Microdistribution of Specific Rat Monoclonal Antibodies to Mouse Tissues and Human Tumor Xenografts

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ABSTRACT

Detailed evaluations of the microdistribution of 125I-labeled monoclonal antibodies (MoAbs) to normal tissue antigens were conducted in BALB/c mice. MoAb 273-34A, which binds to a target molecule on the luminal surface of lung endothelial cells, localizes quickly and efficiently throughout the lung vasculature. MoAb 133-13A, which binds to an antigen on macrophage-like cells expressed in nearly equal amounts in lung, liver, and spleen, localizes most efficiently to spleen and less well to liver and lung. The microdistribution of MoAb 133-13A in liver and spleen is consistent with the antigen distribution in these organs, but in the lung a more diffuse microdistribution is observed, indicating poor access of MoAb to the antigen-positive alveolar macrophages. These findings are consistent with the hypothesis that tight endothelium (lung) represents a significant barrier to extravasation of MoAb into tissue while fenestrated (spleen) and sinusoidal (liver) endothelium are more easily penetrated.

In human tumor bearing nu/nu mice, the microdistribution of MoAb to the ß1 and ß3 subunits of integrin was studied. These MoAbs do not cross-react with murine integrins and thus are tumor-specific in the nu/nu mouse model. Localization of 125I-labeled MoAb 450-11A, which reacts with an intercellular domain of ß1 integrin, is very weak and diffuse. All MoAbs to extracellular domains (mouse 450-9D, 450-30A, and rat 439-9B) localize well to the tumor. Microdistribution of these MoAbs in the 3 different tumors is nonuniform with heavy distribution near the blood vessels, whereas antigen distribution as determined by immunoperoxidase shows a much more uniform pattern throughout the tumors. In experiments with 125I-labeled MoAb 439-9B F(ab')2, the nonuniform pattern of distribution was not changed. Gross and microdistribution of different doses of 125I-labeled MoAb 439-9B were studied. The percent of injected dose per g of MoAb in the tumor at 48 h did not vary significantly (P > 0.1) up to a dose of 500 µg/mouse, and active MoAb was recovered in comparable amounts in the serum from animals in all doses. In contrast, the microdistribution of MoAb at the high dose was different than that at low doses. At doses up to 100 µg/mouse, a perivascular pattern was obtained, whereas at 500 µg/mouse the 125I-labeled MoAb was distributed nearly evenly throughout the tumor. These data indicate that high doses of MoAb penetrate deeply into portions of the tumor that are distant from blood vessels. These data are consistent with the hypothesis that even when MoAbs have penetrated the vessel walls, movement throughout the tumor is restricted. High doses of MoAb overcome this restriction and generate a more uniform MoAb distribution.

INTRODUCTION

Accumulation of MoAb at specific sites in the body is a complicated process involving characteristics of the MoAb as well as the target antigen (1). Whereas early studies focused on high MoAb affinity and tumor specificity, it is becoming clear that other factors may be more important (2, 3).

Recent work by Sands et al. (3) has shown that MoAbs are not evenly distributed in tumor xenografts. Whereas these nude mouse models may not adequately mimic human tumors (4), it is becoming evident that access of antigen to MoAb may limit efficient accumulation. MoAb accumulation is very efficient in situations where tumor antigen is exposed directly to the MoAb (5, 6).

Microdistribution studies using autoradiographic techniques (3, 7, 8) demonstrate that the quality and quantity of tumor vessels affect the MoAb distribution. Theoretical and experimental work of Jain and Baxter (9, 10) has subdivided the potential barriers to MoAb penetration into tumors into 3 processes: (a) distribution in vascular space; (b) transport across microvascular walls; and (c) transport through interstitial space. It is not clear which of these barriers is limiting. Fujimori et al. (11) have developed mathematical models to account for penetration of MoAb and their fragments in tumors.

We have developed several MoAbs to mouse tissues and to human tumor-associated antigens, which can be used to test these predictions. In previous work, it was shown that the gross distribution of these MoAbs supports the hypothesis that transport across vessel walls is the major limiting factor in MoAb accumulation (6). Studies on the microdistribution of the MoAbs reported here strengthen and extend this observation. In addition, it is shown that transport through interstitial space in tumors can be facilitated by high doses of MoAb that effectively drive the antibody deep into tumor sites distal from blood vessels in accordance with predictions of the model of Fujimori et al. (11).

