Determinants of the Antitumor Effect of Radiolabeled Monoclonal Antibodies


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ABSTRACT

The murine B-cell lymphoma 38C13 model was used to study the radiobiological effect of 131I-monoclonal antibody (MAB) therapy compared with dose equivalent external beam irradiation. Continuous exponentially decreasing low dose rate (LDR) γ-irradiation, and multiply fractionated (MF) X-irradiation were compared with dose equivalent 131I-MAB. The relative therapeutic efficacy of radioimmunotherapy, and the relative contribution of (a) low dose rate; (b) whole body irradiation; and (c) microdosimetry to the overall effect were determined. Groups of mice with or without B-cell lymphoma were treated with either (a) 131I-anti-idiotype MAB; (b) 131I-isotype-matched irrelevant control MAB; (c) 5-15 Gy 250 kV X-irradiation given as a single fraction; (d) 2.5-30 Gy 250 kV X-irradiation given in 10 fractions/2 weeks; or by (e) continuous exponentially decreasing γ-irradiation via a 137Cs source, which simulated the effective half-life of the 131I-MAB. In tumor-free mice the LDR dose was approximately 10 Gy for MF and LDR external irradiation, and 11-12 Gy for 131I-MAB. However, the effect of these modes of irradiation on tumor size differed significantly. The cumulative percentage of tumor reduction averaged over 12 days was 0.63 ± 0.05%/Gy for MF, and 1.36 ± 0.061%/Gy for LDR external irradiation (a relative efficacy factor of 1.63 for LDR irradiation; P = 0.01). Assuming homogeneous body distribution, the tumor reduction effect over 12 days for 131I-MAB was 2.064 ± 0.13%/Gy for specific, and 1.742 ± 0.1%/Gy for nonspecific isotope-matched irrelevant 131I-MAB (P = 0.02). When 131I-MAB was compared to LDR external irradiation, the relative efficacy factor was 1.99 (P < 0.001). In summary, there was a dose rate effect on tumor response, which may in part explain the efficacy of radioimmunotherapy. The additional effect of 131I-MAB on tumor response was only partially explained by the cumulative concentration ratio of 131I-MAB tumor/131I-MAB whole body, which was on average 1.7. This relatively low concentration ratio was partly due to tumor-mediated dehalogenation. Thus, the overall tumor response was a function of the total dose, dose rate, and both the specific and nonspecific distribution of 131I-MAB.

INTRODUCTION

Over the past few years, there has been increasing interest in the therapeutic use of radiolabeled monoclonal antibodies. Little is known about the effects of the low dose rate of these radiolabeled MAB or their relative efficacy compared with conventional fractionated external beam irradiation. In order to compare the therapeutic efficacy of RIT, and the relative contribution of (a) low dose rate; (b) whole body irradiation; and (c) microdosimetry to the overall effect, a murine B-cell lymphoma model was utilized (1).

Comparison of dose equivalent MF X-irradiation with continuous exponentially decreasing LDR γ-irradiation (same effective t1/2 as 131I-MAB in this model) allowed for the determination of the relative importance of low dose rate to the effect (tumor size, toxicity) of RIT. The effect of dose equivalent homogeneous continuous exponentially decreasing LDR external whole body irradiation was compared with specific 131I-anti-idiotype MAB and 131I-isotype-matched irrelevant control MAB in order to determine the relative contribution of both specific localized and nonspecific whole body irradiation on overall effect.

