Effects of Radiolabeling Monoclonal Antibodies with a Residualizing Iodine Radiolabel on the Accretion of Radioisotope in Tumors

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ABSTRACT

The effect of using a “residualizing” iodine radiolabel, dilactitol-iodotyramine, for radioimmunolocalization of antibodies to tumors was investigated. This tracer is designed to be lysosomally trapped after catabolism of the labeled antibody. mAbs RS7 and RS11 were used for in vivo and in vitro studies on the uptake and retention of radioisotope into tumor cells. Both are murine IgG1 mAbs with pancarcinoma reactivity, which react with integral membrane glycoproteins. mAb RS7 has been shown to be relatively rapidly catabolized by the antigen-bearing cell line Calu-3, whereas RS11 is catabolized more slowly in the same cells. An 111In- or 82Y-isothiocyanatobenzyl-diethylentriamine pentaacetic acid conjugate was also tested because these radiometals are known to be lysosomally trapped, and iodination via chloramine T was used to provide a baseline. In vivo, a substantial increase in retention of the label by cells was observed when the dilactitol-tyramine DLT- or 111In-labeled mAbs were used, and the improvement gained by the use of these residualizing labels was greater with the use of the rapidly catabolized mAb (RS7) than it was with the more slowly catabolized mAb (RS11). In biodistribution studies in nude mice bearing Calu-3 tumor xenografts, a dramatic improvement in the tumor accretion of the radiolabel was seen with the use of the 123I-labeled DLT- or 82Y-labeled mAbs. For example, at day 7 the percentage of injected dose/g in the tumor was 5.54 ± 1.47% (SD), 38.06 ± 8.04%, and 43.18 ± 19.50% for the conventionally iodinated, DLT- and 82Y-labeled RS7, respectively. Dosimetry calculations performed on the biodistribution data predict increases of approximately 8- and 4-fold in the absorbed dose to tumor with the use of 123I-labeled DLT- and 82Y-labeled mAbs, respectively, compared to the conventional 111In. In contrast to in vivo findings, these results were similar for both RS7 and RS11, suggesting that the use of DLT may be advantageous for most of the mAbs binding to the cell surface, including antibodies that are catabolized relatively slowly. The advantage of 123I-labeled DLT over 82Y is due to the longer physical half-life of the 111In.

INTRODUCTION

The delivery of radiation to tumor cells by radiolabeled mAbs depends on both the ability of antibodies to penetrate solid tumors to reach antigens within them and the processing of the antibodies after antigen binding. mAbs binding to the cell surface can potentially be internalized and catabolized by the cells. Although it is frequently assumed that few mAbs are “rapidly” internalized, we have recently reported that all mAbs binding to the surface of human tumor cells in vitro are gradually catabolized with a half-life of 1–2 days (1, 2). Such a rate of catabolism will have a major effect on radioimmunotherapy with isotopes such as 123I and 82Y, with decay half-lives of 3–8 days. The apparent controversy regarding mAb internalization is readily explained in terms of the time point utilized in different experiments. Thus, only mAbs internalized via clathrin-dependent endocytosis (coated pits) will be internalized efficiently within 1–2 h. Most mAbs are probably internalized by the non-clathrin-dependent pathway, which is much slower (reviewed in Ref. 3). Similar results have been obtained with carcinomas of various histological types, astrocytomas, and melanomas (1, 2).

Once the antibody is catabolized, which occurs within lysosomes, the fate of the radiolabeled catabolic product becomes a key factor. It is known that iodytosine rapidly exits from the lysosome and the cell after its generation after catabolism of conventionally iodinated proteins (4–6). However, other radiolabels are trapped within lysosomes, essentially because of their inability to cross the lysosomal membrane. Iodinated radiolabels have been designed to be lysosomally trapped by taking advantage of their linkage to nonmetabolizable disaccharides (reviewed in Ref. 7). It might be expected that such radiolabels would accumulate within tumor cells to a greater extent than conventional iodine labels, and such results have been demonstrated in vitro (4, 8, 9). However, in vivo results with such residualizing labels have not, to date, been impressive, partly because of accretion in certain normal tissues. The cellobiose-tyramine label was tested by the methods of Ali et al. (9, 10) using an anti-Thy-1.1 mAb and a mouse T-cell lymphoma, but only slightly increased tumor accretion was demonstrated. Demignot et al. (11), using radioiodine-labeled DLT mAb 791T/36 and the human sarcoma 791T, found increased tumor accretion of the DLT label, relative to chloramine T, but also increased accretion in many normal tissues, and there was not a large improvement in tumor/nontumor ratios. However, it seemed worthwhile to perform additional experiments with the DLT label for several reasons. The only direct comparison of the two residualizing labels suggested that DLT might be superior to cellobiose-tyramine (12). The DLT label was tested only with mAb 791T/36, which appears to localize within tumors primarily in the stroma adjacent to the tumor, rather than on the tumor cell surface (13). Clearly, the advantage of residualizing labels should best be seen with the use of mAbs that bind to the cell surface and are catabolized.

