Preclinical Analysis of Intraperitoneal Administration of $^{111}\text{In}$-labeled Human Tumor Reactive Monoclonal IgM AC6C3-2B12^1

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Abstract

An IgMA human tumor cell-reactive monoclonal antibody was developed that reacts with cells of ovarian cancer, colorectal cancer, breast cancer, and certain other malignancies. The monoclonal antibody AC6C3-2B12, which was obtained from a recent recloning, was purified from tissue culture supernatants and analyzed by high-performance liquid chromatography and sodium dodecyl sulfate-PAGE. An animal model was developed in which human tumors grew either as solid peritoneal metastases or as s.c. nodules utilizing the human colorectal carcinoma cell line SW620. The biodistribution of $^{111}\text{In}$-labeled IgM conjugate was studied after i.v. or i.p. administration in nude mice bearing an s.c. xenograft or peritoneal tumor lumps of a human colorectal carcinoma (SW620). IgM administered i.v. cleared rapidly from blood and was deposited mainly in the liver (50% injected dose/g (ID/g)), pancreas (20% ID/g), and kidney (10% ID/g) at 24 h. Tumor deposition was low (<1.0% ID/g) in the s.c. tumor xenograft. In contrast, high tumor targeting (29% ID/g) was found in peritoneal tumor lumps after i.p. administration of $^{111}\text{In}$-labeled IgM. The biological half-life of IgM in the tumor was 100 h. Long peritoneal residence time ($t_1/2 = 67$ h) and low liver uptake (7% ID/g) were observed after i.p. administration. Blood activity was <1% of the injected activity. Tumor:normal organ ratios were high (range, 2—290) from 2 to 144 h after i.p. administration. Whole body autoradiograms at 24 h after i.p. $^{111}\text{In}$-labeled IgM administration confirmed the biodistribution results. In normal beagle dogs, 75% of the i.p.-administered $^{111}\text{In}$-labeled IgM decayed in the peritoneal cavity. The majority of the remaining radioactivity was taken up by mediastinal lymph nodes. Biological half-life in both locations was approximately 137 h. The i.p. administration of intact, specific radiolabeled IgM provides prolonged retention of radioactivity in tumor, low normal tissue uptake, a long peritoneal residence time, and very limited spillover of IgM into the circulation. This approach offers a promising new method for the diagnosis and treatment of certain patients with peritoneal carcinomatosis.

Introduction

Peritoneal carcinomatosis may arise from malignancies that originate in the abdomen such as carcinomas of the ovary or gastrointestinal tract or from distant sites, e.g., carcinoma of the breast (1, 2). Traditional therapy approaches to peritoneal carcinomatosis have included external beam radiation therapy, colloidal ^32P i.p. administrations, and chemotherapy, including i.p. treatments. Treatment with these modalities are, however, rarely curative (1, 2).

The use of radiolabeled antibodies with therapeutic intent, referred to as RIT,^3 has the capability to deliver cytotoxic radiation specifically to tumors while reducing radioactive exposure to normal tissues (3, 4). The first generation of RIT studies utilized polyclonal antibodies. These were replaced in most cases with murine mAbs specific for tumor-associated antigens on the surface of tumor cells. A number of clinical trials have been conducted in patients with various malignancies utilizing mAbs radiolabeled primarily with $^{131}\text{I}$. In many of these trials, tumor radioactivity was <0.1% of the administered activity/g of tumor and insufficient for a therapy effect (5). Moreover, most patients develop HAMAs after administration of mAbs, precluding subsequent administrations of the same agent. HAMAs can divert the newly administered antibodies from their intended targets to the reticuloendothelial system (liver or spleen). This results in rapid excretion of the radioisotope without tumor targeting (6—8). Human Abs have been developed, and available data suggests that these antibodies do not induce anti-antibodies in patients (9). RIT with human mAbs can be given repeatedly without loss of efficacy.

Radioisotopes such as $^{111}\text{In}$ and $^{90}\text{Y}$ are pure $\gamma$ and $\beta$ emitters, respectively. Tumor cell surface-reactive immunoglobulins of human origin labeled with $^{111}\text{In}$ and $^{90}\text{Y}$ may constitute a powerful new generation of in vivo diagnostic or therapeutic agents for patients with peritoneal carcinomatosis. Intravenous $^{111}\text{In}$-labeled antibodies have been utilized successfully in the diagnosis of several malignancies. $^{111}\text{In}$-labeled mAbs may also be utilized to perform dosimetry for subsequent RIT with $^{90}\text{Y}$-labeled immunoglobulin.

