**In Vitro** and **in Vivo** Reactivity of an Internalizing Antibody, RS7, with Human Breast Cancer

Lisa B. Shih, Hong Xuan, Rosarito Aninipot, Rhona Stein, and David M. Goldenberg

Garden State Cancer Center at the Center for Molecular Medicine and Immunology, Newark, New Jersey 07103

Abstract

RS7, a murine IgG1 antibody raised against human lung carcinoma, possesses pancarcinoma reactivity. The antigen defined by this antibody is present in tumors of the lung, stomach, bladder, breast, ovary, uterus, and prostate. Efficient targeting and therapy by radioiodinated RS7 has been demonstrated previously in animals bearing Calu-3 (an adenocarcinoma of the lung) xenografts. In this study, the efficiency of tumor targeting and the efficacy of therapy of this antibody in nude mice bearing the MDA-MB-468 human breast carcinoma were evaluated. The tumor: nontumor ratios of RS7 were 1.9–2.1 times higher than those for Ag8 (the control antibody) on day 14, except for the heart. These values were similar to that of RS7 in the Calu-3 xenograft model. Radioimmunotherapy using 250 μCi of 131I-labeled RS7 in animals bearing ~0.1 cm³ tumors (~10 days old) caused the disappearance of tumors in 6 of 10 animals at 2 weeks postinjection. Tumors eventually disappeared from all animals, and animals remained tumor-free until the termination of the study (11 weeks of duration), except for one animal that developed a transient reappearance of tumor. The tumors in animals that received an equal dose of 131I-labeled Ag8, or unlabelled RS7 or Ag8, either were unchanged or continued to grow. Treatment that used 275 μCi of 131I-labeled RS7 in animals carrying established tumors (1 month old, ~0.2–0.3 cm³) showed that this antibody is effective in controlling the growth of this tumor. Tumors in the treatment group began to disappear between the second and third weeks after the injection of the radiolabeled antibody. Seven of 10 animals remained tumor free at 15 weeks after the injection. Tumors in animals that received an equal dose of control antibody persisted but grew at a slower pace compared to the untreated group. No systemic toxicity was observed.

Introduction

Breast cancer is the most common malignancy among women in North America, accounting for 27% of their cancers (1, 2). In the United States, breast cancer is the leading cause of death among women between the ages of 40 and 55 years. It is estimated that ~182,000 new cases of breast cancer will be diagnosed and 46,000 women will die from this disease annually (1). Treatment modalities such as surgery, radiation therapy, chemotherapy, and hormone manipulation are the common therapy modalities used, but despite the use of new hormonal and chemotherapeutic agents in the past decade, the median survival after recurrence with metastases is only 2 years (3). Thus, there is a continued need for new treatment and early detection modalities.

With the recognition that MAbs can be used to localize a diverse number of tumors, many investigators have focused on the ability of these MAbs to carry various tumoricidal substances for a more selective delivery of an effective dose of these agents to all disseminated cancer sites (4). In this study, the potential of the RS7 antibody as a candidate for the radioimmunodetection and radioimmunotherapy of breast cancer was evaluated. The generation and characterization of the RS7 antibody have been reported previously (5). RS7 is an IgG1 murine MAb with pancarcinoma reactivity and was raised against human squamous cell carcinoma of the lung (6). On the basis of the limited presence of antigen in normal human tissues and the high frequency of expression in malignant breast tissue, this antibody appeared to be a suitable candidate for the target disease. In this study, the reactivity of this antibody with a tumor cell line, the internalization of the antibody, and the tumor targeting of the MAb were determined. The effectiveness of the 131I-labeled MAb as a radiotherapeutic agent in the treatment of human breast cancer xenografts in nude mice was also evaluated. A human mammary adenocarcinoma cell line, MDA-MB-468 (7), was selected for this study. Tumor response after the administration of the specific 131I-labeled RS7 was compared to that of the nonspecific 131I-labeled MAb, as well as to unlabelled RS7.

Materials and Methods

**MAbs.** The production and characterization of the RS7 antibody has been described previously (5). Ag8, an isotype-matched irrelevant mouse myeloma cell line designated P3 × 63 Ag8, was obtained from the American Type Culture Collection (Rockville, MD) and was used as the negative control antibody in this study. The antibodies were purified from ascites fluid by passage through a protein A immunosorbent column (MAPS II, obtained from Bio-Rad, Richmond, CA).