MATERIALS AND METHODS

Cell Lines and MoAbs. Human tumor lines used for xenografts were epidermoid carcinoma A431 (12), lung carcinoma A549 (12), and colon carcinoma SW948 (13). The cells were grown (14) and injected s.c. into nu/nu mice on the right side as described previously (6). Rat MoAb 273-34A specific for a lung endothelial cell surface protein (15), rat MoAb 133-13A specific for a macrophage antigen (16), and rat MoAb 439-9B specific for the human ß1 subunit of the integrin family (14, 17) were used in these studies. Rat MoAb 135-14 was used as a negative control. Target antigen concentrations for these MoAbs have been published previously (6).

Murine MoAb 450-11A and 450-9D to the ß1 subunit of integrin and MoAb 450-30A to the ß3 subunit of human integrin were also used to verify distribution data (18). MoAb P3 IgG was used as a negative control for mouse MoAb.

MoAbs were purified from ascites fluids by ammonium sulfate precipitation, DE52 ion exchange chromatography, and HPLC on a Waters Protein Pak DEAE-5PW column as described previously (6).

Distribution Experiments. MoAbs labeled with 125I or 131I by the chloramine T method were further purified by gel filtration on AcA 34 (6) in 5 mg/ml bovine serum albumin in phosphate-buffered saline. Labeled MoAbs were injected directly after purification or after frozen storage at −20°C for less than 1 week. Binding activity of each labeled preparation was tested by direct binding assays to the appropriate cell lines (19). The fraction of total isotope bound in these assays varied with the antibody Ka and cell density. The values for percent binding...
were MoAb 9B, 9 to 30%; MoAb 13A, 50%; and MoAb 34A, 84%.

Normal B6 BALB/c mice (Oak Ridge colony) or nu/nu mice (Life Sciences, St. Petersburg, FL) bearing tumors of 0.5–1.0 g were used for distribution experiments. At this size, little necrosis is encountered. A431 has keratin-filled interior spaces, whereas A549 and SW948 form multiple bead-like tumors of 2–3 mm in diameter. No studies were done on extremely small tumors. Mixtures of 125I-labeled MoAb and 125I-labeled control MoAb were injected in 0.2 ml of phosphate-buffered saline containing 5 mg/ml bovine serum albumin in the tail vein. At appropriate times, animals were sacrificed for analyses of gross isotope distribution as described previously (6). The ratio for specific accumulation was calculated by dividing the %ID/g for the specific 125I-labeled MoAb by that value for the control 125I-labeled MoAb in each tissue. For the dose experiments, % values were calculated comparing the highest dose to each of the others in a 2-sided Student’s t test. Slices (1–3 mm thick) of kidney, liver, spleen, skin, lung, and tumor were fixed in 10% buffered formalin for 24 h and processed for paraffin embedding (15). Sections (5 μm) on glass slides coated with gelatin and Chrom-alum were dipped in NTB-2 emulsion (Kodak), dried, and stored in a dark desiccated chamber for 1 week to 2 months. Emulsion Chom-alum were exposed in the vascular space (6). Experiments with 3 different 125I-labeled MoAb 9B, 16.2 × 106 cpm, and 2 μg of control 125I-labeled MoAb 135-14, 6.8 × 106 cpm, injected i.v. into adult BALB/c mice. Average of 3 animals sacrificed at 24 h. Antibody in serum retains 2% of original activity.