Biodistribution studies with i.p.-administered radiolabeled murine monoclonal IgG (10—17) or drugs (18, 19) demonstrated that i.p. injections permit greater locoregional concentrations of the radioimmunoconjugate or drug. Because of the large size of the IgM molecule, the i.p. injection of radiolabeled IgM could have several important advantages over systemic i.v. administration of IgM or i.p. administration of IgG molecules. Absence of an endothelial barrier between tumor and radioimmunoconjugate could permit more rapid and intense tumor targeting. IgM molecules, because of their large size, could also permit a longer residence time in the peritoneal cavity than IgG. Moreover, the radiolabeled i.p. IgM may produce less hematological toxicity, which usually is the dose-limiting side effect of RIT. Therefore, the i.p. use of radiolabeled human IgM could potentially overcome most of the current limitations in the management of peritoneal carcinomatosis.

This study demonstrates through biodistribution analysis and autoradiography studies in animal models that i.p. administration of radiolabeled human monoclonal IgM (AC6C3-2B12) results in prolonged presence of radiolabeled IgM in the peritoneal cavity, with little spillover of the radioimmunoconjugate in the peripheral blood. $^{111}\text{In}$-labeled IgM administered i.p. produced high and selective uptake of radioactivity in tumor metastases growing in the peritoneal cavity of nude mice.

Materials and Methods

Production of Antibody

The development of the human monoclonal IgM (AC6C3) was reported previously (20). Human IgM was originally raised against human ovarian carcinoma cells by a heterohybridoma construct with nonsecreting heteromyeloma SPAZ 4 cell line as the fusion partner. The IgM binds primarily to a surface membrane of ovarian carcinoma, but also has affinity for cells of other malignancies, including breast and colon, and in higher concentrations, it also reacts with some malignancies of neuroectodermal origin (20). AC6C3 has been maintained in culture by interval recloning. Human tumor-reactive mAb from a recent cloning is designated AC6C3-2B12.
The labeled immunoconjugates were separated from low molecular weight blood lymphocytes and typed RBC from normal individuals with the above column. A 20-pA aliquot of sample was injected, and the column was eluted with 250 pJ of 0.6 M sodium acetate buffer (pH 5.3) and 250 pA of 0.06 M culture flasks by incubating with 0.05% trypsin for 2 mm at 37°C. The trypsin was washed and incubated in 1:100 dilution of FITC-conjugated goat anti-human IgM for 30 min at 4°C, then washed and resuspended in PBS with 2% (v/v) FCS, 0.02% (w/v) NaN3. Cells were detached from the tissue culture flask by using Versene and were washed with PBS containing 2% (v/v) FCS. Cells were incubated with 100 μl (200 μg) of human IgM or IgM immunoconjugates for 30 min at 4°C. Cells were washed and incubated in 1:10 dilution of FITC-conjugated goat anti-human IgM for 30 min at 4°C, then washed and resuspended in PBS with 2% (v/v) FCS, 0.02% (w/v) Na2S2O4. Cells were fixed with 0.5% (v/v) formalin and examined with an EPICS profile analyzer (Coulter, Hialeah, FL) with a log amplifier attachment. Negative control samples included staining with FITC-conjugated goat anti-human IgM alone and an irrelevant isotype-matched IgM. Additional controls have included staining of nylon wool-purified peripheral blood lymphocytes and typed RBC from normal individuals with the above antibodies.

Tumor Inoculation. Cultured SW620 cells were detached from the cell culture flasks by incubating with 0.05% trypsin for 2 min at 37°C. The trypsin was neutralized with RPMI containing 10% FCS and centrifuged at 800 x g to obtain the cell pellet. Cells were resuspended in sterile PBS at a concentration of 30 x 10^6 cells/ml. Each female athymic nude mouse was given an injection with 0.2 ml of cell suspension (6 x 10^7 cells) through the abdominal wall directly into the abdominal cavity with a 25-gauge needle. Mice were housed in a closed colony and provided sterile food and water. Animal health and tumor growth were monitored daily.