**Cell Line.** The human breast adenocarcinoma MDA-MB-468 was purchased from the American Type Culture Collection. Cells were grown as a monolayer in DMEM (GIBCO, Grand Island, NY) supplemented with 5% horse serum, 5% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine. The cells were routinely passaged after detachment with trypsin-0.2% EDTA.

**Radiolabeling of MAbs.** MAbs were radiodiiodinated with 131I or 125I (New England Nuclear, North Bellerica, MA) by the chloramine T procedure (8). Unbound radiiodide was removed by gel filtration on a PD-10 column (Pharmacia, Piscataway, NJ) equilibrated in 0.04 M sodium phosphate (pH 7.4) with 0.15 M NaCl, 0.02% NaN3, and 1% human serum albumin. Purity of the radiolabeled antibodies, as analyzed by high-performance liquid chromatography with a Bio-Sil SEC gel filtration column (Bio-Rad) and detected by an in-line radioactivity detector (Beckman, Irvine, CA), indicated <5% of free iodide in the preparation. A specific activity of 9.8–10.5 μCi/mg and 8.7–13.6 μCi/mg of 131I-labeled RS7 and 125I-labeled Ag8 were achieved, respectively. Immunoreactivity of the labeled antibody was evaluated on target cells by following the cell binding assay (5). The average percentage of specific binding, bound by 1 × 10⁶ of MDA-MB-468 cells, was 12% for radiolabeled RS7 and ~0% for radiolabeled Ag8. The specificity of the labeled antibody was also examined by incubating the target cells with the labeled antibody in the presence of a saturating dose of the unlabeled antibody. Radioactivity nonspecifically bound to the cell preparation was in the range of 0.8–2.4%, as detected by this method.

**Reactivity of RS7 with the Target Cell, MDA-MB-468.** The reactivity of the RS7 antibody with target cells was evaluated by flow cytometry with an indirect immunofluorescence assay. Target cells (100 μl at 1 × 10⁶ cells/ml) were incubated with serially diluted RS7 at 4°C for 30 min. After washing, the cell-bound antibody was then detected by fluorescein-conjugated goat anti-mouse antibody. The fluorescence intensity and the percentage of positive staining were analyzed by flow cytometry using a FACScan (Becton Dickinson, Mountain View, CA).

**Internalization of RS7.** The internalization of RS7 by the target cells was followed by fluorescence labeling according to the procedure of Prkr et al. (9). MDA-MB-468 cells, at 5 × 10⁵ cells/ml of fresh medium, were added to tissue culture tubes. RS7 and the control antibodies were added to a final concentration of 40 μg/ml. After incubation at 4°C for 1 h, the cells were washed, and the internalization was followed at 37°C. At the time points

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1 Presented at the “Fifth Conference on Radioimmunodetection and Radioimmuno-therapy of Cancer,” October 6–8, 1994, Princeton, NJ. Supported in part by USPHS Grant CA 39841 from the NIH and Grant EDT-16 from the American Cancer Society.

2 Present address: Immunomedics, Inc., 300 American Road, Morris Plains, NJ 07950.

3 To whom requests for reprints should be addressed, at the Garden State Cancer Center at the Center for Molecular Medicine and Immunology, Newark, NJ 07103.

4 The abbreviations used are: MAb, monoclonal antibody; TCA, trichloroacetic acid.
indicated, the cells were cooled to 4°C and washed with 1 ml of cold 0.2%
BSA/PBS (0.01 M phosphate with 0.15 M NaCl (pH 7.2)) and then twice with
PBS. The cells were then treated with 1 ml of Formalde-Fresh (a 10% formalin
solution obtained from Fisher, Fair Lawn, NJ) for 15 min at 4°C. After
washing, antibody present either on the cell surface or inside the cells was
detected by treatment with FITC-labeled goat antimouse antibody (Tago,
Burlington, CA). Fluorescence distribution was evaluated under a BH-2
fluorescence microscope (Olympus, Lake Success, NY). Control antibodies,
such as A103 antibody (an IgG2a antibody that binds to the surface of all
human epithelial cells but does not internalize efficiently), 5E9 antibody (an
antitransferrin receptor antibody, IgG1, with known efficient internalization),
and Ag8 (a negative control immunoglobulin, IgG1), were used to establish
the levels of specific and nonspecific fluorescence.