In previous studies of radioimmunodetection and radioimmunotherapy, we have utilized mAbs RS7 and RS11, which react with integral membrane proteins on the surface of lung carcinoma cells, and other carcinomas (14–17). RS7 was shown to be catabolized relatively rapidly (18), and RS11, an antibody to EGP-2, is catabolized more slowly at a rate similar to that for most mAbs binding to the cell surface (2). Although we have not directly examined the mode of endocytosis involved, it is likely that RS11 internalization is via noncoated vesicles, as discussed previously (1). The faster catabolism of RS7 is compatible with either a coated or noncoated vesicle pathway (19). This experimental system seemed useful for investigating the advantage of residualizing radiolabels. We anticipated that DLT would be most useful with the rapidly catabolized mAb and be less of an advantage with the slowly catabolized mAb. However, our results demonstrated that the use of DLT provided a large advantage with both mAbs.
RESIDUALIZING RADIOLABELS ON mAbs

MATERIALS AND METHODS

mAbs, Cell Lines, and Radiolabeling. The production and initial charac-
terization of RS7 and RS11 (also referred to as RS7-3G11 and RS11-51, re-
spectively) have been described previously (14, 18). Ag8 (American Type
Culture Collection, Rockville, MD), an irrelevant mouse myeloma IgG1 di-
agnized P3 × 63 Ag8, was used as a negative control antibody in this study.
The antibodies were purified from ascites fluid by passage through a protein
A-immunoadsorbent column.

Calu-3, a human adenocarcinoma of the lung cell line, was purchased from
the American Type Culture Collection. The cells were grown as monolayers in
RPMI 1640 (JRH Biosciences, Lenexa, KS) supplemented with 5% fetal
bovine serum, 5% horse 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.

mAbs were radiiodinated with 131I or 125I (New England Nuclear, North
Billerica, MA) by the chloramine-T method (20) or via dilactitol-tyramine
by the method of Strobel et al. (12). Briefly, 5 nmol DLT in 25 µl 0.5 M sodium
phosphate buffer (pH 7.6) were added to 0.5 ml polypropylene vials (number
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by the method of Strobel et al. (12). Briefly, 5 nmol DLT in 25 µl 0.5 M sodium
phosphate buffer (pH 7.6) were added to 0.5 ml polypropylene vials (number
72.730; Sarstedt, Pennsauken, NJ), which had been coated with 60 µg iodogen
(Pierce Chemical Co., Rockford, IL) evaporated from 150 µl dichloromethane.
Five mCi 131I, diluted to 50 µl in the same buffer, were added and incubated
30 min at room temperature. After transfer to a clean vial, 4 units of galactose
oxidase (Sigma Chemical Co., St. Louis, MO) were added in 5 µl of the same
buffer and incubated 45 min at 37°C. After addition of 0.25 mg mAb in 15–50
µl PBS (0.15 M NaCl-0.02 M sodium phosphate (pH 7.2)), 2.0 m sodium
cyanoborohydride was added to a final concentration of 40 mM. After 3 h at
37°C, conjugated mAb was collected by passage over a PD-10 column (Phar-
macia, Piscataway, NJ) with a column buffer of PBS-10 mM NaCl-10 mM
Na2HPO4-0.1% gelatin. The iodination efficiency with this method ranged from
3–6%, resulting in a specific activity of 0.5–1.0 mCi/mg.

111In- and 64Y-labeled mAbs were prepared as described previously (21),
with the use of indium from New England Nuclear and Y from Los Alamos
National Laboratory (Los Alamos, NM). The bifunctional chelate used in
the preparation of these radioimmunoconjugates was the ITC-Bz derivative of
DTPA, provided by ImmunoMedics, Inc., Morris Plains, NJ, and was synthe-
sized following the method of Cummins et al. (22). The preparation and testing
of the antibody conjugates were performed as described by Brechbiel et al.
(23) and Meares et al. (24). Specific activities of 3–5 mCi/mg and 0.1 mCi/mg
were produced with 111In and 64Y, respectively.