MoAb MICRODISTRIBUTION

<table>
<thead>
<tr>
<th>MoAb 13A</th>
<th>MoAb 34A</th>
<th>MoAb 9B</th>
</tr>
</thead>
<tbody>
<tr>
<td>%ID/g</td>
<td>Ratio'</td>
<td>%ID/g</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.7 ± 0.26</td>
<td>0.9 ± 0.041</td>
</tr>
<tr>
<td>Liver</td>
<td>5.2 ± 1.49</td>
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<tr>
<td>Spleen</td>
<td>43.3 ± 7.27</td>
<td>25.6 ± 3.01</td>
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<tr>
<td>Lung</td>
<td>7.6 ± 1.27</td>
<td>2.5 ± 0.48</td>
</tr>
<tr>
<td>Tumor</td>
<td>1.08 ± 0.29</td>
<td>0.8 ± 0.10</td>
</tr>
<tr>
<td>Skin</td>
<td>9.6 ± 1.08</td>
<td>0.6 ± 0.03</td>
</tr>
</tbody>
</table>

Table 1 Gross distribution of Moab 13A, 34A, and 9B in mice*

*All values are expressed as the mean ± SD.

RESULTS

Capillary Wall Effects. We have shown that gross accumulation of MoAb in tumor tissue is most efficient if the antigen is exposed in the vascular space (6). Experiments with 3 different rat MoAbs having similar Ka and target antigen concentrations were compared. One MoAb, 34A, reacts with a Mr, 112,000 glycoprotein found on the luminal surface of lung endothelium; MoAb 13A reacts with a Mr, 100,000 glycoprotein found on macrophages in the lung, liver, and spleen; and the third MoAb, 9B, reacts with human carcinoma xenografts. Data in Table 1 confirm earlier gross distribution data (48-h postinjection) for these 3 MoAb systems.

MoAb 34A localizes very efficiently to lung (%ID/g = 276.1), whereas MoAb 13A localizes well to spleen (43.3) but less well to lung (7.6) and liver (5.2) MoAb 9B specific for tumor cells is marginal in localization at 48 h with a %ID/g value of 3.5. Tissue slices from these animals were processed for microdistribution analyses. Photomicrographs of autoradiographs of these sections are shown in Fig. 1. MoAb 34A is distributed evenly throughout the lung capillary system (Fig. 1a) as described previously (6), whereas MoAb 9B to the human tumor A431 xenograft is localized to areas of the tumor (Fig. 1a) very close to blood vessels and, in some cases, the external margin of the tumor. MoAb 13A is found in highest concentration distributed throughout the red pulp of the spleen (Fig. 1d), whereas only a small amount of MoAb 13A is found in the lung (Fig. 1e). MoAb 13A is also found in focal areas of the liver and sinusoids (Fig. 1j). Little MoAb was detected in non-target tissue in any of these systems.

These data support the hypothesis that capillary endothelium is a major barrier to MoAbs. MoAb 34A to the lung antigen does not need to pass through any cell barrier and is efficiently localized. MoAb 13A localizes evenly in the spleen where capillaries have fenestrated walls allowing easy egress of MoAb, but in contrast MoAb 13A is distributed unevenly in lung where the capillary endothelium has relatively tight junctions. MoAb 9B is distributed very near capillaries locally in the tumor xenograft, but it should be noted that this MoAb has penetrated the capillary walls since target antigen is expressed only in the tumor. Thus, although some penetration of tumor capillary walls occurs over time, other factors limit spread of the MoAb throughout the tumor.