Catabolism of the Cell-bound Antibody. Cellular catabolism of the cell-
bound RS7 antibody was followed using reported procedures (10, 11). Briefly,
MDA-MB-468 cells were suspended in fresh medium at a final concentration
of 2.5 × 10^5 cells/mL and were plated in 96-well plates at 100 μL/well. Cells
were allowed to attach for 24 h and then were incubated with the labeled
antibody for 2 h at 37°C in fresh medium. Approximately 5 × 10^5 cpm of
[125I]labeled RS7 was used for each well. After binding, the cells were washed
repetitively with medium to remove the unbound antibody, and the total
cell-bound radioactivity (i.e., 0 time point) was determined after the cells were
detached by treatment with 2 N NaOH and collected with a cotton swab.
Triplicate wells were used for each time point. In general, ~2–3% of the added
radioactivity could be bound. To determine nonspecific binding, 40 μg/mL of
unlabeled RS7 antibody (a dose that is more than sufficient to saturate the
surface antigen) was added to control wells 30 min before the addition of the
labeled antibody. The nonspecifically bound radioactivity was generally <10%
of the total cell-bound activity. The remainder of the cells were then resus-
pended in 200 μL of fresh medium and incubated at 37°C for cellular process-
ing. At 2, 4, 21, and 45 h, 100 μL of the supernatant (which was half of the
supernatant) were collected and counted to determine the percentage of radio-
activity that was released. After counting, the same supernatant was
then treated with 5 ml of cold 10% TCA solution to precipitate the intact antibody
(or large fragments of IgG) in the presence of 1 mg of bovine IgG as carrier
protein. After centrifugation at 4000 × g for 15 min in a Sorvall RC2-B
centrifuge, the precipitate was counted to estimate the fraction of "intact" IgG
(large fragments of IgG would also be precipitable with TCA). The percentage
of intact IgG was determined by comparing the precipitable radioactivity to the
total. The cell fraction was washed twice, harvested as described above, and
counted. Thus, at each time point, the percentage of cpm retained by the cells
and the percentage of cpm released into the supernatant were determined. The
released cpm was further divided into intact and degraded components.

Rate of Antibody Internalization. The rate of antibody internalization
was determined by following the procedure of Opreasko and Wiley (12) using
radioiodinated MAb as a tracer. Briefly, MDA-MB-468 cells were plated in
96-well plates (Dynatech, Chantilly, VA) at 30,000 cells/well and allowed to
attach for 24 h. The cells were washed and incubated with [125I]-labeled RS7 at 4°C
for 2 h. After removal of the unbound radioactivity, the cells were incubated
at 37°C for internalization. At various time intervals, sets of cells (triplicates
for each time point) were chilled immediately in an ice water bath to prevent
further internalization. The cells were washed once with cold medium (200
μL), and the surface-bound radioactivity was then removed by treatment with
200 μL of 0.1 M acetate-0.1 M glycine (pH 3.0) at 4°C. At the 0 time point, no
internalization was anticipated, in that most of the radioactivity should remain
on the cell surface. Therefore, the percentage of radioactivity removable at
time 0 was used to determine the efficiency of the acid treatment. In this case,
the acid treatment was able to remove ~85–90% of radioactivity. Radioactivity
that was not removed by this procedure was considered to be internalized and
was normalized by the percentage of cpm that could not be removed from the
cells at time 0. The CPM_intracellular/CPM_surface ratio was plotted versus time,
and the rate of internalization was determined from the slope of the line.

In Vivo Studies. Mice were infected in 6–8-week-old female nu/nu
mice (Harlan-Sprague-Dawley, Indianapolis, IN) by s.c. injection of 2.3 × 10^7
cultured cells, which reach an average size of ~0.1 cm^3 after 10 days and
~0.2–0.3 cm^3 after 1 month. The animals were randomly divided into groups
for the in vivo studies.