MATERIALS AND METHODS

Experimental Design. Groups of 9- to 12-week-old female C3H/HeN mice with (7 days after s.c. injection of 1 × 106 38C13 cells) or without B-cell lymphoma were treated with either (a) 131I-anti-idiotype MAB; (b) 131I-isotype-matched irrelevant control MAB; (c) 5-15 Gy 250 kV X-irradiation given as a single fraction; (d) 2.5-30 Gy 250 kV X-irradiation given in 10 fractions/2 weeks; or by (e) continuous exponentially decreasing γ-irradiation via a 137Cs source, during which time cages were moved a calculated distance daily from a 137Cs source in order to mimic the effective t1/2 of the 131I-anti-idiotype MAB in this model. Three experiments using 131I-anti-idiotype and two with isotype-matched irrelevant control MAB were performed. One experiment using single fraction X-irradiation, two multifractionated X-irradiation experiments, and four experiments using continuous exponentially decreasing γ-irradiation from a 137Cs source were performed. Two of the 137Cs experiments used dose levels of 10-30 Gy and two used doses ranging from 2.5 to 17.5 Gy. All experiments used 5-10 mice/dose level and contained a control (untreated) group. Additional groups of 4-10 mice without tumors were treated in parallel at all dose levels studied. Tumor size and survival were measured. Total body activity was counted daily and whole body doses were calculated, assuming total absorption of the nonpenetrating component using Medical Internal Radiation Dose (MIRD) formulas (2). External whole body, rather than localized tumor site irradiation was utilized because of the relatively high whole body dose component of 131I-MAB.

Mice. Nine to 12-week-old female C3H/HeN mice were obtained from Simonsen Laboratories (Gilroy, CA). Mice were tested and found to be negative for antibodies to mouse hepatitis virus, Sendai virus, and Mycoplasma pulmonis. The mice were given Lugol’s solution (5.0 ml Lugol’s stock/400 ml H2O; stock: 10 g KI, 5 g elemental iodine in 100 ml H2O) in their water 3 days before initiation of therapy and for 2 weeks thereafter. Routine necropsies with standard histopathological studies were performed on all externally irradiated mice. Mice were sacrificed for humanitarian reasons when moribund and to preclude autolysis of tissue. In several experiments, biodistribution studies were performed by sacrificing animals at 1, 24, 48, 72, 96, 120, 148, and 172 h after injection of radiolabeled 131I- and/or 125I-MAB. The weight and activity of the entire mouse as well as dissected organs were measured, and the percentage of injected dose/g was calculated.

Tumor and Cell Line. 38C13 is a carcinogen (7,12-dimethyl benz(a)anthracene)-induced B-cell tumor which was produced in a C3H/eB mouse depleted of T-cells (3). This tumor and its in vitro adapted cell line express IGM on the cell surface. It is a high grade lymphoma composed of phenotypically transformed small lymphocytes. The animals were given injections s.c. in the flank of 1 × 106 38C13 cells in a 0.1-ml volume. By day 7 most animals had discrete palpable tumors with a mean diameter of 9.3 mm, and widespread disease as evidenced by generalized adenopathy and histopathological involvement of lymphohematopoietic tissues. Within a given experiment mice were randomly assigned to treatment groups.

Antibody Production, Purification, and Testing. Monoclonal anti-idiotype antibodies were made as previously described (1). Anti-idiotype (IgG1) MAB was directed to unique antigenic determinants within the variable region of the surface immunoglobulin expressed on...
The purity of the final preparation was >95% as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Iodination and Radioimmunooactivity. Antibodies were labeled with $^{131}$I by using the chloramine-T method (4). Briefly, MAB, Na$^{131}$I (specific activity, 7.10-8.68 Ci/mg; DuPont, Wilmington, DE), and chloramine-T (1-4%; 1%/2 mg MAB) in 0.5 M phosphate-buffered saline were mixed together for 5 min. The reaction was stopped by adding sodium thiosulfate at 4 times the concentration of chloramine-T. The $^{131}$I-MAB was separated from the reactants by using a gel filtration column (Pharmacia, Sweden). The specific activity was measured, and it was 5.94, 9.32, and 10.53 mCi/mg for $^{131}$I-anti-idiotype MAB 4C8, and 9.08-9.95 mCi/mg for $^{131}$I-isotype-matched irrelevant control MAB. The immunoreactivity of the eluant was determined with a solid phase immunoabsorption technique. Cyanogen bromide-activated Sepharose 4B beads (Sigma, St. Louis, MO) were coupled to purified 38C13 idiotype. Various concentrations of beads were incubated in duplicate with 900 μl $^{131}$I-MAB (5 ng/ml). The volume was adjusted to 1000 μl by using RPMI 1640 (Applied Scientific, San Francisco, CA) with 1% bovine serum albumin (Miles Laboratories, Inc., Elkhart, IN). Following incubation at room temperature for 1 h on a rotating mixer, tubes were centrifuged for 2 min at 16,000 × g. Supernatant (750 μl) was removed for counting. Total, bound, and free counts were measured. Total/bound counts were plotted as a function of 1/bead volume. Using this method, the immunoreactivity (1/y intercept × 100) of labeled MAB preparations was 58.8-83.3%. Mice were given injections shortly thereafter of the $^{131}$I-MAB i.v. through the tail vein. At the highest doses of $^{131}$I-MAB, mice received up to 100 μg MAB. Similar amounts of unlabelled MAB had no significant effect on established flank tumors in previous experiments.

