51 were generated by the same hybridization procedure using a crude membrane preparation, derived from a surgically removed human primary squamous cell carcinoma of the lung, as immunogen. MH-99, an anti-epithelial surface antigen IgG, MAb (11), kindly provided by Dr. M. J. Mattes, NP-4, an anti-CEA IgG, MAb (12), and Ag8 (American Type Culture Collection, Rockville, MD), an irrelevant mouse myeloma IgG, designated P3 X 63 Ag8, were used as control antibodies in this study. The antibodies were isolated from ascites fluid by passage through a protein A-immunosorbent column (Bio-Rad, Richmond, CA).

The F(ab')2 fragments were produced by digestion with pepsin (Sigma Chemical Co., St. Louis MO; 12 μg/ml, in 0.1 M sodium citrate, pH 3.7 at 37°C for up to 2 h). For purification of F(ab')2, the preparation was dialyzed against PBS to remove small particles, then separated from undigested IgG by chromatography on a protein A column.

Screening Procedures. Procedures for selection of MAbs and for identification of antibody specificity included ELISA performed in 96-well polystyrene microtiter plates coated with tumor or normal tissue membrane preparations, indirect immunofluorescent assays analyzed by flow cytometry, and immunoperoxidase staining of cryostat sections. These procedures were performed according to methods described previously (6).

Western Blot. SDS gel electrophoresis (13) of antigens, followed by transfer to nitrocellulose filters and immunostaining with MAbs, was done according to the method of Towbin et al. (14).

Radiolabeling of Monoclonal Antibodies. RS5-4H6 was radiolabeled with the 125I-Bolton-Hunter Reagent (New England Nuclear, North Billerica, MA) according to the original procedure of Bolton and Hunter (15) due to complete loss of immunoreactivity following iodination of this MAb with chloramine T. Other MAbs and antibody fragments were radioiodinated with 131I or 125I (New England Nuclear) by the chloramine T method (16). Unbound radiiodine was removed from antibody-bound iodine by gel filtration over a PD-10 column (Pharmacia, Piscataway, NJ) that had been equilibrated in 0.04 M sodium phosphate, pH 7.4, with 0.15 M NaCl, 0.02% NaN3, and 1% human serum albumin. Radiolabeled antibody was characterized by high-performance liquid chromatographic analysis using a Zorbax GF-250 column (Dupont, Wilmington, DE), and analyzed for immunoreactivity using a cell binding assay.

Immunoreactivity. Assessment of immunoreactivity of the MAbs after radiolabeling was performed by cell binding assays. Lung tumor cells were washed with PBS and adjusted to 1 x 10⁷ cells/ml. Washed...
cells (100 μl) were mixed with 25 μl of labeled MAb (approximately 50,000 cpm) and 25 μl of either an unlabeled irrelevant IgG or 25 μl of the unlabeled specific MAb. A minimum of 100-fold excess unlabeled MAb was used. Following a 1-h incubation at 4°C with shaking, the incubation mixture was diluted with 8 ml of PBS containing 1% horse serum. Cells were pelleted for 5 min at 400 × g, and counted. Incubations were performed in triplicate and results calculated as percentage specific binding.

Biotinylation of Monoclonal Antibodies. Purified MAbS were biotinylated using sulfosuccinimidyl 6-(biotinamide)hexanoate (NHS-LC-Biotin; Pierce, Rockford, IL). NHS-LC-Biotin was incubated with MAb (2.0 mg/ml) at a molar ratio of 50:1 for 6 h at room temperature, followed by dialysis against PBS to remove low molecular weight side products. Comparative titrations of the MAbs binding to Calu-3 (analyzed by flow cytometry) before and after biotinylation, indicated full retention of binding.

In Vivo Localization. Female nude mice were used. Tumors were propagated by either s.c. injection of 0.2 ml of a 10% suspension of minced tumor or s.c. inoculation of 1 × 10^7 washed tissue culture cells. The mice were used for biodistribution studies when tumors reached a size of 0.1-0.5 g. Radioiodinated antibodies or fragments were injected i.v. into the tumor-bearing animals. The animals were sacrificed at the time indicated in each experiment and the radioactivity of both 125I and 125I in the tumor, liver, spleen, kidneys, lungs, and blood was determined after correction for physical decay and downscatter in a 2-channel gamma-scintillation counter. The data are expressed as percentage of injected dose per gram of tissue and tumor/nontumor ratios. Results are the mean ± SD of four to six animals per data point.

