Monoclonal Antibody Targeting of Human Non-Small Cell Carcinoma of the Lung

Rhona Stein, Robert M. Sharkey, and David M. Goldenberg

Center for Molecular Medicine and Immunology, at the University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07103

Abstract

Murine monoclonal antibody RS5-4H6 is an IgG1, which was raised against a crude membrane preparation of Calu-3, a human adenocarcinoma of the lung cell line. MAb RS5-4H6 reacts with the majority (14 of 15) of surgical specimens of non-small cell carcinomas of the lung by immunoperoxidase staining. Reactivity with this antibody was not limited to tumors of the lung but rather exhibited pancarcinoma reactivity, staining 87% of tumors from all organs tested. In this study we examined the potential of radiolabeled RS5-4H6 to target Calu-3 xenografts in nude mice. The monoclonal antibody was found to localize preferentially to the heterotransplanted tumors, with 6.6% of the injected dose/g accumulating in the tumor at 7 days, a 3-fold higher level than an anticalcitriol monoclonal antibody which was used as a negative control.

Introduction

Radiolabeled MAbs1 have been utilized experimentally in the last several years as agents capable of specifically locating or attacking cancer cells in a number of malignancies, including melanoma (1), colon carcinoma (2, 3), and breast carcinoma (4). The in vivo use of radiouclide-labeled antibodies to several types of tumor-associated antigens or products has proven to be a reliable method for defining primary, recurrent, metastatic, and occult carcinoma (5). Immunotoxins, composed of monoclonal antibodies and toxins either from bacteria or plant sources, are also showing promise as specific anticancer agents; these conjugates combine the specificity of the MAb with the cytotoxicity of the toxin (6). These promising techniques are just beginning to be exploited in lung cancer (7–12). MAbs, against previously undefined antigens, were utilized as antibody targets in these studies.

RS5-4H6, a murine MAb, was produced in our laboratory following immunization of BALB/c mice with a membrane preparation of an adenocarcinoma of the lung cell line, Calu-3 (13). The membrane preparation was prepared from solid tumors grown as xenografts in athymic (nude) mice. This approach was used to increase the probability of expression of antigens which may be expressed in decreased amounts when cells are grown in tissue culture. MAB RS5-4H6, an IgG1, was selected on the basis of its binding to the immunogen, its lack of reactivity with normal human liver membranes and WBC, and its reactivity with frozen sections of lung tumor specimens. Immunohistology showed a selectivity for normal and neoplastic lung epithelium, as well as other cancers. The RS5-4H6-antigen demonstrates a pancarcinoma distribution in a majority of lung carcinomas (non-small cell), breast carcinomas, colon carcinomas, renal carcinomas, and ovarian carcinomas. Most other normal human tissues were not stained, but occasional cells of the stomach, salivary, and other glands, as well as kidney tubules, were reactive. Although MAb RS5-4H6 stained 87% of the cancer tissues tested from the various organs, strong reactivity (greater than 25%) was seen on only 3 of 22 cancer cell lines studied by flow cytometry. MAB RS5-4H6 binds to an antigen with an apparent molecular weight greater than 300,000.

Due to the high frequency of RS5-4H6 antigen expression in a wide variety of tumor types and its limited presence on normal human tissue, we have selected this antibody for initial studies of tumor localization in vivo.

Materials and Methods

Cell Culture. Human carcinoma cell lines derived from lung (Calu-3 and SK-MES-1) and colon (LS 174T and LoVo) were purchased from the American Type Culture Collection (Rockville, MD). The cells were grown as monolayers in either RPMI 1640, Dulbecco’s modified Eagle’s medium, or minimal essential medium (Gibco Laboratories, Grand Island, NY), supplemented with 10% fetal bovine serum, penicillin (100 μg/ml), and streptomycin (100 μg/ml). The cells were routinely passaged after detachment with trypsin-0.2% EDTA.

Monoclonal Antibodies. The production and initial characterization of RS5-4H6 has been described previously (13). Briefly, BALB/c mice were immunized with a membrane preparation of Calu-3, a human lung adenocarcinoma cell line grown in nude mice. Hybridization was performed according to the method of Galfre et al. (14), using the nonsecreting mouse myeloma cell line SP 2/0-Ag14 as a fusion partner and polyethylene glycol 1000 as the fusing agent. NP-4, an anti-CEA IgG1 monoclonal antibody (15) and Ag8 (ATCC, Rockville, MD), an irrelevant mouse myeloma IgG1 designated P3X63Ag8, were used as control antibodies in this study. The antibodies were isolated from ascites fluid by passage through a protein A immunosorbent.

NP-4 was radioiodinated with 125I (New England Nuclear, North Billerica, MA) by the chloramine-T method (16). RS5-4H6 was radioiodinated with the 125I-Bolton-Hunter reagent (New England Nuclear) according to the original procedure of Bolton and Hunter (17).

Immunoperoxidase Staining. Immunoperoxidase staining was performed on 5–6-μm-thick cryostat sections of frozen autopsy and surgical specimens. The sections were fixed with 2% buffered formaldehyde and stained using the avidin:biotin:horseradish peroxidase complex method (Vector Laboratories, Burlingame, CA) according to the protocol suggested by the manufacturer.