Calculation of Dose and Dose Rate Equivalents to RIT Using $^{131}$I-Anti-Idiotype Monoclonal Antibody. The activity (μCi) of mice given injections of $^{131}$I-MAB was determined immediately following injection and daily by using a dose calibrator (Capintec Radioisotope Calibrator CRC-7 using the $^{131}$I setting). The effective $t_{\text{eff}}$ and average retention time of mice for different doses of $^{131}$I-anti-idiotype was determined, and the total whole body dose was calculated by integrating the area under the retention curve, and assuming total absorption of the non-penetrating radiation component with a homogeneous distribution (2). $^{131}$Cs Continuous Exponentially Decreasing Low Dose Rate $\gamma$-Irradiation. Using a 120 Ci $^{131}$Cs source with a predetermined calibration range (μGy/h) at 1- to 50-foot distances, cages were moved a calculated distance from the source daily in order to mimic the effective $t_{\text{eff}}$ of $^{131}$I-4C8 over a 2-week period. Dose rates (≤28.8 cGy/h) and total dose levels were equivalent to those of RIT experiments. Dosimeters (2/ cage) were placed underneath cage bottoms, and removed at the end of the 2-week irradiation period for reading.

250 kV X-Irradiation. Mice were irradiated, using a Plexiglas holder by a 250-kV X-ray machine, 15 mA (Philips, Netherlands), using a 0.35-mm copper filter. The total dose was given either as a single dose (fraction), or in 10 fractions over 2 weeks (Monday–Friday).

Measurement of Tumor Size and Survival. Tumors were measured in three dimensions with calipers. Tumor dimensions were recorded every other day. Tumor size was expressed as a volume transformation ($VT$) defined as $\sqrt{\text{length} \times \text{width} \times \text{height}}$ in mm. This equation was used because it (a) allowed for the reduction of large volumes to smaller numbers ($VT$) with more uniformity in associated error, and (b) increased the linearity of the tumor response data. Tumor response data were calculated as (a) median $VT$, (b) percentage of daily control (untreated) $VT$, and (c) cumulative tumor response $\text{c}(t) = 100 \times \frac{\int_{0}^{t} [VT(t) - VT(t)]}{VT(t)}$ (A)

where $VT(t)$ is the median tumor size in the control group on day $t$, and $VT(t)$ is the median $VT$ in the treated group $i$ at time $t$. The integration was performed from day $0$ to day $d$. Use of this integrated function minimized the time dependency of observed effects. The reproducibility (SE) of the tumor response data is shown in Tables 1 and 2. Mice were observed daily in order to obtain long-term survival data. Tumor-free mice were used to determine the LD$_{50/30}$ so that tumor-related deaths would not contribute to this measurement.

RESULTS

Effect on Survival. The effect of SF 250 kV X-irradiation, MF 250 kV X-irradiation, continuous exponentially decreasing LDR $\gamma$-irradiation, and $^{131}$I-MAB on survival in tumorless mice was studied. The SF LD$_{50/30}$ was less than 5 Gy, approximately 10 Gy for MF and LDR, and 11-12 Gy for $^{131}$I-MAB. There was no statistically significant difference between MF, LDR, or $^{131}$I-MAB in survival. All irradiated animals (with the exception of those treated with SF $\geq 10$ Gy) died of bone marrow failure with bacteremia and/or hemorrhage, and most had macroscopic tumor at the time of death.