Assessment of immunoreactivity after radiolabeling was performed with the
use of a direct cell-binding assay (14).

In Vitro Antibody Retention Experiments. As described previously in
detail (1), confluent cells in 96-well plates were incubated with 5 × 105 cpm
of labeled antibody for 2 h at 37°C, then washed four times to remove unbound
labeled antibody. Tissue culture medium (0.2 ml) was added and incubation
was continued for various times as indicated in “Results.” Under these con-
ditions, the cells remain healthy and continue to divide for at least 3 days. At
desired times, 0.1 ml of media was collected, and after additional washing,
the cells were solubilized with 2.0 M NaOH. After determining the cpm in the
medial precipitate was collected by centrifugation. Nonspecific antibody binding was
precipitated with 5

In Vivo Biodistribution Studies. Tumors were propagated in female mi/nu
mice (Harlan Sprague-Dawley, Indianapolis, IN) at 6–8 weeks of age by s.c.
implantation of tumor cell suspension in the leg of the mouse. The mice were
used for in vivo biodistribution studies approx

RESULTS

Retention and Processing of mAbs on Calu-3 Cells in Culture. Calu-3, an adenocarcinoma of the lung cell line, was used as a model
system for evaluating the retention and cellular processing of radio-
abeled mAbs by tumor target cells with the use of mAbs RS7 and
RS11. Comparisons were made between the binding and processing of
the two mAbs radiiodinated by either the conventional chloramine-T
procedure or with the use of the residualizing adduct dilactitol-
ynine. The mAbs were also labeled with 111In with the use of
ITC-Bz-DTPA. The results of the processing studies of the three
radioconjugates of each of these mAbs are summarized in Table 1.

The data indicate a difference in the retention of the two mAbs
when radiiodinated by the conventional chloramine-T procedure.
The processing of 125I-labeled RS7 was faster than 125I-labeled RS11,
consistent with the results of earlier studies on the relative rates of
internalization of the two mAbs (2, 18). This was especially apparent
at 21 h when only 22.9 ± 0.8% (SD) of initially bound RS7 remained
bound to the cells, whereas with RS11, 47.9 ± 2.4% remained.
When the residualizing labels were used, the percentage of radiolabel
retained by the cells increased substantially. With 125I-labeled DLT and
111In-labeled mAbs, approximately 60% of initially bound cpm re-
ained at 21 h. Nonspecific binding averaged 4.8% (ranging from 2.4
to 7.8%). Both 125I-labeled DLT and 111In labeling yielded approxi-
mately equal retention of the labels with either mAb RS7 or RS11,
indicating that the rate of antibody internalization and catabolism does
not affect the cellular retention of the radiolabel when residualizing
labels are used.

Table 1. Processing of radioconjugates by Calu-3 cells

<table>
<thead>
<tr>
<th>mAb Label</th>
<th>4 h</th>
<th>21 h</th>
<th>45 h</th>
<th>69 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS7 125I (chloramine-T) 79.28 ± 2.69 22.91 ± 0.82 9.93 ± 0.98 4.70%</td>
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<tr>
<td>125I-labeled DLT 74.37 ± 9.03 64.90 ± 6.00 52.35 ± 0.79 45.39 ± 2.33</td>
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<tr>
<td>111In 77.00 ± 11.35 60.11 ± 18.20 49.11 ± 12.16 42.72 ± 11.77</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS11 125I (chloramine-T) 85.38 ± 1.70 47.86 ± 2.39 33.27 ± 7.09 69.4%</td>
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</tr>
<tr>
<td>125I-labeled DLT 80.27 ± 6.03 58.64 ± 6.39 48.48 ± 5.22 41.65 ± 5.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>111In 82.51 ± 7.90 67.03 ± 13.90 56.28 ± 8.84 51.30 ± 8.08</td>
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</tr>
</tbody>
</table>

* No SD is reported because this time point was not determined in the second
eperiment.

The percent of originally bound mAbs retained by cells at the indicated times were
determined as described in “Materials and Methods.” Values represent the mean ± SD of
two independent experiments, each done in triplicate. The designation 125I (chloramine-T)
represents labeling of the mAbs with the use of the conventional chloramine-T procedure.