In the biodistribution studies, 10 μCi of control antibody (125I-labeled Ag8)
and 25 μCi of specific antibody (131I-labeled RS7) were coinjected i.v. into the
tumor-bearing animals. At the times indicated, blood was removed from the
anesthetized animals by heart puncture. The animals were then sacrificed, and
the radioactivity of both radioisotopes in the tumor, lungs, liver, spleen,
kidney, heart, bone, and blood was determined, after correction for physical
decay and downsampling, in a γ-scintillation counter. The results are the mean ±
SD of five animals per group.

In therapy experiments, groups of animals (ranging from 6 to 10) received
injections of 250 μCi of 131I-labeled RS7 or Ag8 via the tail vein. Control
animals received unlabeled RS7 or Ag8 at the equivalent protein dose as that
of the treated groups (i.e., ~30 μg). The tumor growth rate was determined by
measuring the length, width, and thickness of each tumor with a caliper and
was expressed in cubic centimeters. Measurements were taken before injection
of the radiolabeled antibody and then on a weekly basis. Toxicity was mea-
sured by change of body weight.

Dosimetry. The approximate radiation dose absorbed by the tumor and
other tissues was calculated from the biodistribution data obtained over the
course of 14 days, as described previously (13). The tumor radiation dose was
determined by computing a trapezoidal integration to avoid overestimation of
the cumulative dose, with a zero time value of 0 being assumed. However, for the
normal tissues, the rad (cGy) dose was calculated by following the exponential
integration, and the zero time point value was extrapolated according to the
exponential curve.

Results

Reactivity of RS7 with MDA-MB-468 Cells. The reactivity of RS7 against MDA-MB-468 cells is shown in Fig. 1. RS7 antibody at
a level as low as 0.5 μg/mL is capable of producing positive staining on
most cells. In addition to its strong reactivity, this antibody was
also internalized efficiently by MDA-MB-468 cells, in a fashion
similar to that observed in the Calu-3 cell line. In this study, the rate
and extent of antibody internalization was investigated by labeling
the cells with un conjugated antibody, followed by detection with fluores-
cine-labeled second antibody. At the 1-h time point, a high percentage
of antibody has been internalized, with a trace of the membrane
staining remaining (Fig. 2). The patterns of fluorescence distributions
of control antibodies A103 and 5E9 were used as the reference for cell
surface binding and internalization studies, respectively (data not
shown). The internalization of the antibody was further demonstrated
by a quantitative measurement that used 125I-labeled RS7 as a tracer
with MDA-MB-468 cells. Cells were incubated with the radioiodi-
nated RS7 for 2 h at 4°C. The cells were washed to remove the unbound radioactivity and then incubated at 37°C to allow internal-
ization. At specific time points, the cells were chilled in an ice bath to
prevent further cellular uptake, and the surface-bound antibody was
then removed by acid treatment. Acid treatment of the 0 h time point,
where all the antibody is present on the cell surface, allows the
removal of ~85–90% of surface-bound antibody. The rate of RS7

![Fig. 1. Binding of RS7 to MDA-MB-468 cells in flow cytometry. The linear fluores-
cence scale, as depicted on the top of the figure, was followed; , baseline fluores-
cence level that was established by the binding of the irrelevant Ag8 antibody. The
fluorescence intensity of antibody binding that is greater than this level is considered
positive. A, cells were treated with 25 μg/mL of Ag8 control antibody. B, cells were treated
with 0.5 μg/mL of RS7. Most of the cells in the latter case were positively stained.
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internalization ($K_o$) on MDA-MB-468 cells was found to be 0.0147 min$^{-1}$ ($r = 0.9928$), as calculated from the slope of CPM$_{intracellular}$/CPM$_{surface}$ versus time (Fig. 3). The rate of internalization of RS7 by MDA-MB-468 cells was slightly slower than that observed previously for Calu-3 lung carcinoma cells (0.0232 min$^{-1}$; $r = 0.9757$; Ref. 5). Nevertheless, this rate of internalization suggested that under saturating conditions, 50% of the cell-bound antibody can be internalized within 70 min at 37°C, as calculated from the slope at the point where the CPM$_{intracellular}$/CPM$_{surface}$ ratio equals 1.