Effect on Tumor Size. Fig. 1 shows data from one experiment in which groups of mice received either 0, 5, 10, 15, 22.5, or 30 Gy of MF X-irradiation, and demonstrates the method used to analyze tumor response data. Median tumor sizes expressed as a volume transformation ($VT$) were utilized instead of mean sizes because of the occasional occurrence (≤10%) of spontaneous regression in untreated animals, and long-term survival in several treated (cured) animals. Each point represents the median tumor size ($VT$) from five mice in an experimental subset. Fig. 1A shows tumor size ($VT$) as a function of time $[VT(t)]$ in Equation A. In Fig. 1B the same data are plotted as the percentage of the daily control (untreated) tumor size $\frac{100 \times VT(t)}{VT(t)}$.

Effect on Survival. The effect of MF 250 kV X-irradiation, continuous exponentially decreasing LDR $\gamma$-irradiation, and $^{131}$I-MAB on survival in tumorless mice was studied. The SF LD$_{50/30}$ was less than 5 Gy, approximately 10 Gy for MF and LDR, and 11-12 Gy for $^{131}$I-MAB. There was no statistically significant difference between MF, LDR, or $^{131}$I-MAB in survival. All irradiated animals (with the exception of those treated with SF $\geq 10$ Gy) died of bone marrow failure with bacteremia and/or hemorrhage, and most had macroscopic tumor at the time of death.

MF versus LDR. Fig. 2 shows tumor response as a function of total dose for MF and LDR radiation. The averaged (normalized for time) cumulative percentage per day tumor size ($VT$) decrease $[c(t)/d]$ from 0–12 days is plotted as a function of total dose in Gy. Using a zero intercept linear model, the slopes (averaged cumulative percentage of tumor size decrease/ Gy) are significantly different ($P = 0.01$), with LDR irradiation 1.63 times more effective than MF. Interestingly, there was a paradoxical small increase in tumor size at low doses as shown by the negative data points. A direct comparison of tumor size ($VT$; percentage of control) at days 8 and 12 for MF and LDR irradiation is shown in Table 1. There was a significant ($P < 0.02$) difference between MF and LDR at all dose levels.

$^{131}$I-MAB: Specific Anti-Idiotype versus Irrelevant Isotype-matched Control MAB. Tumor response as a function of total dose is shown in Fig. 2 for $^{131}$I-anti-idiotype (specific) and isotype-matched irrelevant (nonspecific) MAB. Again, the averaged cumulative percentage per day of tumor size ($VT$) decrease for days 0–12 is plotted as a function of total dose. The slopes are significantly different ($P = 0.02$), although specific antibody is only approximately 18% more effective than non-specific antibody in tumor reduction.

Comparison of SF, MF, LDR, and $^{131}$I-MAB. Tumor size ($VT$) as a function of time for SF, MF, LDR, and $^{131}$I-MAB is shown in Fig. 3 for total doses of 5, 10, and 15 Gy. Each data point is the median tumor size ($VT$) for a group of 5–10 mice.

$^{131}$I-MAB: Specific Anti-Idiotype versus Irrelevant Isotype-matched Control MAB. Tumor response as a function of total dose is shown in Fig. 2 for $^{131}$I-anti-idiotype (specific) and isotype-matched irrelevant (nonspecific) MAB. Again, the averaged cumulative percentage per day of tumor size ($VT$) decrease for days 0–12 is plotted as a function of total dose. The slopes are significantly different ($P = 0.02$), although specific antibody is only approximately 18% more effective than non-specific antibody in tumor reduction.

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from a single experiment. At all 3 dose levels, the different irradiation schemata may be ranked in terms of efficacy as follows: $^{131}$I-MAB > LDR > MF. At the 5-Gy dose level, SF X-irradiation was similar to both MF and LDR irradiation in effect on tumor size, whereas at 10 and 15 Gy SF was at least as effective as $^{131}$I-MAB, but mice died in 4–6 days of gastrointestinal toxicity. The data have been summarized in Table 2, where slope and SE of the slope are shown for MF, LDR, specific, and nonspecific $^{131}$I-MAB. MF versus LDR, LDR versus specific $^{131}$I-MAB, and specific versus nonspecific $^{131}$I-MAB were all statistically significantly different, with relative efficacy factors (ratio of slopes) of 1.63, 1.99, and 1.18, respectively. The relative efficacy factor for MF versus $^{131}$I-MAB was 3.25.