Preparation of Immunonoconjugate. 2B3M-DTPA derivative was prepared according to a method described previously (21). For antibody conjugation, the 2B3M-DTPA chelator was converted into an ITC-derivative by reacting the amino benzyol group of DTPA with thiophosphogene. The product was purified by column chromatography by using a Florisil gel column and eluted with CH3CN:H2O (30:8). The solvent was lyophilized, and the dried ITC-derivative of 2B3M-DTPA was stored at -20°C. The ITC-2B3M-DTPA was reacted with free amino groups of lysine residues of the antibody to form a thiourea linkage between the antibody molecule and the chelator. mAb (1 x 10^-5 mmol) was reacted with ITC-2B3M-DTPA (1.0 x 10^-4 mmol) at a molar ratio of 1:10 in 1 ml of 0.2 m bicarbonate buffer (pH 8.4) at 4°C for 12 h. Before radiolabeling, chelate immunoconjugates were purified from unconjugated DTPA by filtration using Centricron-30 devices (Amicon Corp., Beverly, MA). HPLC analysis of chelate IgM conjugates was performed on a Zorbax Bioseries GF-250 column. A 20-μl aliquot of sample was injected, and the column was eluted with 0.2 m sodium phosphate buffer (pH 7.2) at a flow rate of 1.0 ml/min. The fractions were monitored by a UV detector at a wavelength of 280 nm.

Radiolabeling of IgM 2B3M-DTPA Conjugates. A 20-μl aliquot of pure 111InCl3 (3.0 mCi; New England Nuclear, Boston, MA) was equilibrated with 250 μl of 0.6 m sodium acetate buffer (pH 5.3) and 250 μl of 0.06 M sodium citrate buffer (pH 5.5). Two hundred-fifty μl of IgM conjugate (2 mg) solution in PBS were added to buffered 111InCl3, mixed well, and incubated at room temperature for 45 min. The labeling mixture was challenged with a 100-fold excess of free DTPA before column chromatography. The labeled immunoconjugates were separated from low molecular weight compounds by Sephadex G-50 gel column (1.5 x 20 cm) chromatography by using 0.05 M PBS as eluant. The labeled immunoconjugates were collected and assayed in a dose calibrator (Capintec CRC-15R; Ramsey, NJ), and labeling efficiency was analyzed by TLC and ITLC. A silica gel-coated fiber glass strip (1 x 10 cm) was loaded with a 1-μl sample of radioimmunoconjugate and developed by saline as a mobile phase. In this system, the radiolabeled immunoconjugate stays at the origin, whereas labeled DTPA moves with the solvent front. The silica gel TLC strip was spotted with radiolabeled immunoconjugates and developed by using a mixture of 10% ammonium acetate aqueous and methanol (1:1) solution as a mobile phase. The ITLC/TLC strips were cut into three segments and counted in a gamma counter (Cobra II; Packard Instrument Co., Meriden, CT).

Serum Stability. An in vitro serum stability study of 111In-labeled immunoconjugate was performed after radiolabeling and purifying the product on Sephadex G-50 (1.5 x 20 cm) as described. The purified, labeled antibody (100 μl) was added to 200 μl of fresh human serum in triplicate. The mixture was incubated at 37°C for 48 h. The serum samples were analyzed on ITLC or TLC as described above. Labeled antibody stayed at the origin, whereas small, radiolabeled moieties moved along the solvent front.

Radioimmunoglobulin Administration to Nude Mice. Before animal administration, the radioimmunoconjugate solution was filtered through a 0.2 μm sterile filter, and the antibody-bound radioactivity was analyzed by TLC and size-exclusion HPLC. The 111In-labeled IgM conjugates were used within 2 h of preparation and delivered by i.p. or i.v. injection in a total volume of 0.2 ml of PBS. Doses of 20, 50, and 100 μCi of 111In-labeled immunoconjugates were administered i.p. per mouse 17 days after tumor inoculation of human colorectal carcinoma SW620 cells for biodistribution, autoradiography, and whole body retention, respectively. For i.v. administration, 20 μCi of 111In-labeled IgM conjugates were injected via the tail vein.

Biodistribution in Nude Mice. Those mice with s.c. tumors were selected for i.v. injection and those with peritoneal cavity metastases were selected for i.p. injection. Mice that had peritoneal cavity tumor xenografts from the SW620 tumor cell line were euthanized at 2-, 24-, 72-, 120-, and 144-h postinjection. Mice that had s.c. tumor xenografts from SW620 colon carcinoma cells were euthanized at 24- and 72-h postinjection. The normal tissues and the tumor lumps or xenograft were excised, weighed, and assayed for content of radioactivity in a gamma counter (Cobra II; Packard, Inc.). Results were expressed as % ID/g. An injectate sample was used as a decay control.