Because it is assumed that after the uptake of bound MAb by cells, intracellular processing takes place, which eventually degrades the antibody, the processing of RS7 by MDA-MB-468 cells was evaluated (10, 11). In this study, the presence of degraded antibody in the cell preparation is used as an indication of antibody internalization. Radioiodinated RS7 was allowed to bind with the cells at 37°C for 2 h. After removal of the excess antibody, the cell-bound radioactivity and the activity released into the supernatant were followed for 45 h (Fig. 4). Radioactivity associated with the cells (which includes the activity both on the surface and within the cells) or released into the supernatant was measured. The radioactivity released into the supernatant was further characterized as intact antibody or degraded fragments by TCA precipitation. Degraded fragments, due to their small size, are not precipitated by TCA; thus, the radioactivity present in the supernatant that is nonprecipitable by TCA is considered to be degraded as the result of antibody internalization. As shown in Fig. 4, the radioactivity was released gradually into the supernatant. At 21 h, <30% of the radioactivity remained cell bound, whereas a significant amount of the radioactivity was released. A large percentage of the released radioactivity was found in the TCA-nonprecipitable fraction (i.e., degraded), which indicated that the antibody must have been internalized by the cells and was consequently degraded. On the basis of these observations, it is concluded that RS7 is efficiently internalized and processed by MDA-MB-468 cells but at a rate that is slightly slower than that in lung cancer cells [i.e., Calu-3 cells, in which ~80% of the cell-bound antibody was degraded by 21 h (14)]. The slower degradation of RS7 by MDA-MB-468 cells may be due to the slower rate of internalization ($K_o$) of antibody in this system, as well as differences in intracellular trafficking affecting its rate of catabolism.

**Biodistribution.** The distribution of radioiodinated RS7 in nude mice bearing MDA-MB-468 tumor xenografts was determined 1, 3, 7, and 14 days after coadministration of $^{125}$I-labeled Ag8 and $^{131}$I-labeled RS7. The percentage of injected dose of $^{125}$I-labeled RS7 per gram of tumor on days 1–14 was between 6.9 and 16.2% (Table 1). This level of tumor uptake was approximately 2-fold higher than that of the specific antibody. As shown in Fig. 5, the tumor:non-tumor ratios of the specific antibody continue to increase over the period of study, whereas that of the nonspecific antibody was either maintained at the same level or decreased over the 14-day period. The tumor:heart ratio of both specific and nonspecific antibodies on days 1 and 3 was found to be greater than at the later time points. This observation is believed to be associated with the large SD observed at these two time points.

The estimated radiation dose to the tumor and other tissues, shown in Table 2, was calculated based on the distribution study over 14 days and the masses of all the tissues. During a 14-day period, a total dose of ~2175 cGy could be delivered to the tumor by 250 µCi of $^{131}$I-labeled RS7, whereas the dose delivered to the heart, kidney, spleen, and liver was ~25% of this level. The radiation dose delivered to the lungs (860 cGy/250 µCi) was slightly higher than other normal tissues. This result may have been caused by the slow blood clearance, combined with the high quantity of blood in the lungs. The radiation dose delivered to the tumor by the nonspecific antibody, Ag8, was only 50% of the level of the specific antibody, whereas the dosages delivered to normal organs were similar regardless of the specificity of the antibody. These observations further suggest that RS7 is specific in delivering radiation to the tumor but behaves similarly to the control antibody in the normal tissues. The cumulative absorbed dose was also normalized to blood to correct for the effect of blood pool radiation. Under these conditions, the dosages in normal organs were similar for the two antibodies, whereas the tumor dose was 1.8-fold greater with RS7.