The additional effect of $^{131}$I-MAB on tumor response is only partially explained by the cumulative concentration ratio of $^{131}$I-MAB tumor/$^{131}$I-MAB whole body, which averaged approximately 1.7. Table 3 shows cumulative organ doses derived from a single exponential fit of tracer concentration data of a single experiment (2 mice for each of 5 observation days) using specific $^{131}$I-MAB. The units are in percentage of dose per g for the intercept, 1/day for the slope, and percentage of dose-day/g for the cumulative dose. The average tumor dose is indeed higher (0.258) than the average whole body dose (0.149), secondary to both specific localization and prolonged retention of $^{131}$I-MAB in the tumor. Concentration ratios for specific/nonspecific radiolabeled MAB for tumor, liver, and blood as a function of time are shown in Table 4. Higher intratumoral concentrations

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**Table 1** Tumor size (VT) at day 8 and 12 for multiply fractionated versus low dose rate irradiation

<table>
<thead>
<tr>
<th>Gy</th>
<th>Median tumor size ± SE (mm)</th>
<th>% of control</th>
<th>Median tumor size ± SE (mm)</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>MF 59.5 ± 7.2 (100) 0.80</td>
<td>75.6 ± 10.6 (100) 0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDR 59.0 ± 6.0 (100) 0.80</td>
<td>72.7 ± 9.8 (100) 0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-7</td>
<td>MF 58.8 ± 4.2 (98.8) 0.02</td>
<td>81.8 ± 5.5 (108.2) 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDR 53.1 ± 4.7 (90.0) 0.02</td>
<td>68.0 ± 5.3 (93.5) 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-10</td>
<td>MF 51.6 ± 3.1 (86.7) 0.001</td>
<td>63.6 ± 4.6 (84.1) 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDR 37.0 ± 5.3 (62.7) 0.001</td>
<td>40.7 ± 6.1 (56.0) 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-15.8</td>
<td>MF 43.8 ± 3.8 (73.6) 0.001</td>
<td>51.5 ± 4.7 (68.1) 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDR 29.8 ± 5.8 (50.5) 0.001</td>
<td>37.9 ± 6.1 (52.1) 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- P values are based on a 2-tailed t-test for unpaired sample mean differences with 18 degrees of freedom.

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**Table 2** Cumulative percentage of tumor size (VT) decrease from days 0–12

<table>
<thead>
<tr>
<th>Slope</th>
<th>SE</th>
<th>P</th>
<th>Relative efficacy factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF</td>
<td>0.635</td>
<td>0.055</td>
<td>0.01</td>
</tr>
<tr>
<td>LDR</td>
<td>1.036</td>
<td>0.061</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Specific $^{131}$I-MAB</td>
<td>2.064</td>
<td>0.133</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nonspecific $^{131}$I-MAB</td>
<td>1.742</td>
<td>0.100</td>
<td>0.02</td>
</tr>
</tbody>
</table>
ANTITUMOR EFFECT OF RADIOLABELED MONOCLONAL ANTIBODIES

Table 3 Cumulative organ doses with specific 131I-MAB

<table>
<thead>
<tr>
<th>Organ</th>
<th>Intercept (A)</th>
<th>Slope (X)</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>0.042</td>
<td>-0.163</td>
<td>0.258</td>
</tr>
<tr>
<td>Liver</td>
<td>0.048</td>
<td>-0.368</td>
<td>0.130</td>
</tr>
<tr>
<td>Body</td>
<td>0.051</td>
<td>-0.342</td>
<td>0.149</td>
</tr>
</tbody>
</table>

Table 4 Concentration ratios specific/nonspecific 131I-MAB

<table>
<thead>
<tr>
<th>Time</th>
<th>Specific</th>
<th>Nonspecific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>1.43</td>
<td>0.88</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.85</td>
<td>0.81</td>
</tr>
<tr>
<td>Day 4</td>
<td>0.82</td>
<td>0.81</td>
</tr>
</tbody>
</table>

of specific radiolabeled MAB (expressed as percentage of dose/g) persisted for only 1 day. The values in this table reflect the shorter retention time of the specific antibody. The shorter retention time of specific 131I-MAB compared with nonspecific 131I-MAB on day 0. Mice (4 mice for each time point) were sacrificed 1, 2, and 4 days following 131I-MAB injection. The weight and activity of the entire mouse as well as dissected organs were measured, and the percentage of injected dose/g was calculated. Cumulative organ doses for tumor, liver, and whole body were derived from a single exponential fit (Ae^{-x}) of an experiment (2 mice for each time point). The intercept (A: percentage of dose per g), slope (X; I/day), and cumulative organ dose (Ae^{-x} / X) percentage of dose-day/g) are shown below.