The processing of 125I-labeled RS7 was faster than 125I-labeled RS11, consistent with the results of earlier studies on the relative rates of
internalization of the two mAbs (2, 18). This was especially apparent
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indicating that the rate of antibody internalization and catabolism does
not affect the cellular retention of the radiolabel when residualizing
labels are used.
cells were incubated for up to 3 days in tissue culture medium containing a mixture of SDs of triplicates were all S0.381* of the total cpm/well for RSI Land so. 11% for RS7. At varying times, cells were washed and collected and the percentage cpm specifically bound was determined. Nonspecific binding was determined by including a large excess of unlabeled mAb in control wells, and the specific binding was calculated by subtraction.

At varying times, cells were incubated with the mAbs labeled with either 125I or 111In. The Ab was either RS11 (A) or RS7 (B). At varying times, cells were washed and collected and the percentage cpm specifically bound was determined. Nonspecific binding was determined by including a large excess of unlabeled mAb in control wells, and the specific binding was calculated by subtraction. SDs of triplicates were all ≤0.36% of the total cpm/well for RS11, and ≤0.11% for RS7. C, from the same experiment, the value plotted is (% 111In specifically bound)/(%125I specifically bound). The increase in this ratio with time indicates greater retention of indium in comparison with iodine.

As reported previously (1), a relatively small fraction of bound antibody was released intact from the cells. The majority of released cpm was found in a degraded form (trichloroacetic acid or methanol soluble fraction; data not shown). At 3 days, the percentage of bound antibody released in intact form (i.e., not catabolized) varied from 10 to 27% of the initially bound antibody, depending on the conjugate. In general, this value was reached within the first day, in contrast to the release of degradation products, which continued to increase during the 3-day time course.

**In Vitro Experiments Using a Prolonged Antibody Incubation.**

In vivo, an antibody remains at high levels in the circulation for several days, although it gradually decreases due to clearance from the blood. To more closely mimic the in vivo situation, in vitro experiments were performed in which the cells were incubated with the mAbs continuously for up to 3 days. This protocol can potentially result in the accumulation of large amounts of radiolabel/cell. At various times, wells were harvested to determine the accumulation of cell-bound cpm. A double label study was performed comparing the residualizing label, 111In, with conventionally conjugated 125I in the same wells. To ensure that uptake was via antigen-specific binding, and not due to fluid-phase uptake of mAb present in the medium, control wells contained a large excess of unlabeled mAb; this was effective at inhibiting mAb uptake by >90%. The results, shown in Fig. 1, demonstrate that accumulation of 125I increased from 2 to 24 h but reached a plateau after 24 h. In contrast, 111In continued to accumulate for 3 days, the duration of the experiment, with both mAbs. Note, however, that the advantage of the residualizing label, although clearly seen, is less pronounced in this experiment than it was in the earlier in vitro experiments. This can be attributed to the fact that 125I uptake increases markedly from 2 to 24 h, probably due to cell multiplication, which makes the retention of 111In less prominent.

The ratio of the percentage of specifically bound cpm of the two radioisotopes (% 111In specifically bound/%125I specifically bound) is presented in Fig. 1C. This graph shows that the advantage of the residualizing label is considerably greater for RS7 than it is for RS11 because the ratio increases by 2.4-fold with RS7 and only 1.2-fold for RS11, from 2 to 72 h. Therefore, this experiment also suggests that residualizing labels should produce more of an advantage with RS7 than with RS11.

**In Vivo Studies.** A comparison of the in vivo targeting of mAbs RS7 and RS11 conjugated with the conventional and residualizing radiolabels was performed in nude mice bearing xenografts of Calu-3. An unreactive mAb, Ag8, was included as a control. These biodistribution studies were performed to assess whether the increases in tumor cell retention observed in vitro when the residualizing labels (131I-labeled DLT and radiometal) were used translate into higher accretion of radiolabel at the target site in vivo. In addition, the contribution of the rate of mAb internalization and catabolism to radioisotope accretion and tumor:nontumor ratios was investigated by comparing the results obtained with the use of the two mAbs. The results are summarized in Fig. 2. Marked improvements in the tumor accretion of radiolabel were seen with the use of the 131I-labeled DLT- or 90Y-labeled mAbs in comparison to the conventional 131I-labeled antibodies. For example, at day 7 the %ID/g in tumor was 5.54 ± 1.47% (SD), 38.06 ± 8.04%, and 43.18 ± 19.50% for the...
conventional $^{131}$I-, $^{131}$I-labeled DLT-, and $^{88}$Y-labeled RS7, respectively. No significant differences in tumor accretion were observed between RS7 and RS11 with any of the labels. For certain data points, the SDs for tumor accretion of mAbs were relatively high. This variation among tumors could not be fully explained by variation in tumor size or by any other characteristic that has been identified; thus, variation can be attributed only to unknown variation in the physiological properties of the tumors.