Flow Cytometry. Tissue culture cell lines were tested for reactivity with MAbs using an indirect immunofluorescence assay. Washed cells (100 μl) were mixed with 25 μl of MAb (50 μg/ml) and incubated at 4°C for 30 min. The cells were then washed twice with Dulbecco’s phosphate-buffered saline without calcium and magnesium containing 1% horse serum, followed by the addition of 100 μl of fluorescein-conjugated goat anti-mouse IgG (Tago, Inc., Burlingame, CA). The tubes were incubated for 30 min at 4°C. The cells were washed twice as above and analyzed by flow cytometry using an Ortho Spectra III flow cytometer (Ortho Diagnostics Inc., Westwood, MA).

Animal Studies. Female nude BALB/c mice were used. Tumors were propagated by s.c. injection of 0.2 ml of a 10% suspension of minced tumor. The mice were used for the biodistribution study when tumors reached a size of approximately 0.5 g (7 weeks). 125I-RS5-4H6 was mixed with 131I-NP-4 and injected i.v. into the tumor-bearing animals. The animals were sacrificed on day 7 following the radioantibody injection.


2 To whom requests for reprints should be addressed, at the Center for Molecular Medicine and Immunology, 1 Bruce Street, Newark, NJ 07103.

3 The abbreviations used are: MAb, monoclonal antibody; CEA, carcinoembryonic antigen.
The radioactivity of both $^{131}$I and $^{125}$I 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/g of tissue, localization ratio (ratio of specific versus irrelevant IgG), and tumor/nontumor ratios.

Results

Immunoperoxidase Staining of Frozen Tissue Sections. The immunoperoxidase technique was used to study reactivity of frozen tissue sections with MAb RS5-4H6. The antigen recognized by this MAb was found to be tumor associated. These results are summarized in Table 1 and presented in more detail elsewhere (13). MAb RS5-4H6 stained all specimen of the adenocarcinoma, squamous cell carcinoma and adenosquamous cell carcinoma of the lung which were tested. Positive reactivity was not limited to tumors from the lung, but rather was observed with tumors from all organs tested, including breast, colon, lung, kidney, and ovary. Overall, 87% of the cancer tissues tested were reactive. RS5-4H6 also stained normal lung epithelium in some specimens, but the antigen density appeared higher in tumor cells. Most other normal human tissues were unreactive, except for glands in the breast and kidney tubules. Occasional cells of colon, sebaceous glands of the skin, and other glandular tissue showed reactivity with this MAb.

In Vivo Distribution. A biodistribution experiment was performed in Calu-3 tumor-bearing nude mice. As shown in Table 2, RS5-4H6 shows strong reactivity with this cell line although reactivity is low or negative with most other lines (13). NP-4, an anti-CEA monoclonal antibody, exhibited the opposite reactivity, reacting with other cell lines (LoVo and LS 174T) but showing negligible binding to Calu-3 cells.

MAb RS5-4H6 was labeled with $^{125}$I by the Bolton-Hunter technique due to loss of immunoreactivity following iodination with chloramine-T (data not shown). Using the $^{125}$I-Bolton-Hunter reagent, 15% of the radioactivity was incorporated into antibody, yielding a specific activity of 3.36 $\mu$Ci/µg, a ratio of 0.25 mol of $^{125}$I-Bolton-Hunter reagent per mol of RS5-4H6.

The Calu-3 tumor-bearing mice were inoculated i.v. on day 0 with both $^{125}$I-RS5-4H6 (25 $\mu$Ci) and $^{131}$I-NP-4 (10 $\mu$Ci). The animals were sacrificed on day 7 and the radioactivity of the tumor, organs, and blood were counted. The percentage of injected dose per g localizing in the tumor and organs are presented in Fig. 1. RS5-4H6 was found to localize preferentially to the heterotransplanted tumors with 6.6 ± 2.3% (SD) of the injected dose/g localizing in the tumor after 7 days, compared to values of 1.6 ± 0.8% to 2.4 ± 0.9% localizing in the other organs.

The comparative distribution in RS5-4H6 and NP-4, presented in Table 3, shows that the RS5-4H6 tumor accumulation was due to antibody specificity rather than nonspecific accumulation, since NP-4 did not show similar accumulation in the tumor. Tumor/nontumor ratios of 2.9 to 4.9 are seen in the organs with RS5-4H6, whereas NP-4 tumor/nontumor ratios were in the range of 1.3 to 2.4. The localization ratio, which relates the percentage of injected dose/g of the specific MAb (RS5-4H6) to that of the control MAb (NP-4), indicates that RS5-4H6 attained a 3.1-fold higher level in the tumor than the control anti-CEA, MAb.