Microdistribution in Different Xenografts. In order to show that the microdistribution in A431 xenografts was not unique to this highly keratinizing tumor, experiments on 2 other tumor xenografts were done. Data in Table 2 show the gross distribution of 125I-labeled MoAb 9B in mice bearing A549 or SW948 tumors, both of which express the β6 subunit of integrin (14). Microdistribution of 9B in each of these tumors is not uniform and is restricted to areas near blood vessels (Fig. 2, a–c). This can be seen at higher magnification (Fig. 2, d–f) where some blood vessels can be identified by the presence of red blood cells. In contrast, the antigen distribution as determined by immunoperoxidase staining in these tumors is much more uniform (Fig. 2, g and h), extending over the entire cellular portion of the tumor. Keratinized or highly necrotic areas do not stain. In experiments done at 48 h (Fig. 2) or at 105 h (data not shown), the distribution is similar. Furthermore, up to 20% of the original injected dose of active 125I-labeled MoAb 9B can be detected circulating at the time of sacrifice, indicating that MoAb is still present in the blood. These results indicate that uneven microdistribution is not unique to a particular tumor or due solely to uneven local antigen concentrations or depletion of circulating MoAb. Again it should be noted that MoAb localized in the tumor has penetrated the capillary wall, but does not move very far into the tumor mass.
Fig. 1. Microdistribution as a function of different capillary endothelium. Comparison between 125I-labeled MoAb deposition (a-f) and immunoperoxidase stain (g and i). Nu/nu mice bearing A431 tumors were given injections of 125I-labeled MoAb 9B; tumor (a), lung (b), or liver (c). BALB/c mice were given injections of 125I-labeled MoAb 13A; spleen (d), lung (e), or liver (f) or with 125I-labeled MoAb 34A; lung (g) or liver (i). h, a standard stain of lung with no autoradiography. The animals were all sacrificed at 48 h postinjection. Details of 125I-labeled MoAb specific activity are in the legend to Table 1. All magnifications are the same (300 μm bar).
Microdistribution of Mouse MoAb to Integrin Subunits. Several attempts were made to alter the microdistribution of MoAb in A431 tumors. One possible explanation for the uneven microdistribution of 125I-labeled MoAb 9B in A431 tumors is that rat MoAb does not penetrate tissue in the mouse due to unidentified host factors. Murine MoAb 450-9D, which competes for epitopes with the rat MoAb 9B, and mouse MoAb 450-30A1, which binds to the human α5 subunit of integrin (associated with β1 in these tumors), were tested for their penetration into tumors. Both mouse MoAb 450-9D (Fig. 3b) and mouse MoAb 450-30A1 (Fig. 3d) give the same microdistribution of the mouse MoAb as for the rat MoAb 9B (Fig. 3a), indicating no major species effect in localization. In these experiments, we included MoAb 450-11A1 (Fig. 3c), which binds to the cytoplasmic domain of the β1 subunit (18). This MoAb showed no significant gross localization to the tumor (data not shown), indicating that an epitope in the cytoplasm of these tumor cells does not mediate localization.

Microdistribution as a Function of MoAb Dose. Data for the gross distribution of 4 concentrations of MoAb 9B IgG in nu/nu mice bearing the A431 xenograft are shown in Table 3. Table 4 shows the corresponding data for MoAb 9B F(ab')2. The data are consistent with previous experiments and further show that increases in amount of MoAb injected have little effect (P > 0.1 for all comparisons) on gross distribution of either whole MoAb 9B or F(ab')2 9B, although F(ab')2 has lower uptake in tumors and faster clearance from the circulation. Furthermore, active 125I-labeled MoAb 9B IgG is found circulating at 48 h at similar levels in each of the different doses. The fractions of original MoAb activity recovered in the serum at 48 h were 20.3, 16.4, 15.4, and 12.4%, respectively, for the doses of 2, 50, 113, and 500 μg. In contrast to the gross distribution, which varies only slightly with dose, microdistribution in this experiment indicates that high-dose MoAb penetrates more deeply into the tumor (Fig. 4). MoAb 9B at doses of 2, 50, and 113 μg/mouse give the peripheral pattern observed in Fig. 4, a–c, whereas the 500-μg dose (Fig. 4d) gives a diffuse microdistribution. The number of grains at this dose appears to be lower even though the %ID/g is similar for all doses. In order to quantitate these differences, sections of tumor photomicrographs were analyzed for grain density as a function of distance from the nearest blood vessel. Some subjective judgement is made in choosing a representative area of the tumor, but variations in total number of grains are normalized in the histogram. Data in Fig. 5 show that with the 500-μg dose of MoAb, an even distribution of grains from the vessel margin to distal portions of the tumor is observed. At the lower dose (50 μg) of MoAb, most of the grains are concentrated within 20–30 μm of the vessels. Seventy % of the low-dose MoAb is found within 20 μm of the vessel margin, whereas only 40% of the high-dose MoAb is within 20 μm.

DISCUSSION

Previous work (3) has shown that uptake of MoAb is high in renal cell carcinoma xenografts that are well-vascularized with relatively permeable vessels. In contrast, poorly vascularized tumors accumulate less MoAb. We have shown primarily that uptake of radioiodinated antibody by a target inside the vascular space is very efficient relative to uptake of MoAb, which must pass through capillary walls to reach their target antigen (6).