The results of these studies are shown in more detail in Figs. 3 and 4, which show the percentage of the injected dose of radiolabel/g of normal tissue and the tumor:non-tumor ratios for these tissues for the RS7 studies over the 14-day time course. Similar results were observed with the radiolabeled RS11 (data not shown). As seen in Fig. 3, the % ID/g of radiisotopes were relatively low for all three labels in the normal tissues. This indicates that although the $^{131}$I-labeled DLT and $^{88}$Y labels are accumulating in the tumors to levels approximately 7–8 times greater than those observed with the use of conventional $^{131}$I-labeled mAbs (Fig. 2), the accretion in normal tissues is not changed as much by the use of the residualizing labels. The effect of increased tumor accretion without matching increases in normal tissue accretion is the generation of increases in tumor:non-tumor ratios (Fig. 4). Tumor:liver ratios at day 7 are 3.40 ± 0.69 (SD) with $^{131}$I-(conventionally)labeled RS7 compared to 16.26 ± 4.12 with $^{131}$I-labeled DLT-RS7 and 15.49 ± 4.66 with the use of $^{88}$Y-labeled RS7. Maximal tumor:non-tumor ratios were seen in the muscle with 90.64 ± 25.49 and 84.00 ± 31.62 observed at day 7 after injection of $^{131}$I-labeled DLT-RS7 and $^{88}$Y-labeled RS7, respectively, compared to 11.60 ± 2.42 with the use of conventional $^{131}$I-labeled RS7. At day 7, the lowest ratios were seen in the blood, at 0.87 ± 0.19, 8.84 ± 1.86, and 8.05 ± 2.73, for the conventional $^{131}$I-labeled, $^{131}$I-labeled DLT, and $^{88}$Y-labeled RS7, respectively.

The increases over time in the tumor:non-tumor ratios were similar for the $^{131}$I-labeled DLT and $^{88}$Y-labeled mAbs, the only exception being bone, where the tumor:non-tumor ratio decreases after day 3 with the $^{88}$Y label but not with the $^{131}$I-labeled DLT-antibodies. This most likely results from the known bone-seeking properties of the yttrium, which has become dissociated from the chelator. The accretion of yttrium by bone seen here is only significantly different from that of the iodine labels at the latest time point (14 days after injection) and would not contribute a significant radiation dose to the bone when $^{90}$Y is used therapeutically, due to the short physical half-life (64 h) of $^{88}$Y.

Residualizing labels will accumulate not only in tumor cells to which the antibody binds but also in any normal tissues that are responsible for antibody clearance and catabolism. The organs most active in IgG catabolism/g of tissue are the liver, spleen, and possibly the kidney (26). Although the kidney was noted to have increased accretion of IgG (or its degradation products), the results are difficult to interpret because the kidney may accumulate degradation products that are generated in other organs (26). Differences in the accretion of the radiolabels in various normal organs are addressed in Fig. 5, which shows the accumulation of $^{131}$I-labeled DLT-RS7 in various organs in the Calu-3-bearing nude mice, in comparison to RS7 labeled conventionally with $^{131}$I. Elevated localization ratios, defined as (%ID/g $^{131}$I-labeled DLT mAb)/(%ID/g conventional $^{131}$I-labeled mAb), are seen in the liver, spleen, and kidney, as expected (26). Although the liver, spleen, and kidney accrete more radiiodine with $^{131}$I-labeled DLT than with the conventional $^{131}$I label, the increases in the ratios are not as large as those observed in the Calu-3 tumor. Thus, the tumor:non-tumor ratios are increased even for these organs. Fig. 5 suggests that $^{131}$I-labeled DLT slowly accumulates to a small extent in the bone because the localization ratio increased from day 7 to day 14.

Fig. 4. Tumor:non-tumor ratios for radiolabeled RS7 in nude mice bearing Calu-3 tumors. Mice with tumors were injected i.v. on day 0 with conventional $^{131}$I-labeled-RS7 (O), $^{131}$I-DLT-labeled RS7 (C), or $^{88}$Y-labeled RS7 (A) and sacrificed successively as described in Fig. 1. Tumor:non-tumor ratios were calculated from the data presented in Figs. 2 and 3. Sm Int, small intestine; Lg Int, large intestine.
RESIDUALIZING RADIOLABELS ON mAbs

Fig. 5. Relative accumulation of 131I-labeled DLT-RS7 in tumor and normal mouse tissues. The value plotted is the (% injected dose/g of tissue with 131I-labeled DLT-RS7)/(% injected dose/g of tissue with conventional 131I-labeled RS7), to show the preferential accumulation of DLT in certain tissues. For tissues that do not retain either label preferentially, the ratio will be 1.0. The ratios are derived from the data presented in Figs. 2 and 3. Int., intestine.