Discussion

While diverse cancers have been targeted with radiolabeled monoclonal antibodies, tumors of the lung have not been studied extensively by this approach. RS5-4H6, a MAb raised in our laboratory against non-small cell carcinoma of the lung, was selected for an initial study on the feasibility of using MAbs to localize lung tumors in vivo. This MAb was selected due to its high frequency of reactivity with surgical specimens of adenocarcinoma and squamous cell carcinoma of the lung and its limited expression on normal tissues. In the present study, a double-label protocol was used, whereby $^{125}$I-RS5-4H6 and $^{131}$I-NP-4, an anti-CEA MAb, were compared. RS5-4H6 was found to localize preferentially to the heterotransplanted lung tumors, with 6.6% of the injected dose/g localizing in the tumor at 7 days. The tumor accumulation was shown to be due to antibody specificity since the labeled NP-4 exhibited a 3-fold

Table 1 Immunohistological reactivity of RS5-4H6 with human tumor sections

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>No. positive/no. tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung tumors</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>5/5</td>
</tr>
<tr>
<td>Adenosquamous cell carcinoma</td>
<td>2/2</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>7/7</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>0/1</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>4/5</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>3/5</td>
</tr>
<tr>
<td>Kidney carcinoma</td>
<td>4/4</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>2/2</td>
</tr>
</tbody>
</table>

Table 2 Reactivity of MAbs with human tissue culture cell lines

<table>
<thead>
<tr>
<th>Target cell line</th>
<th>Reactivity (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RS5-4H6</td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td></td>
</tr>
<tr>
<td>Calu-3</td>
<td>78.6*</td>
</tr>
<tr>
<td>SK-MES-1</td>
<td>2.8</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td></td>
</tr>
<tr>
<td>LoVo</td>
<td>5.3</td>
</tr>
<tr>
<td>LS 174T</td>
<td>3.8</td>
</tr>
</tbody>
</table>

* Percentage of cells with positive fluorescence minus background fluorescence detected in absence of first antibody.

Table 3 Comparative distribution of labeled antibodies in tumor-bearing nude mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tumor/nontumor ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RS5-4H6</td>
</tr>
<tr>
<td>Liver</td>
<td>3.7 ± 1.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.9 ± 2.7</td>
</tr>
<tr>
<td>Lungs</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>Blood</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Tumor</td>
<td>3.1 ± 0.8</td>
</tr>
</tbody>
</table>
lower level of accumulation in the Calu-3 tumors.

The results of the radioimmunolocalization study performed using RS5-4H6 were comparable to recent reports from other laboratories on the targeting of lung tumor xenografts in nude mice (7–10), although a few laboratories achieved lower tumor localization ratios (11, 12). In all cases, except SM1 (which reacts with small cell carcinoma only and not non-small cell carcinoma of the lung (10)), cross-reactivity with some, if not all, normal epithelial tissue was noted. 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 with a molecular weight of over 300,000.

The effectiveness of MAbs for clinical application in detection and therapy of cancers will depend on the sensitivity and specificity of these probes. The antibodies must be specific to the tumors, i.e., not generally reactive with normal tissue. However, absolute specificity is probably not required. Quantitative differences in antigen concentration or in the accessibility of the antigens to MAb may yield sufficiently high accretion of MAb in the tumors relative to nontumorous organs to have clinical value. To date, absolute specificity has not been shown to be achievable, and even if such a MAb were identified, its use would almost certainly sacrifice sensitivity since antigens of this limited range would be likely to be present at low levels in the tumor. Tumor-associated antigens which may also be present in normal epithelial tissues at quantitatively restricted levels or at locations which may be inaccessible to MAb, such as in the lumen of glands, may be preferable, since a higher antigen density can presumably yield a higher level of MAB accumulation in the tumor.

An improvement in the ratio of accretion of labeled MAb in the tumors compared to nontarget tissues may be attainable by improving MAb clearance from the blood pool or by engineering a MAb cocktail by mixing MAbs with different targeting properties. Antibodies reactive with internal antigens (cytoplasmic or nuclear) have been shown to localize to tumors by accumulating in necrotic areas with a lack of accumulation in the viable areas of the tumor. An example of this was suggested by Chan et al. (18) in their study of the localization of lung cancer using a radiolabeled MAb directed against a nuclear oncogene product. Conversely, Endo et al. (8) demonstrated that the MAb in their localization study (MAb 8) was absent from the central necrotic area of the tumor but did accumulate in the surrounding viable zone. Combinations of antibodies with such differences would likely yield an improved accretion of labeled antibody in the tumor mass.

Improved blood pool clearance of labeled MAbs is under investigation by several methods, including the use of antibody fragments (9), second antibody (19), and chemical modification (20). These methods require study in a lung cancer model system. Although enhanced clearance of labeled MAb will yield lower accumulation in target organs, it may also provide less chance for interaction with the tumor and therefore may also lower tumor accretion. In view of the high frequency of RS5-4H6 antigen expression on a wide variety of tumor types, its limited expression on normal human tissue, and its ability to localize to tumor in vivo, MAB RS5-4H6 may have potential use in the diagnosis and therapy of cancer. Further studies on the chemistry of the antigen recognized by this MAB and on its tumor-targeting potential are in progress.

References

Monoclonal Antibody Targeting of Human Non-Small Cell Carcinoma of the Lung

Rhona Stein, Robert M. Sharkey and David M. Goldenberg


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/50/3_Supplement/866s

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.