**Radioimmunotherapy.** Nude mice bearing MDA-MB-468 tumor xenografts (~0.1 cm$^3$) were inoculated with 250 µCi of $^{131}$I-labeled antibodies. Animals that received the specific antibody demonstrated a dramatic response to the treatment. In 6 of 10 mice, the tumors regressed at 2 weeks postinjection (Fig. 6). Tumors...
disappeared from all animals by the fourth week postinjection, and the animals remained tumor free until the termination of the study (i.e., 12 weeks postinjection), except for one animal that developed a transient reappearance of tumor. The reason for this transient reappearance of tumor, which later disappeared, was not clear. On the contrary, most of the tumors in the animals that received equal radioactivity of nonspecific antibody (i.e., 131I-labeled Ag8) either persisted or continued to grow. A transient tumor regression was observed between 2 and 5 weeks after injection. The tumor growth in mice receiving injections of 30 μg of unlabeled RS7 or Ag8 was not inhibited; the tumors reached an average size of approximately six times that at the onset of the study.

Therapy was also evaluated on established tumors of ~0.2–0.3 cm³ in size (~1 month after cell inoculation). When animals were given 131I-labeled RS7 at 275 μCi/mouse, a significant difference of tumor growth from the untreated control group was observed (Fig. 7). Tumors in this group began to disappear as early as 2 weeks after injection, and complete disappearance was noted by the fourth week. Seven of 10 animals remained tumor free at the termination of the study (i.e., 15 weeks after the treatment). At this time point, tumors in the untreated control group had increased ~15-fold; final sizes were ~2–3 cm³ (2 tumors became ulcerated on the ninth week). Mice that received an injection of 275 μCi of 131I-labeled Ag8 also showed decreased tumor growth compared to the untreated group. Although the tumors in this group never disappeared completely and began to return to their pretreatment size by the sixth week after injection, the difference in tumor growth rates between the specific antibody (RS7) and the nonspecific antibody (Ag8) was not significant (P < 0.2). The therapeutic effect produced by the nonspecific antibody may have been due to the high sensitivity of this tumor to whole-body irradiation. Indeed, the specific effect of RS7 compared to Ag8 was more apparent at the lower radiation dose. None of the treatments caused significant systemic toxicity in the animals because there was no significant change in the body weight of the animals (Fig. 8).

Table 1  Tissue distribution of 131I-labeled RS7 and 125I-labeled Ag8 in nude mice bearing MDA-MB-468 breast tumor xenografts

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Tissue</th>
<th>Days postradioantibody injection</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS7</td>
<td>MDA-MB-468</td>
<td>16.2 ± 3.3²</td>
<td>16.1 ± 3.0⁰</td>
<td>10.5 ± 1.7⁷</td>
<td>6.9 ± 1.3⁴</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>5.3 ± 0.6</td>
<td>3.5 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>1.2 ± 0.4</td>
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<td></td>
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<tr>
<td>Spleen</td>
<td>6.1 ± 1.2</td>
<td>3.1 ± 0.9</td>
<td>1.9 ± 0.9</td>
<td>1.2 ± 0.3</td>
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<td></td>
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<tr>
<td>Kidney</td>
<td>4.3 ± 0.2</td>
<td>3.6 ± 0.4</td>
<td>2.3 ± 0.03</td>
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<tr>
<td>Lungs</td>
<td>6.6 ± 0.8</td>
<td>5.4 ± 0.6</td>
<td>3.3 ± 0.2</td>
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<tr>
<td>Heart</td>
<td>3.7 ± 0.5</td>
<td>3.6 ± 0.9</td>
<td>3.2 ± 0.4</td>
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<tr>
<td>Bone</td>
<td>2.9 ± 0.4</td>
<td>1.9 ± 0.1</td>
<td>1.1 ± 0.03</td>
<td>0.8 ± 0.2</td>
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<td>Blood</td>
<td>12.2 ± 1.9</td>
<td>10.9 ± 0.9</td>
<td>7.2 ± 0.6</td>
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<tr>
<td>Ag8</td>
<td>MDA-MB-468</td>
<td>7.1 ± 2.4</td>
<td>9.2 ± 1.5</td>
<td>5.4 ± 1.5</td>
<td>4.4 ± 0.7</td>
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<tr>
<td>Liver</td>
<td>2.8 ± 0.2</td>
<td>2.5 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>1.6 ± 0.1</td>
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<td>Spleen</td>
<td>2.9 ± 1.4</td>
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<td>1.9 ± 0.3</td>
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<td>Kidney</td>
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<td>5.3 ± 0.8</td>
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<tr>
<td>Heart</td>
<td>1.9 ± 0.1</td>
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<tr>
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<td>6.8 ± 0.2</td>
<td>4.9 ± 1.3</td>
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</table>