RESULTS

Production and Selection of Monoclonal Antibodies. The MAbs described in this paper are products of three fusions of immune BALB/c splenocytes with the nonsecreting mouse myeloma cell line SP2/0-Ag14. RS5-4H6 was raised against a membrane preparation of a Calu-3 xenograft grown in nude mice and has been described previously (6). For the production of RS7-3G11 and RS11-51, BALB/c mice were immunized with a crude membrane preparation directly from surgically removed squamous cell carcinoma of the lung without prior culturing of the cells. Hybridomas resulting from these fusions were screened by ELISA assays against the membrane preparations used as immunogen (primary screen) and membrane preparations of normal liver (secondary screen). Stable hybridomas reactive with the immunogen but not the normal liver were further screened by flow cytometry for lack of reactivity with normal peripheral white blood cells, and by immunohistochemistry for the ability to stain tissue sections of lung tumors without staining normal connective tissue.

As a result of this screening three MAbs, RS5-4H6, RS7-3G11, and RS11-51, were selected which demonstrated reactivity with lung tumor-associated antigens. These MAbs were subcloned by limiting dilution. Isotyping by Ouchterlony double diffusion indicated that these three MAbs are of the IgG1, k isotype.

Reactivity of Monoclonal Antibodies against Various Cell Types. Table 1 compares the binding of the three MAbs to a panel of human cell lines and peripheral white blood cells using a flow cytometric assay. As detected by flow cytometry, the antigens were expressed on tissue culture cell lines to varying degrees. RS11-51 was reactive with all tumor lines tested, whereas RS5-4H6 and RS7-3G11 showed greater selectivity. Distinct subpopulations of cells were not observed. A relatively low level of reactivity such as 14.2% reactivity of RS11-51 with A549 indicates weak reaction in all cells, that is a small shift in the fluorescent intensity of the cell population as a whole with 14.2% of the cells falling into the region defined as positive. The profile of reactivity was similar but not identical for RS5-4H6 and RS7-3G11. Normal lung fibroblasts (CCD-18Lu) and peripheral blood granulocytes, lymphocytes, and monocytes do not express the antigens recognized by these monoclonal antibodies.

Reactivity of Monoclonal Antibodies against Human Tissue. The three MAbs were tested by immunohistology on frozen sections from a variety of normal and tumor tissues. As shown in Table 2, MAbs RS5-4H6, RS7-3G11, and RS11-51 were found to be reactive with essentially all the non-small cell carcinomas of the lung specimens which were tested, without regard to histological classification. Small cell carcinomas of the lung were not tested due to a failure to procure this tissue type as fresh samples. Breast, colon, renal, and prostatic carcinomas were also reactive immunohistologically with these MAbs. Except for epithelial tissue, which reacted to varying degrees with the three MAbs, normal tissues did not stain. The intensity of cross-reactivity with normal epithelial tissue is RS11-51 > RS7-3G11 > RS5-4H6; that is, RS11-51 shows less intensity of cross-reactivity with normal epithelial tissue than the other two MAbs. The cut-off marker is set so that background fluorescence equals from 1 to 5%.

| MAb reactivity with human tissue culture cell lines and normal peripheral blood cells by flow cytometry |
|-------------------------------------------------|-------------|-------------|-------------|
| Target cells | RS5-4H6 | RS7-3G11 | RS11-51 |
| Lung adenocarcinoma A549 | 0.5 | 0.1 | 14.2 |
| Calu-3 | 57.1 | 79.0 | 93.8 |
| Lung squamous cell carcinoma Calu-1 | 20.3 | 0.0 | 24.8 |
| SK-MES-1 | 0.0 | 51.8 | 61.3 |
| Lung small cell carcinoma NCI-H69 | 0.0 | 0.0 | 26.3 |
| Lung fibroblast CCD-18 LU | 0.0 | 0.0 | 0.1 |
| Breast carcinoma BT-20 | 80.5 | 36.9 | 96.5 |
| MCF7 | 71.9 | 95.2 | 96.7 |
| Colon carcinoma HT-29 | 0.5 | 6.1 | 49.4 |
| LoVo | 0.0 | 0.1 | 10.3 |
| LS174T | 0.0 | 0.2 | 51.6 |
| SW1116 | 0.0 | 0.4 | 63.9 |
| Prostatic carcinoma Du 145 | 75.8 | 84.6 | 43.9 |
| PC-3 | 12.6 | 35.3 | 76.9 |
| Peripheral blood Granulocytes | 0.0 | 0.0 | 0.0 |
| Monocytes | 0.0 | 0.0 | 0.0 |
| Lymphocytes | 0.0 | 0.0 | 0.0 |