Whole-body Autoradiography. Tumor-bearing mice with i.p. tumor lumps receiving 50 μCi of 111In-labeled IgM were sacrificed at 24 h, and the extremities were removed. The body was fixed in carboxymethyl cellulose (4%) and frozen. The frozen block was mounted on a cryomicrotome (Hacker Instruments, Fairfield, NJ), and 30-μm thick coronal sections were made. Photographs were taken at each section. The sections were freeze dried, followed by placement on X-ray film (X-OMAT AR; Eastman Kodak Co., Rochester, NY) for 48 h and developed. Photographs and autoradiographs of each section were compared.

Whole-body Retention. Whole-body retention of radioactivity was determined by measuring the whole nude mouse in a dose calibrator (Capintec CRC-7). Four mice were measured at nine different time points beginning with t = 0 to t = 144 h. Measurements were corrected for 111In-labeled decay.

Radioimmunoglobulin Administration to Beagle Dogs. 111In-labeled IgM conjugates were injected i.p. to two female normal beagle dogs. A volume of 5-ml PBS containing 0.5 mg of IgM was injected. The total radioactivity administered was 1 mCi/dog. A catheter was inserted transcutaneously into the peritoneal cavity. Proper positioning of the catheter tip was verified by radiocontrast and abdominal X-ray.

Biodistribution Studies in Dogs. Blood and urine samples were analyzed for radioactivity at 2-h postinjection and then daily for 6 days. Wholebody scintigraphy studies were performed 1, 24, 48, 96, and 144 h after administration. Regions of interest identified to calculate half-life in body compartments containing high amounts of radioactivity. Seven days after administration, animals were euthanized. Samples of all major organs were taken, weighed, and counted for radioactivity in a gamma counter.

Results

Quality Control Analysis. Table 1 shows the results of the quality control analysis of the radiolabeled chelate immunoconjugate. The structure of radiolabeled IgM conjugate is shown in Fig 1. An average of four DTPA molecules are attached to four lysine residues/IgM.

4 S. Pathak, personal communication.
Table I Quality control analysis of radioimmunoconjugates
Protein-bound fraction after 24h and 48 h incubation at 37°C in human serum.
Reactivity with irrelevant isotype matched human mAb 4.7%.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>111In-labeled 2B3M-DTPA IgM (AC6C3-2B12)</th>
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</thead>
<tbody>
<tr>
<td>DTPA/IgM</td>
<td>4.0</td>
</tr>
<tr>
<td>Specific activity</td>
<td>1.0 mCi/mg</td>
</tr>
<tr>
<td>Radiochemical purity</td>
<td>ITLC 98%, TLC 98%</td>
</tr>
<tr>
<td>Serum stability</td>
<td>ITLC 96%, ITLC 94%</td>
</tr>
<tr>
<td>Immunoreactivity</td>
<td>73%</td>
</tr>
<tr>
<td>FACS analysis</td>
<td>61%</td>
</tr>
</tbody>
</table>

![Fig. 1. Structure of radiolabeled 2B3M-DTPA IgM conjugate.](image-url)

The fluorescence-activated cell sorting analysis of AC6C3-2B12 and AC6C3-2B12 conjugates indicated 82 and 61% binding activity, respectively. The mean log fluorescence intensity for AC6C3-2B12 and AC6C3-2B12 conjugates was 13.2 and 5.2, respectively. An irrelevant isotype-matched human antibody reacted with only 4.7% of SW620 carcinoma cells. Nylon wool-purified lymphocytes and typed RBC from normal individuals did not show fluorescence in fluorescence-activated cell sorting analysis (data not shown).