Similar results were observed in the other experiments described here, and also in experiments utilizing normal BALB/c mice (data not shown). This accretion is likely to be due to the macrophage content of the bone marrow.

The possible contribution of the rate of mAb internalization and catabolism to radioisotope accretion and tumor:nontumor ratios was investigated by directly comparing the results obtained with the use of the two mAbs. Tumor:liver ratios of the mAbs are shown in Fig. 6. No significant differences in tumor:nontumor ratios were observed between RS7 and RS11 with the 131I-labeled DLT or 88Y labels. With the conventional 131I-labeled mAbs, at day 14 after injection, the tumor:liver ratio with RS7 was lower than that with RS11. This difference, which was also seen in other normal tissues, is due to faster normal blood clearance of 131I-RS11 rather than increased tumor accretion. Thus, the marked advantage obtained with residualizing labels is seen equally well with both mAbs.

Dosimetry. Cumulative absorbed radiation doses were calculated from the biodistribution data of the above studies (Table 2). 88Y was used in the biodistribution experiments in lieu of 131I, which would be used therapeutically. This substitution was made because the γ emissions of 88Y allow counting of the tissues in the γ counter. Mean cumulative absorbed doses were computed for 88Y with the use of the 88Y data because the distribution of the two isotopes of yttrium can be assumed to be the same. To compare the three studies, the absorbed doses were normalized to a blood dose of 1500 cGy, a level which has been observed to be approximately the maximal tolerated dose in this system. The data are summarized in Table 3. The administered doses of these conjugates necessary to achieve a 1500-cGy absorbed dose to blood are 242 μCi conventionally 131I-labeled-RS7, 368 μCi 131I-labeled DLT-RS7, and 170 μCi 88Y-labeled RS7. Although the max-

Table 2 Mean cumulative absorbed dose in tissues

<table>
<thead>
<tr>
<th>Dose to tissue (cGy/mCi)</th>
<th>131I-labeled CT mAb</th>
<th>131I-labeled DLT mAb</th>
<th>88Y-labeled DTPA mAb*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS7</td>
<td>Calu-3</td>
<td>4561</td>
<td>24051</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>6208</td>
<td>4077</td>
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<tr>
<td></td>
<td>Liver</td>
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<tr>
<td></td>
<td>Large intestine</td>
<td>1159</td>
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<td>Liver</td>
<td>1595</td>
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</tr>
<tr>
<td></td>
<td>Lungs</td>
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<td>1817</td>
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<tr>
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<td>Muscle</td>
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<td>Small intestine</td>
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<td>Spleen</td>
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<tr>
<td>RS11</td>
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<tr>
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<td>Spleen</td>
<td>659</td>
<td>799</td>
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</tbody>
</table>

* CT, labeling performed with the use of the conventional chloramine-T procedure.

b Calculated from 88Y-labeled DTPA-RS11 biodistribution data.
Table 3 Mean cumulative absorbed dose normalized to 1500 cGy dose to blood

<table>
<thead>
<tr>
<th></th>
<th>Dose to tissue (cGy)</th>
<th>¹³¹I-labeled CT** mAb</th>
<th>¹³¹I-labeled DLT mAb</th>
<th>⁹⁰Y-labeled DTPA mAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS7</td>
<td>(242 µCi)**</td>
<td>(368 µCi)</td>
<td>(170 µCi)</td>
<td></td>
</tr>
<tr>
<td>Calu-3</td>
<td>1,102</td>
<td>8,849</td>
<td>5,138</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1,500</td>
<td>1,500</td>
<td>1,500</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>229</td>
<td>302</td>
<td>363</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>377</td>
<td>1,041</td>
<td>510</td>
<td></td>
</tr>
<tr>
<td>Large intestine</td>
<td>228</td>
<td>213</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>452</td>
<td>913</td>
<td>799</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>497</td>
<td>669</td>
<td>539</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>119</td>
<td>128</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>207</td>
<td>347</td>
<td>228</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>319</td>
<td>844</td>
<td>526</td>
<td></td>
</tr>
<tr>
<td>RS11</td>
<td>(297 µCi)</td>
<td>(436 µCi)</td>
<td>(150 µCi)</td>
<td></td>
</tr>
<tr>
<td>Calu-3</td>
<td>1,250</td>
<td>10,905</td>
<td>4,283</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1,500</td>
<td>1,500</td>
<td>1,500</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>223</td>
<td>369</td>
<td>342</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>526</td>
<td>1,459</td>
<td>496</td>
<td></td>
</tr>
<tr>
<td>Large intestine</td>
<td>166</td>
<td>318</td>
<td>177</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>673</td>
<td>1,667</td>
<td>832</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>644</td>
<td>741</td>
<td>648</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>143</td>
<td>208</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>195</td>
<td>349</td>
<td>221</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>317</td>
<td>1,041</td>
<td>481</td>
<td></td>
</tr>
</tbody>
</table>