* Percent of cells with positive fluorescence minus background fluorescence.
Table 2 Immunohistological reactivity of MAbs with frozen tissue sections

<table>
<thead>
<tr>
<th>Tissue</th>
<th>RS5-4H6</th>
<th>RS7-3G11</th>
<th>RS11-51</th>
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<td>3</td>
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<tr>
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<td>1</td>
<td>3</td>
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<tr>
<td>Spleen</td>
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</table>

* Reactivity is defined as follows: -, <5% of tissue is stained; +, 5–50% of tissue or light stain in 5–100% of tissue; ++, 50–100% of tissue stained with high intensity.

than that of the tumor mass. This, along with the fact that the staining of the lung tumors is throughout the zone of tumor cells whereas, the normal lung alveolar staining just lines the edge of the structure have the combined effect of magnifying the staining differential in lung tumor compared to normal lung. The normal kidney staining with these MAbs was localized to the collecting tubules. Generally, approximately 15–30% of the tissue was stained, but the intensity of stain was strong and was therefore recorded as ++. MAb RS5-4H6 was significantly more reactive with kidney cancer than RS7-3G11, while the reverse was true for carcinoma of the prostate where RS7-3G11 reacted strongly and RS5-4H6 was unreactive. RS11-51 reactivity was strong in both kidney and prostate cancers. In the normal pancreas RS5-4H6 stained only the ducts, which represents approximately 5% of the tissue. RS7-3G11 stained both ducts and acini (~80% of tissue) but not islets, although the stain was light.

In the colon the staining differential between RS11-51 and the other two MAbs is especially evident. In the colon carcinoma specimens (except for one sample with RS7-3G11) only low level antigen expression is observed with MAbs RS5-4H6 and RS7-3G11. In the normal colon RS5-4H6 and RS7-3G11 react only weakly with the specimens noted as positive, and in these only the lumen of the glands are stained. Conversely RS11-51 has intense reactivity with all tumors, 100% of cells staining, and normal glands of colon have similar intensity. Other areas of normal colon are unstained.

In breast tumors heterogeneous staining was observed with MAb RS5-4H6, with 10–50% of the cells staining in the specimens graded "+,", however the intensity of stain was as great as the "++" specimen, indicating a heterogeneous population of cells some with high antigen density and some lacking antigen. In normal breast only the lumen of glands stained with

Fig. 1. Immunohistological staining of carcinoma tissue sections. Frozen surgical specimens of a squamous cell carcinoma of the lung stained with the MAbs indicated. A, RS5-4H6; B, RS7-3G11; C, RS11-51; D, Ag8. Hematoxylin counterstain, × 40.
RS5-4H6 and RS7-3G11. The intensity of stain was not as great as in most tumor specimens but direct comparison is difficult due to morphological differences.

Characterization of Antigens. Western blot analysis was performed using the membranes from Calu-3 tumors grown in nude mice. The three MAbs were found to react with molecules of different biochemical properties. RS5-4H6 reacts with a molecule of apparent molecular weight greater than 300,000 (6). RS11-51 showed binding to a band with a molecular weight equivalent to that of the MH-99 antigen (Fig. 2), which has been previously shown to be 38,000 (17). This band was only present when electrophoresis was run in the absence of reducing agents, the same property displayed by MH-99. RS7-3G11 antigen binding could not be detected by this technique.

In Vivo Distribution. In order to identify animal tumor models appropriate for in vivo targeting studies, cryostat sections of tumor xenografts were immunostained with biotinylated MAbs. Direct staining with biotinylated MAbs, rather than the indirect standard method used for screening MAb reactivity with human tissues, was performed to avoid the problem of high background generated from endogenous mouse immunoglobulins present in the heterotransplanted tumors. RS5-4H6, RS7-3G11, and RS11-51 all reacted with Calu-3 (adenocarcinoma of the lung) tumor xenografts, whereas both NP-4, an anti-CEA monoclonal antibody, and Ag8 showed negligible reactivity with this tumor. When these MAbs were tested for reactivity against GW-39, a CEA-expressing, signet-ring carcinoma of the colon of human origin (3), only NP-4 and RS11-51 were reactive. This result is analogous to the flow cytometry data which showed negligible reactivity of RS5-4H6 and RS7-3G11 with cultured colon cancer cell lines, even though 5/9 colon cancer surgical specimens were reactive with each of these MAbs. GW-39 was not included in the flow cytometry study since this line is not being propagated at present in tissue culture.