³ Percentage of injected dose/g ± SD; n = 5 animals.
⁴ Tumor uptake of RS7 was compared to the control antibody. The significant difference was calculated by Student's t-test.
⁵ P < 0.01.
We have found that a pancarcinoma antibody, RS7, labeled with $^{131}$I, can effectively control the growth of a human breast tumor xenograft in nude mice. The antigen defined by RS7 is a glycoprotein of $M_r$ 46,000–48,000, which was recently classified as the Cluster 13 epithelial/carcinoma antigen (15). On the basis of the apparent molecular weight before and after deglycosylation, and the peptide sequence, this antigen may be the protein product of the GA733–1 gene, which belongs to the GA733 gene family (15). This antibody is rapidly internalized by lung carcinoma cells (Calu-3) in vitro at a rate of 0.0232 min$^{-1}$. However, a slower rate of internalization was observed in ME180 (a cervical carcinoma, at 0.012 min$^{-1}$) and MDA-MB-468 (0.0147 min$^{-1}$) cells.

Our studies using RS7 as an agent for radioimmunotherapy in the MDA-MB-468 nude mouse model demonstrated the feasibility of this treatment modality against breast carcinoma. With a single injection of 250 $\mu$Ci $^{131}$I-labeled MAb RS7, a complete regression of the tumors (group of 10) of 0.1 cm$^3$ size was seen, and the effects lasted for an 11-week observation period (except for one animal). With a single injection but at a slightly higher dose of 275 $\mu$Ci, RS7 is able to cause regression of established tumors of −0.2–0.3 cm$^3$. At 15 weeks after injection of the radiolabeled antibody, 7 of 10 animals had no measurable tumor present. These results show that RS7 antibody in the $^{131}$I-labeled form is effective in controlling the growth of a human breast tumor xenograft. These effects appear to be greater than what was observed in the lung cancer model (5). It is recognized that the effectiveness of radiolabeled antibody therapy is dependent on several parameters, such as antigen expression, accessibility of the antigen, vascularity of the tumor, and sensitivity of the tumor to the radiation. The greater effect that was observed in the MDA-MB-468 model than the lung carcinoma using the same antibody is probably due to the sensitivity of the cell line. This phenomenon was also observed with the irrelevant antibody Ag8, where a greater nonspecific effect of this antibody was demonstrated in this tumor model than in the lung tumor system. In addition to tumor sensitivity, the nonspecific uptake due to a different vascular permeability in tumors may also account in part for the efficacy of the nonspecific antibody. Furthermore, RS7 is internalized and catabolized more rapidly in the lung carcinoma model (based on in vitro observations). Thus, the residence time of the $^{131}$I antibody in the Calu-3 tumor may be relatively brief (due to more rapid antibody degradation or other mechanisms, such as tumor cell-mediated dehalogenation) in comparison to the MDA-MB-468 tumor.

Because RS7 is internalized by the target cells, this antibody may be a promising candidate for other toxic immunoconjugates, particularly immunotoxins. It has been well documented that internalization of immunotoxin is an absolute requirement for antitumor activity (16). Internalization of small-molecular-weight drug immunoconjugates has also been considered to be advantageous (17), although effective drug immunoconjugates of a noninternalizing (or slowly internalizing) antibody have been documented (18). The antitumor effects observed in these cases may have been due to the release of the drugs before entry into the target cells. Radiolabeled antibodies do not need to be internalized, because they exert their toxic effects on adjacent cells. However, it is important to carefully select the radionuclides to be used with an internalizing antibody. Radionuclides that tend to be retained inside the cell, once internalized, such as $^{111}$In or $^{90}$Y, may be preferred over those with a shorter residence time, such as $^{131}$I.