* CT, labeling performed with the use of the conventional chloramine-T procedure.
** Calculated from ⁹⁰Y-labeled DTPA-RS11 biodistribution data.
*** Numbers in parentheses, the administered dose necessary to achieve 1500 cGy absorbed dose to blood.

Table 3 Mean cumulative absorbed dose normalized to 1500 cGy dose to blood

Table 3 Mean cumulative absorbed dose normalized to 1500 cGy dose to blood

The relationship between radiometals and DLT must be considered because both labels increase the cellular retention of radioisotope. It was recently reported that radiometals bound to strong chelators such as ITC-Bz-DTPA are trapped within cells after catabolism of the antibodies to which they were conjugated (8) and that they are trapped within lysosomes (28). Although no radiolabel appears to be trapped permanently within lysosomes (7, 8), the retention of DLT and ITC-Bz-DTPA seemed to be very similar in a direct comparison (8). Thus, it could be predicted that these two labels would produce very similar antibody localization results, and this is confirmed in the present study. It has been widely accepted that the difference in tumor localization between radiometals and a conventional iodine label is primarily due to deiodination. However, as discussed previously in detail (8), experimental evidence points directly to an explanation based on differences in the processing of the catabolic products rather than deiodination.

Because chelated radiometals have been utilized in a large number of investigations and sometimes have been compared with a conventional iodine label, such results can potentially provide additional insight into the advantage of residualizing labels. However, this analysis is complicated by two factors: (a) most earlier studies used relatively unstable chelators, mainly the cyclic anhydride or mixed anhydride of DTPA. These chelators release a substantial amount of free metal, which then binds to transferrin and is taken up by the liver (29). Work with these chelators did demonstrate increased accumulation in tumors, relative to an iodine label (30, 31), but in regard to normal tissue uptake the results obtained with these unstable chelators are difficult or impossible to compare with our experiments; and (b) a considerable variety of the newer, stronger metal chelators have been developed (29, 32, 33) with some significant differences between them. Although all chelators may release very small amounts of free metal, they may differ in other characteristics, including lysosomal retention. In view of these considerations, only a few comparisons of strong metal chelators with iodine labels are clearly relevant to the present study.

Sharkey et al. (34) compared ITC-Bz-DTPA with iodine using an anticarcinoembryonic antigen mAb. Their results demonstrated increased tumor accretion of the metal, but there was also increased normal tissue accretion, and dosimetry calculations indicated that yttrium was probably inferior to iodine for purposes of radioimmuno-therapy. The most extensively characterized mAb tumor system has been mAb B72.3 and the LS174T colon carcinoma, in which a wide variety of benzyl-DTPA derivatives have been tested (35, 36). In this case, it was reported that there was no significant difference in biodistribution between a strong metal chelator and iodine. This result can perhaps be attributed to the fact that the B72.3 antigen is not a cell surface antigen but rather a secreted mucin, and that the mAb is, therefore, not internalized and catabolized. This consideration may also apply to the results with carcinoembryonic antigen antibodies (34) because the carcinoembryonic antigen occurs in both secreted and membrane-bound forms (37). In this context, we note that different benzyl-DTPA derivatives may vary in their level of lysosomal retention, but this of course can be seen only with mAbs that are efficiently internalized and degraded. Stein et al. (15) compared iodine and indium using mAb RS7 on ME180 cervical carcinoma tumors in a system essentially identical to that used here except for the different target cell. In that study, indium produced higher absolute tumor accretion and higher tumor:non-tumor ratios for some organs but lower ratios for the liver and spleen. In summary, although the use

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** M. J. Mattes and R. D. Blumenthal, unpublished data.
of indium does generally result in increased tumor accretion, the
dramatic difference in tumor:nontumor ratios described here has not
been described in other experimental systems. Thus, the advantage of
residualizing labels will depend on the particular mAb and on the
particular tumor target.