The MAbs were iodinated, with resulting specific activities of 3.36 mCi/mg for RS5-4H6 and 9.4-16.8 mCi/mg for RS7-3G11, RS11-51, and RS11-51 F(ab')2 fragments. High-performance liquid chromatographic analysis indicated homogeneous preparations with minimal (<2%) free iodine in the preparations. The average percentage of specific binding to Calu-3 cells was 61% for intact RS11-51, 38% for RS11-51 F(ab')2, 42% for RS7-3G11, and 14% for RS5-4H6, indicating the presence of reactive MAb in the preparations. Nonspecific control MAbs exhibited 0.0% specific binding to Calu-3 cells.

The Calu-3 bearing nude mice were administered 10–25 μCi of either 125I or 131I-labeled antibodies i.v., and sacrificed on day 7. The radioactivity of the tumor, organs, and blood were counted. The percentage of injected dose per gram localizing in the tumor and organs on day 7 for three experiments is presented in Fig. 3. The three antibodies were found to localize preferentially to the heterotransplanted tumors. At 7-day post-

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Fig. 2. Western blot. A Calu-3 membrane preparation was subjected to electrophoresis on a 10% polyacrylamide gel in SDS (without reducing agents) followed by transfer to nitrocellulose paper and immunostaining with the MAbs indicated. A, MH-99; B, RS11-51; C, Ag8. Adjacent lanes of the gel contained protein standards; left margin, molecular weights (x 10⁶).

Fig. 3. Biodistribution of iodinated MAbs in nude mice bearing Calu-3 tumors. The animals were sacrificed on day-7 postinoculation and tumor, organ, and blood radioactivity was counted. The percentage of the injected dose per gram of tissue was calculated from this data. A, 25 μCi (7.4 μg) 125I-RS5-4H6 and 10 μCi (0.6 μg) 131I-NP-4, average tumor size is 0.56 ± 0.35 g. B, 25 μCi (1.9 μg) 131I-RS7-3G11 and 10 μCi (0.6 μg) 125I-Ag8, average tumor size is 0.12 ± 0.06 g. C, 25 μCi (2.5 μg) 131I-RS11-51, average tumor size is 0.48 ± 0.15 g.
injection the localization of the three MAbs into Calu-3 tumors was similar with 6.6 ± 2.3%, 8.6 ± 2.3%, and 6.9 ± 1.8% of the injected dose per gram ± SD localizing in the tumor for RS5-4H6, RS7-3G11, and RS11-51, respectively. This represents approximately three times the incorporation of control immunoglobulins, 2.8 ± 0.1% using Ag8 and 2.1 ± 0.5% with NP-4.

Due to the strong reactivity of RS11-51 with both Calu-3 and GW-39 tumor xenografts as demonstrated by staining of frozen sections with biotinylated MAbs (Fig. 4), this MAb was selected for use in an experiment which compared the in vivo localization in Calu-3 lung tumor-bearing nude mice with the localization in GW-39 tumor-bearing nude mice. The percentage injected dose per gram of 131I-RS11-51 localizing in the tumor, organs, and blood on day-7 postinjection of labeled antibody is shown in Fig. 5. The results of 131I-RS11-51 biodistribution in Calu-3-bearing nude mice (shown in Fig. 3) are replotted here for comparison. The accretion of labeled RS11-51 in GW-39 was 3.5-fold higher than in Calu-3, with 24.4 ± 7.9% of the injected dose per gram localizing in the tumor at 7-day postinjection, yielding a tumor/liver ratio of 18.6 ± 2.3. As a comparison using 131I-Ag8, 5.8 ± 1.0% of the injected dose per gram localized in GW-39 at day-7 postinjection, resulting in a tumor/liver ratio of 2.2 ± 0.1.