DISCUSSION

Little is known about dose rate effects of radioimmunotherapy on tumor response and toxicity. A variety of studies have demonstrated an inverse relationship between dose rate and mean lethal dose (LD_{50}) (8-11). Nevertheless, it has generally been accepted that there is decreased effect with decreased dose rate and increased exposure time (12-14), which has been attributed to increased repair of sublethal damage and cellular proliferation (14). Our data however fail to confirm this tenet, and demonstrate instead increased effect with decreased dose rate.

To date there are no published studies on the in vivo dose rate effects for lymphoma, and little is reported about dose rate effects in the range expected for radioimmunotherapy with mononuclear antibodies (0.05-0.2 Gy/h or 0.00083-0.0033 Gy/ min), (15-22). Our data demonstrate a significant increase in the tumor response to low dose rate irradiation (≤28.8 eGy/h), as shown by the increased efficacy of continuous exponentially decreasing LDR irradiation compared with MF X-irradiation at equivalent dose levels. 131I-Anti-idiotype MAB was approximately twice as effective as LDR, on the basis of whole body dose equivalents, presumably because of the concentration ratio of 131I-MAB tumor/131I-MAB whole body, which was on average approximately 1.7. 131I-Anti-idiotype MAB was 3.25 times as effective as dose equivalent MF because of the combined effect of the low dose rate irradiation and specific uptake of 131I-anti-idiotype MAB by tumor. Although there was a statistically significant difference (P = 0.02) between specific (anti-idiotype) and nonspecific (irrelevant) 131I-MAB on tumor response, the relative efficacy factor was only 1.18. The surprisingly small difference between specific and nonspecific 131I-MAB may be explained by (a) the lack of sustained high concentrations of 131I-anti-idiotype at the tumor site because of antigenic modulation and rapid dehalogenation (effective t_{1/2} = 3.4 days for nonspecific 131I-MAB, and 2.5 days for specific 131I-MAB in tumor-bearing animals); (b) a relatively higher dose rate for the specific 131I-MAB; and (c) nonspecific uptake of nonspecific antibody because of differences in vascular permeability between tumor and normal vasculature, and possible differential effects of whole body irradiation on tumor vasculature, with subsequent increased permeability of tumor endothelial cells. Thus, the total effect of RIT on both tumor growth and toxicity is a function of the total dose, dose rate, and both specific and nonspecific 131I-MAB distribution.

Our conclusions and the relative efficacy factors generated above are obviously limited to this particular animal model and antibody. Had there been little or no antigenic modulation and hence less dehalogenation, or increased specific uptake of 131I-MAB by tumor, the results would have been correspondingly different. Nevertheless, we believe that these findings are clinically relevant. Clinical trials with radiolabeled pan B and anti-idiotype MAB are currently ongoing at several locations. The majority of patients with non-Hodgkin's lymphoma have disseminated disease, as do the mice at the time of treatment, making it difficult to separate whole body distribution (nonspecific distribution) from specific distribution in widely disseminated (diffusely distributed) malignant disease. For example, bone marrow toxicity may be secondary to the nonspecific whole body dose, and to binding of the antibody to malignant cells (and to a lesser extent to normal marrow cells) in the bone marrow (23).