In this study, we have analyzed the gross and microdistribution of 3 MoAb systems in an attempt to determine the mechanisms underlying exclusion of MoAb from tumor tissue. The data support the following conclusions: (a) tight endothelial junction capillaries represent major barriers to efficient accumulation of MoAbs; (b) although the capillary wall limits the %ID/g that accumulates in a tumor, another barrier exists in the tumor tissue that restricts MoAb penetration to areas distant from blood vessels; and (c) high MoAb concentrations promote a more even distribution of MoAb in poorly vascularized tumor xenografts, presumably due to better penetration of the MoAb in the extravascular fluid.

MoAb 34A, which binds to a protein P112 preferentially expressed in lung capillary endothelium, does not need to penetrate the endothelial cell barrier to reach its antigen. Thus with this MoAb, localization to the lung is fast, efficient, and uniform (6, 15). The uniform distribution throughout the lung is achieved even at low doses of MoAb, which do not saturate the available antigenic sites (6).

MoAb 13A, which binds to a target protein P100 on macrophages in lung, liver, and spleen, represents an intermediate situation. This MoAb localizes efficiently in the spleen where capillary endothelium is fenestrated (18, 20). It has a microdistribution similar to that for P100 as determined by immunohistochemistry (16). In contrast, MoAb 13A does not localize well to the lung even though P100 concentrations in lung and spleen are similar and the lung is encountered prior to the spleen in the i.v. injection used here. The lung capillary system is “tight” with little space between endothelial cells (18, 20). Localization in the liver, which also contains some P100, is restricted to sinusoids adjacent to large vessels. Penetration into the tightly packed hepatocytes does not occur; however, interpretation of autoradiographic data in the liver may be in question due to the large concentration of dehalogenating enzymes found here. Early success of Scheinberg and Strand (21) in targeting antibodies to viral gp70 to virus producing leukemia cells in the spleen were probably due to the easy access of MoAb to the spleen cells. A similar situation has been encountered in efficient targeting anti-Thy-1 MoAb in spleen (22).

In 3 different human tumor xenografts, inefficient, uneven distribution of low-dose MoAb 9B was observed. Small effects of antigen concentration were noted in that A549 tumors have poorer localization of 9B than do A431 and SW948 tumors, which express 5–10-fold more of the target protein β1 subunit (14). Similar small effects (i.e., <2-fold) have been noted by others (23).

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Table 2: Gross distribution of 125I-labeled MoAb 9B in nu/nu mice bearing different human tumor xenografts.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>%ID/g ± SE</th>
<th>Ratio ± SE</th>
<th>%ID/g ± SE</th>
<th>Ratio ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>1.10 ± 0.65</td>
<td>0.84 ± 0.24</td>
<td>1.38 ± 0.37</td>
<td>1.02 ± 0.067</td>
</tr>
<tr>
<td>Liver</td>
<td>1.05 ± 0.81</td>
<td>0.78 ± 0.26</td>
<td>1.23 ± 0.40</td>
<td>0.93 ± 0.016</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.68 ± 1.17</td>
<td>0.84 ± 0.19</td>
<td>1.41 ± 0.40</td>
<td>1.10 ± 0.030</td>
</tr>
<tr>
<td>Lung</td>
<td>1.82 ± 1.01</td>
<td>1.11 ± 0.36</td>
<td>2.50 ± 0.44</td>
<td>1.18 ± 0.090</td>
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<tr>
<td>Tumor</td>
<td>5.43 ± 1.84</td>
<td>3.40 ± 1.6</td>
<td>4.60 ± 1.47</td>
<td>3.12 ± 0.50</td>
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<td>Skin</td>
<td>1.38 ± 0.94</td>
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<td>1.67 ± 0.52</td>
<td>1.06 ± 0.021</td>
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<tr>
<td>Serum</td>
<td>7.66 ± 4.22</td>
<td>0.69 ± 0.29</td>
<td>10.38 ± 3.1</td>
<td>0.97 ± 0.015</td>
</tr>
</tbody>
</table>

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All values are expressed as the mean ± SD.
Fig. 2. Microdistribution of $^{131}$I-labeled MoAb 9B in different tumor xenografts. Autoradiography (a–f) of A431 tumor (a and d), SW948 tumor (b and e), and A549 tumor (c and f) at 48 h postinjection. a–c are at low magnification (300 µm bars), d–h are at higher magnification (bars). g and h, staining of the tumor sections with immunoperoxidase to determine antigen distribution. See legends to Tables 1 and 2 for MoAb specific activities.
at the tumor site is limited due to the capillary wall barrier. The gross accumulation of radiolabeled MoAb in tumors may be enhanced over that seen in the β⁺ system by higher affinity MoAbs and more stable labeling methods that prevent dehalogenation (24, 25).