The time course of tumor/nontumor ratios of RS11-51 and F(ab')2 fragments of RS11-51 in nude mice bearing Calu-3 and GW-39 tumors are compared in Figs. 6 and 7. As with intact RS11-51, the accretion of RS11-51 F(ab')2 into GW-39 is greatly increased compared to the accretion in Calu-3. At day-3 postinjection the percentage of injected dose per gram of the F(ab')2 fragment is 0.39 ± 0.12% in Calu-3 tumor compared to 5.65 ± 1.5% in GW-39 tumor, resulting in dramatically increased tumor/nontumor ratios using the GW-39 model. This is seen by the fact that the scale on Fig. 7 is six times that of Fig. 6. Tumor/liver ratios of 11.2 ± 1.5 and 55.8 ± 18.7 were observed for incorporation of the RS11-51 F(ab')2 fragment into Calu-3 and GW-39, respectively. Maximal tumor/nontumor ratios were seen in the spleen with 24.9 ± 4.4 and 139.5 ± 68.0 observed at day-3 postinjection of RS11-51 F(ab')2 and 9.7 ± 3.2 and 33.4 ± 9.4 observed at day-14 postinjection of intact RS11-51 in the two tumor models. The organs with minimal tumor/nontumor ratios were the kidneys when RS11-51 F(ab')2 was injected with 5.2 ± 1.2 and 37.8 ± 15.5 at day-3 postinjection; and the lungs when intact RS11-51 was used with 4.2 ± 1.2 and 16.2 ± 4.3 observed at day-14 postinjection in Calu-3 and GW-39-bearing animals, respectively.

DISCUSSION

Three murine MAbs, RS5-4H6, RS7-3G11, and RS11-51, all IgG1, have been raised against human non-small cell carcinoma of the lung and characterized. Based on immunohistochemical reactivity with a panel of normal and tumor frozen tissue sections, the MAbs exhibit pancarcinoma reactivity. Immunostaining was observed in breast, colon, renal, and prostatic carcinomas as well as carcinomas of the lung. The MAbs differ in that they bind to distinct antigens as shown by Western blot analysis and flow cytometric analysis of cultured tumor cells. By Western blot analysis RS5-4H6 reacts with an antigen of molecular weight greater than 300,000 (6). RS11-51 reacts with an antigen of approximately 40,000 molecular weight, and the RS7-3G11 antigen could not be detected by this technique.
The results of $^{131}$I-RS11-51 biodistribution in Calu-3 or GW-39 tumors. Nude mice bearing GW-39 were inoculated with $^{131}$I-RS11-51 (25 µCi, 1.9 µg), and sacrificed on day-7 postinoculation. Average tumor size is 0.51 ± 0.19 g. The results of $^{131}$I-RS11-51 biodistribution in Calu-3-bearing nude mice (shown in Fig. 3) are replotted here for comparison.

Since we observed similarity in tissue distribution (18) and migration in reduced and unreduced SDS gels, between the antigens detected by MH-99 and RS11-51, it is likely that the RS11-51 antigen is closely related to or identical to the epithelial surface antigen with which MAbs MH-99 and 17-1A react (17). Competitive binding or sequential immunoprecipitation experiments are necessary for proof.

A difference in reactivity with cultured human cell lines was observed. RS11-51 stained all tumor cell lines which were tested. RS5-4H6 and RS7-3G11 showed low or negative reactivity with the four colon lines tested and the binding to cell lines derived from lung, breast, and prostatic cancers ranged from negative to strongly positive, but did not follow the same pattern for both MAbs.

The three MAbs all cross-reacted with normal epithelial tissue in immunohistological staining studies, but the intensity of staining varied. RS11-51 exhibited stronger reactivity in normal epithelium than RS7-3G11 or RS5-4H6, especially in the colon. Although the methods employed here do not allow quantitation of antigen expression, it appeared that antigen density in tumors was higher than in normal epithelial tissues. Problems in quantitation include the different morphology of normal versus tumor specimens, as well as variable quality of tissue specimens.

To assess whether these MAbs are potentially useful for clinical applications in tumor imaging and therapy, targeting studies were performed in nude mouse xenograft models. Nude mice bearing heterotransplanted human tumors are currently widely used in investigations of murine MAb targeting to human tumors. The results of radioimmunolocalization studies performed using radioiodinated RS5-4H6, RS7-3G11, and RS11-51 in the Calu-3 model demonstrated that these MAbs targeted the human lung cancer xenograft. The MAb accumulation in tumor was shown to be due to antibody specificity rather than nonspecific accretion since labeled control MAbs exhibited a threefold lower level of accumulation in the tumors.