Furthermore, our findings regarding the relative importance of very low dose rate irradiation, and the whole body dose contribution, may explain in part the therapeutic efficacy of RIT. For example, complete responses in B-cell lymphoma patients treated with 131I-anti-idiotype MAB have been reported with tumor doses as low as 8.5 Gy (5). Similarly, a surprisingly high response rate was observed in patients with hepatomas treated with 131I-antiferritin (peak dose rate, 0.045-0.050 Gy/h; total tumor dose 10-12 Gy by day 15 (24), and in patients with both hepatomas and Hodgkin's disease treated with 90Y-antiferritin [peak dose rate, 0.16 Gy/h; total tumor dose, 20-35 Gy (25)]. In general, much higher doses of conventionally fractionated external beam radiation therapy would be required in order to achieve similar clinical responses. Of note, Pierquin (26) obtained improved tumor control in patients with squamous cell carcinoma of the head and neck with 1.1-1.8 Gy/h
(10 Gy/day; 60–70 Gy total) as compared with conventionally fractionated external beam radiation therapy.

An “inverse” dose rate effect has been reported in some cell lines with increased cell killing occurring at decreased dose rates (same total dose) (27, 28). This occurred only when dose rates were sufficient to stop cell growth after exposure times long enough for cell cycle redistribution (28). Williams and Dillehay (22) have shown increased cell killing in vitro with exponentially decreasing dose rates compared with dose equivalent constant dose rates. It has been postulated that the sensitization associated with exponentially decreasing dose rates may be dependent upon an initial dose rate above a given threshold, that may depend in part on the inherent radiosensitivity of the particular tumor cell line. Two possible mechanisms have been proposed to explain this effect: (a) time-dependent dose-dependent direct sensitization of cells (22), and (b) accumulation of cells in G2 (14, 22, 27, 29) which is a very radiosensitive phase of the cell cycle (14). Other factors that may affect this phenomenon of sensitization are reoxygenation during protracted exposure (14) and possible unexplained effects on repair of sublethal damage.

Our findings are consistent with those of Wessels et al. in which RIT of colorectal and renal cell carcinoma xenografts was found to be more effective than high dose rate external beam irradiation on a per rad basis (18–21). They have postulated that the targeting of a viable subpopulation of cells by RIT, that is largely responsible for tumor volume doubling, may contribute to the increased efficacy of RIT (21). Indeed, our data (Table 3) indicate that an increased cumulative tumor concentration (131I-MAB tumor/131I-MAB whole body = 1.73) is insufficient to explain the increased tumoricidal effect, if the intratumoral concentration of 131I-MAB is homogeneous. Other factors that may help explain the paradox between tumor response with RIT and the predicted response based on conventional external beam radiation therapy are (a) significant tumor distribution heterogeneity (as evidenced by thermoluminescent dosimeter measurements and autoradiographs of tumors) after RIT (30), and (b) poorly understood effects of total body irradiation. Complete response rates of 67–85% have been reported in lymphoma patients treated with 1.5–3.0 Gy total body irradiation (0.15–0.30 Gy fractions + a boost to the involved field) (31–34). Furthermore, in lymphoma, whole body irradiation has been shown to be more therapeutically efficacious in mice than local irradiation alone (35–37).

In our experience, with very few exceptions, it was not possible to give a curative dose of radiation without killing the animals of bone marrow failure. Similarly, bone marrow toxicity has been the primary limiting factor that has determined the therapeutic dose of 131I- and 90Y-MAB administered to patients. Vaughan et al. (38, 39) calculated the minimal requirements for effective therapy with 131I- and 90Y-labeled monoclonal antibodies injected i.v., assuming an average accumulation of 0.005% of the administered dose/g of tumor, and a maximum reasonable whole body dose of 2 Gy. They concluded that the tumor uptake should be increased by at least a factor of 10 for effective therapy without lethal whole body irradiation. Similarly, Atcher et al. (15) reported that dose rates from 131I and 99mTc in vivo were suboptimal and inadequate for curative therapy. With autologous bone marrow transplantation it may now be possible to give curative doses without dose-limiting bone marrow toxicity. Studies are currently under way using the 38C13 model with bone marrow transplantation in order to further optimize the treatment regimen.

For RIT treatment-planning purposes, however, the effect of RIT cannot be accurately predicted on the basis of total dose and average tumor concentration. Rather, dose rate, a whole body irradiation component, and heterogeneous tumor distribution of radiolabeled MAB must be taken into account.

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Determinants of the Antitumor Effect of Radiolabeled Monoclonal Antibodies

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