Even if the %ID/g can be maximized, a second problem, the microdistribution of MoAb in the tumor, remains, especially for therapy of solid tumors (26). Accumulation of MoAb near the vessel margin has been documented in the GW-39 tumor using dual tracer microautoradiography (27). Uneven distribution of MoAb in tumors cannot be accounted for solely by poor penetration of capillaries. There is ample evidence that MoAbs do get out of tumor capillaries. The β⁺ antigen is not expressed in capillaries and therefore the 9B MoAb that accumulates in the xenograft must penetrate with some efficiency. The fact that active MoAb 9B to tumor antigen remains circulating for up to 5 days after injection even at low doses indicates that slow egress of MoAb from capillaries should be adequate. It is more likely that blood flow restriction and intercellular fluid dynamics in the tumor (28) cause the uneven microdistribution. The mathematical model of Fujimori et al. (11) for MoAb penetration of tumors incorporates factors for MoAb, size, concentration, and Ka, as well as vascular permeability and intratumor pressure. These equations predict an "antigen barrier" that can be lowered by high concentrations and lower affinity MoAb. In our experiments, we observed a more even distribution of ¹²⁵I-labeled MoAb 9B throughout A431 xenografts when administered at higher concentrations in agreement with the model. The number of silver grains at the highest dose of ¹²⁵I-labeled MoAb appears to be lower even though the %ID/g for all doses is similar. Even if the gross distribution data give falsely high levels of accumulation at high doses, the difference in distribution is still documented by the normalized comparison in Fig. 5. It is possible that a given MoAb with lower affinity may yield an even distribution in a particular system if the dose can be optimized. Detailed studies on this approach

<p>| Table 3 Gross distribution of different doses of ¹²⁵I-labeled MoAb 9B in nu/nu mice bearing A431 tumors* |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Percent ID/g (μg dose)</th>
<th>Ratioa</th>
<th>Percent ID/g (μg dose)</th>
<th>Radiob</th>
<th>Percent ID/g (μg dose)</th>
<th>Ratioa</th>
<th>Percent ID/g (μg dose)</th>
<th>Radiob</th>
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</thead>
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<tr>
<td>Kidney</td>
<td>0.93 ± 0.11</td>
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<td>1.01 ± 0.04</td>
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<td>Liver</td>
<td>0.95 ± 0.07</td>
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<td>Spleen</td>
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<td>1.03 ± 0.14</td>
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</tr>
<tr>
<td>Lung</td>
<td>1.11 ± 0.06</td>
<td>0.97 ± 0.10</td>
<td>1.24 ± 0.09</td>
<td>0.99 ± 0.07</td>
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<tr>
<td>Tumor</td>
<td>5.3 ± 2.8</td>
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<td>5.9 ± 0.60</td>
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<td>Skin</td>
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<tr>
<td>Serum</td>
<td>12.7 ± 5.9</td>
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<td>10 ± 5.4</td>
<td>0.87 ± 0.12</td>
<td>0.91 ± 0.04</td>
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* Nu/nu mice bearing A431 tumors were given injections of 2 μg ¹²⁵I-labeled MoAb 9B diluted with varying amounts of unlabeled MoAb 9B. Animals were sacrificed at 48 h postinjection and processed for gross and microdistribution of MoAb. Gross distribution was based on 3 mice/group except the 2-μg dose (5 animals). All values are expressed as the mean ± SD.