The results of these radioimmunolocalization studies performed in Calu-3 were comparable to recent reports from other laboratories on the localization of the lung tumor xenografts in nude mice (19-22), although a few laboratories achieved lower tumor localization ratios (23, 24). In all these reports of MAbs reactive with non-small cell carcinoma of the lung, cross-reactivity with some, if not all, normal epithelial tissue was noted in immunohistochemical staining of frozen tissue sections.

Shani et al. (23) utilized KS 1/4, a murine MAb, recognizing a M, 40,000 antigen expressed in epithelial malignancies as well as being highly expressed in normal epithelial tissue (25), and compared the biodistribution of the antibody labeled with four different radionuclides in nude mice bearing human lung adenocarcinoma. These workers concluded that radioiodine-labeled intact KS 1/4 was impractical for visualizing solid tumors. Dazord et al. utilized $^{131}$I-Po66, which recognizes an antigen with a molecular weight in the range of 47,000–50,000, also with cross-reactivity to epithelial tissue, for injection into nude mice bearing xenografts of human lung squamous cell carcinoma (19). The results with Po66 were similar to the localization of the MAbs we report on in this paper except that the preferential localization in tumor was not seen until day 9. Endo et al. (20) studied the localization of MA8, recognizing an antigen of M, 48,000, to a xenografted human small cell carcinoma of the lung. This MAb was raised against small cell carcinoma of the lung but exhibited greater reactivity to non-small cell carcinoma of the lung, and cross-reactions with normal tissue and cancers from other organs was reported to be limited, although reactivity with normal lung was observed. With MA8, again the level of labeled antibody accretion into tumor was similar to that with RS5-4H6, RS7-3G11, and RS11-51. Jitsukawa et al. performed a similar study using a rat anti-non-small cell carcinoma of the lung MAB 2H7, recognizing a M, 40,000 antigen (24). This antibody also exhibited cross-reactivity with other carcinomas and some normal epitel-
lial tissue. Using an adenocarcinoma of the lung xenograft in nude mice, tumor antibody accretion was approximately 2–3-fold higher than in nontumor tissue, but the ratio of MAb 2H7 tumor accretion to that of a nonspecific MAb tumor accretion was only 1.5.

These antibodies all recognize antigens in the molecular weight range of 40,000–50,000, and therefore are not reacting with the same antigen as RS5-4H6, which recognizes an antigen of over 300,000, but may be related to RS11-51. It is also apparent that none of the MAbs exhibits absolute tumor specificity, but rather that some cross-reactivity with normal epithelial tissue is present.

The comparative biodistribution of RS11-51 IgG in GW-39 and Calu-3 indicated a 3.5-fold higher antibody accretion (percentage injected dose per gram) in GW-39 with corresponding increases in tumor/nontumor ratios, and an even more dramatic increase when F(ab')2 fragments were used. The reasons for the higher antibody accretion in GW-39 remain to be established. Although the physiology of GW-39 and Calu-3 tumors were not studied, differences in histopathology are present (signet ring carcinoma, GW-39, versus moderately well differentiated adenocarcinoma, Calu-3). Viability differences were also noted. GW-39 tumors were generally 100% viable and necrotic areas were often seen in Calu-3 tumors. These results indicate that comparisons of the localization of different antibodies which are drawn from data obtained from different tumor models cannot yield meaningful conclusions on the relative potential of MAbs in the clinical setting. These studies do play a role in providing comparative information on the targeting capabilities of different monoclonal antibodies within the same system, and yield preclinical information necessary to justify a clinical trial.

Although these experiments are useful indicators of the ability of specific MAbs to localize and be retained by tumors, predictions of subsequent clinical localization and therapy must be qualified since significant differences exist between the model and the clinical situation. Factors such as the consequences of cross-reactivity with antigens in normal human tissues and blood, the effect of the site of tumor (e.g., in nude mouse models regardless of tumor's site of origin), and heterogeneity among clinically presented tumors are not approximated by the model.

Further evaluations of the MAbs discussed in this paper, RS5-4H6, RS7-3G11, and RS11-51, are warranted in light of the high frequency of antigen expression on a wide variety of tumors and the ability to target tumors in vivo. Potential applications exist for the use of these MAbs in cancer imaging and therapy, and for serological detection of tumor-associated antigens.

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