Table 2 Mean cumulative absorbed dose in tissues through day 14 postinjection

<table>
<thead>
<tr>
<th>Tissue</th>
<th>cGy/mCi</th>
<th>Normalized to blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ag8</td>
<td>RS7</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>4320</td>
<td>8700</td>
</tr>
<tr>
<td></td>
<td>700</td>
<td>1280</td>
</tr>
<tr>
<td>Bone</td>
<td>1110</td>
<td>1250</td>
</tr>
<tr>
<td></td>
<td>181</td>
<td>184</td>
</tr>
<tr>
<td>Heart</td>
<td>2480</td>
<td>2460</td>
</tr>
<tr>
<td></td>
<td>404</td>
<td>362</td>
</tr>
<tr>
<td>Kidney</td>
<td>2240</td>
<td>2240</td>
</tr>
<tr>
<td></td>
<td>365</td>
<td>330</td>
</tr>
<tr>
<td>Liver</td>
<td>2030</td>
<td>2430</td>
</tr>
<tr>
<td></td>
<td>331</td>
<td>358</td>
</tr>
<tr>
<td>Lung</td>
<td>3260</td>
<td>3420</td>
</tr>
<tr>
<td></td>
<td>531</td>
<td>504</td>
</tr>
<tr>
<td>Spleen</td>
<td>1890</td>
<td>2250</td>
</tr>
<tr>
<td></td>
<td>308</td>
<td>331</td>
</tr>
<tr>
<td>Blood</td>
<td>6140</td>
<td>6790</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

To avoid overestimation of the cumulative tumor dose, the cGy dose in the tumor was computed using trapezoidal integration. A zero time value of 0 was assumed. However, for the normal tissues, the dose was calculated by following the exponential integration, and the zero time point value was extrapolated according to the exponential curve.
with an internalizing MAb. Radionuclides with a short energy path, such as electron-capture radionuclides ($^{125}$I) and α-emitters ($^{212}$Bi), may also be suitable radioconjugates for internalizing antibodies.

Recently, Brummendorf et al. (19) reported the radioimmunolocalization results of two newly developed antibodies, 12H12 and BM2. These two antibodies recognized different epitopes on the mucin glycoprotein TAG-72. The percentage of injected dose per gram and the tumor:nontumor ratios observed with these MAbs were similar to the ranges with RS7. Currently, a large number of antibodies against different epitopes of human neoplastic breast tissue have been investigated. Among them, anticarcinoembryonic antigen antibodies, such as Immu-4 (20) and BW 431/26 (21), and anti-milk fat globule antibodies, such as Mc5 (22), have entered clinical testing in either imaging or therapy trials. Other antibodies in clinical investigations include anti-TAG-72 antibodies, such as B72.3 and CC49 (23–25), anticell surface antigens, such as MAb B6.2 (26, 27), and various others, including L6 and 791T/36 (28–30). These previous studies have demonstrated the feasibility of using radiolabeled antibodies in the early detection of breast carcinoma and have indicated the potential of using such antibodies as carriers of cytotoxic agents (e.g.,

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**Fig. 6.** Radioimmunotherapy of 10-day-old MDA-MB-468 tumors using 250 μCi of $^{131}$I-labeled RS7. The size of the tumors and the body weight of the animals were monitored on a weekly basis. The percentage of tumor growth of the individual tumor is shown. Animals received either the unlabeled RS7 or Ag8 at 30 μg/animal or radiolabeled RS7 or Ag8 at 250 μCi/animal. The rate of tumor growth of animals given injections of radiolabeled RS7 was significantly different from that of animals given injections of unlabeled antibodies ($P < 0.01$).

**Fig. 7.** Radioimmunotherapy of 1-month-old MDA-MB-468 tumors using 275 μCi of $^{131}$I-labeled RS7. The percentage of tumor growth of individual tumors is shown. Animals were either untreated or received radiolabeled RS7 or Ag8 at 275 μCi/animal. Groups of 10 animals were used. The rate of tumor growth of animals given injections of radiolabeled RS7 was significantly different from the untreated animals ($P < 0.0005$) but less significant from the control antibody Ag8 ($P < 0.2$).
toxins, drugs, or radionuclides) for therapy (4). In the light of these encouraging clinical applications, and the promising characteristics of RS7 (such as the efficient internalization and the high frequency of antigen expression in breast cancer), this antibody may be a suitable candidate for immunocug conjugate therapy.

References

In Vitro and in Vivo Reactivity of an Internalizing Antibody, RS7, with Human Breast Cancer

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