Although DLT and benzyl-DTPA seem to be very similar in terms
of retention within lysosomes, significant differences between these
labels remain. One difference is in the accretion of yttrium by bone.
Such bone accretion can occur only after the metal escapes from the
chelator; therefore, it is relatively minor when strong chelators such as
benzyl-DTPA are used. Some release of yttrium does still occur, and
this material appears to be efficiently taken up by bone. Sharkey et al.
(34) concluded that bone accretion was an obstacle to the use of
benzyl-DTPA chelates of yttrium. This factor, therefore, represents an
advantage of DLT over yttrium. The release of yttrium can be reduced
with the use of even stronger chelators, namely the macrocycles (32),
but such chelators are inconvenient to use because radiolabeling is
less efficient. Our data indicate that bone accretion is probably not
entirely due to uptake of free metal but also due to accumulation of
residualizing labels in the bone. Such accumulation was seen with
DLT, although at a level much less than in liver, spleen, and kidney.
This accumulation is unlikely to be due to uptake in the bone itself,
and we tentatively attribute this to uptake by bone marrow macro-
phages. It would be expected that benzyl-DTPA-yttrium would sim-
ilarly accumulate in bone. Thus, bone accretion can occur by two distinct mechanisms. However, it should be emphasized that the level
of bone marrow accretion of the residualizing labels is very low and,
therefore, unlikely to represent a toxicity problem.

The major difference between yttrium and iodine, however, is seen
not in the tumor:nontumor ratios but only in the dosimetry and is due
to the difference in decay half-life. Thus, an approximately 2-fold
difference in radiation delivered to the tumor is seen with estimated
equitoxic doses. It is apparent that, due to relatively slow uptake and
accumulation of the radiolabel within the tumors, a 3-day radiation
half-life is not long enough to take full advantage of the retention of
the label at the tumor site. There are few if any other residualizing
radiolabels with relatively long half-lives (1–2 weeks) that have been
utilized. One potential candidate is $^{177}$Lu (38). However, it cannot be
assumed that lutetium or other metals will behave identically to the
benzyl-DTPA chelate of indium or yttrium, and results may depend on
the particular chelator used.

Although we expected that the advantage of a residualizing label
would be greater for a rapidly catabolized mAb, RS7, than it would be
for a slowly catabolized mAb, RS11, such a difference was not seen.
In retrospect, this is perhaps not surprising. The difference in the rate
of catabolism of these two mAbs (seen in vitro) is only approximately
2-fold, which perhaps is a small difference compared to the other
processes that are involved. Although we attempted to modify our in
vitro experiment to more closely simulate the in vivo situation, by
keeping the labeled mAb in the medium for 2–3 days, these results did
not appear to more closely parallel the in vivo data. This discrepancy
may be due to differences in the physiological state of the cells when
grown in nude mice or in tissue culture. For example, differences in
the rate of membrane turnover would have major effects on the rate
of antibody processing. Because RS11 has consistently been observed
to be processed in a manner similar to the majority of “typical”
membrane-reactive mAbs on a variety of cell lines (2), we expect that
most mAbs reacting with the surface of Calu-3 will be processed
similarly.

Additional use of DLT for radioimmunotherapy is complicated
partly by relatively low conjugation efficiency. The efficiency of
protein iodination obtained in these studies, 3–6%, is sufficient for
radioimmunodetection experiments in mice and for in vitro experi-
ments but not for clinical studies. However, we have recently im-
proved the efficiency to approximately 33%, simply by increasing the
concentration of mAb used by a factor of 20. Additional improve-
ments in efficiency are under investigation.

In summary, the prolonged retention of $^{131}$I-labeled DLT mAbs in
tumor cells, the 8-day half-life of the $^{131}$I, and the relatively low levels
of accretion in normal tissues combine to make radioiodinated DLT an
adduct of great potential for radioimmunotherapy. The effective-
ness of this methodology appears not to be limited by whether a mAb
is rapidly catabolized by antigen-expressing cells and should be of
general applicability.

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Effects of Radiolabeling Monoclonal Antibodies with a Residualizing Iodine Radiolabel on the Accretion of Radioisotope in Tumors