a The ratio is calculated as %ID/g of MoAb 9B divided by the %ID/g for ¹²⁵I-labeled MoAb 135-14. The control MoAb was at 2 μg/dose (500,000 dpm) for all experiments.
with different MoAbs to β4 are underway. These data indicate that active MoAb continues to circulate for up to 5 days after injection even at low doses of MoAb that could not saturate all of the epitopes. Furthermore, the fraction of total injected dose that remains in the circulation at 48 h is not greatly dependent on dose up to 500 μg/mouse. Sharkey et al. (29) and Worlock et al. (30) also noted this effect. This result is not predicted by the mathematical model (11) although even higher doses may saturate antigen sites as the model predicts. Indeed, doses over 2 mg of MoAb give poorer localization ratios and high blood concentrations, which indicate antigen saturation.5

Epstein et al. (31) have shown efficient uptake of the anti-histone MoAb TNT in necrotic tissue implicating efficient egress from vessels and localization to intercellular antigen. Our MoAb 450-11A to the cytoplasmic domain of the β4 subunit did not localize well in the tumor even in necrotic areas of extremely large tumors (>2 g) (data not shown). The difference in these results may be due to other factors such as antigen concentration or degree of necrosis specific to the individual systems.

It may be possible to increase tumor vascular permeability with X-ray (32), interferon, interleukin-2 (33), or hypothermia (34), although these treatments may have side effects (20, 28). Since MoAbs do penetrate capillaries, but seem to find resistance in the tumor extracellular space, these approaches may have only limited advantages. In summary, large concentrations

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**Figure 4.** Microdistribution of 125I-labeled MoAb 9B in A431 tumors as a function of dose. Nu/nu mice bearing A431 tumors were given injections of 125I-labeled MoAb 9B diluted with unlabeled MoAb 9B (see Table 3 for specific activities) and sacrificed at 48 h postinjection. Total MoAb dose: 2 μg (a); 50 μg (b); 113 μg (c); or 500 μg (d). Magnification, 300 μm bars.

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**Figure 5.** Quantitation of distance of MoAb from blood vessels at 2 different doses. Five pictures from experiments using doses of 50 (○) or 500 μg (●) of MoAb 9B (Fig. 4, b and d) were analyzed for distance of silver grains from a well-defined blood vessel margin. Data were averaged and normalized to the same number of total grains counted for each dose.

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**Table 4.** Gross distribution of 125I-labeled MoAb 9B F(ab')2 in nu/nu mice bearing A431 xenografts (%ID/g)°

<table>
<thead>
<tr>
<th></th>
<th>4 μg</th>
<th>300 μg</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
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<tr>
<td>Kidney</td>
<td>1.04 ± 0.03</td>
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<td>Liver</td>
<td>0.76 ± 0.21</td>
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<td>Spleen</td>
<td>0.91 ± 0.21</td>
<td>0.35 ± 0.09</td>
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<tr>
<td>Lung</td>
<td>1.24 ± 0.06</td>
<td>0.69 ± 0.12</td>
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<tr>
<td>Tumor</td>
<td>4.13 ± 0.56</td>
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<tr>
<td>Serum</td>
<td>4.32 ± 0.22</td>
<td>1.96 ± 0.91</td>
</tr>
</tbody>
</table>

° Nu/nu mice bearing A431 tumors were given i.v. injections of 4 μg 9B F(ab')2, 35.2 × 10⁶ cpm or 300 μg F(ab')2, 45 × 10⁶ cpm. Animals were sacrificed at 24 h or 48 h postinjection [2 animals/group except for 300 μg F(ab')2/48 h 3 animals]. Fraction of original binding activity remaining in serum: 4 μg/24 h = 80%; 4 μg/48 h = 52%; 300 μg/24 h = 80%; and 300 μg/48 h = 62%. Specific activities of control 125I-labeled MoAb 135-14 fragments were too low to give significant data for a ratio comparison in these experiments. All values are expressed as the mean ± SD.
of relatively low-affinity MoAb may allow penetration to poorly vascularized areas of tumors giving a more even distribution and more efficient therapy, but cross-reaction with normal vascularized areas of tumors giving a more even distribution of relatively low-affinity MoAb may allow penetration to poorly tissue may prove limiting.

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Microdistribution of Specific Rat Monoclonal Antibodies to Mouse Tissues and Human Tumor Xenografts

Stephen J. Kennel, Rita Falcioni and Jim W. Wesley


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