HPLC analysis of IgM immunoconjugates showed one major peak (99%), with a retention time identical to that of a standard human IgM. The immunoconjugates were radiolabeled with 111In, and the labeled product was purified by Sephadex G-50 column chromatography. The column purification step removed any unbound isotope, resulting in a final product with >98% of the radioactivity bound to IgM conjugates (high molecular weight protein on TLC and ITLC). Colloids or aggregates were not observed during the radiolabeling procedure or when the sample was tested by HPLC analysis. The immunoreactivity of 111In-labeled AC6C3-2B12 conjugates was determined by a direct cell-binding assay by using antigen excess conditions. AC6C3-2B12 conjugates demonstrated 73% binding, whereas irrelevant IgM showed only 27% binding to the SW620. The reactivity of AC6C3-2B12 conjugates with heptocellular carcinoma cell line, used as a control, was 32%. The specific activity of the final product was 1.0 mCi/mg of antibody. The serum incubation of radioimmunoconjugates illustrated the stability of the 111In-labeled AC6C3-2B3M-DTPA complex under physiological conditions. After a 48-h incubation, 94% of the radioactivity still rested with the IgM conjugates.

**Biodistribution in Nude Mice.** Fig. 2 shows the biodistribution of 111In-labeled IgM injected i.p. in nude mice bearing human colorectal (SW620) tumor lumps. Tumor uptake was at least two-fold higher than all normal tissues at all time points. The maximum was 29% ID/g of tumor at 24 h. The spleen had the second highest uptake with 12% ID/g at 2 h. Pancreas uptake ranged from 9 to 11% for the first three time points and decreased to <5% for the last two time points.

![Fig. 2. Biodistribution of 111In-labeled 2B3M-DTPA IgM conjugates administered intraperitoneally in nude mice with peritoneal carcinomatosis of human colorectal carcinoma (SW620) at 2 (A), 24 (B), 72 (C), 120 (D), and 144 h (E) (n = 4 mice/time point). Columns, % ID/g; bars, SD.](image-url)
Fig. 3. Biodistribution of i.v.- and i.p.-administered $^{111}$In-labeled 2B3M-DTPA IgM conjugates in nude mice bearing intraperitoneal tumor lumps (■; $n = 4$ mice/time point), or s.c. xenografts (●; $n = 3$ mice/time point) of human colorectal carcinoma (SW620). Columns, % ID/g; bars SD.

Whole-body Autoradiography. Fig. 5 shows three whole-body cross-sections of a mouse at different depths compared with their corresponding autoradiograms (bottom panel). The dark areas on the autoradiograms indicate the presence of $^{111}$ln. Tumor borders are the darkest regions. The liver, pancreas, and kidney also showed $^{111}$In-labeled IgM uptake, but it was relatively less intense than the tumor lumps. In the anterior slice, the mediastinal lymph node picked up

Whole-body Retention. Fig. 4 shows the data plotted for the whole-body retention of radioactivity in nude mice, which was measured for over a period of 7 days. The half-life of the radioimmunglobulin was approximately 67 h for the whole body. The combination of physical and biological half-lives for $^{111}$In-labeled IgM gives an effective half-life of 33.7 h.

Table 2 Tumor:Normal tissue ratios

<table>
<thead>
<tr>
<th>Organ</th>
<th>2 h</th>
<th>24 h</th>
<th>72 h</th>
<th>120 h</th>
<th>144 h</th>
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<tbody>
<tr>
<td>Heart</td>
<td>116</td>
<td>116</td>
<td>144</td>
<td>98</td>
<td>96</td>
</tr>
<tr>
<td>Lung</td>
<td>6</td>
<td>11</td>
<td>12</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Stomach</td>
<td>8</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Intestine</td>
<td>10</td>
<td>15</td>
<td>10</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Spleen</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Muscle</td>
<td>26</td>
<td>62</td>
<td>41</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>Femur</td>
<td>26</td>
<td>47</td>
<td>27</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>Kidney</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Blood</td>
<td>57</td>
<td>290</td>
<td>272</td>
<td>317</td>
<td>241</td>
</tr>
<tr>
<td>Liver</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
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</tr>
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</table>

(n = 4 mice/time point). Tumor:blood, tumor:heart, tumor:femur, and tumor:muscle ratios were extremely high (>47 at 24 h), indicating low uptakes of radioactivity in these normal tissues. Tumor:lung ratios were lower, between 6 and 19. This might be explained by radioactivity in mediastinal lymph nodes contained in the lung specimen. Tumor:pancreas, tumor: liver, tumor: kidney, and tumor: spleen ratios had the lowest values of all the normal tissues sampled, between 2 and 5. The remainder of the organs located around the peritoneal cavity, i.e., stomach and small intestines, showed higher tumor: normal tissue ratios, between 7 and 33.
Fig. 5. Photographs and autoradiographs of slices of whole mouse mounts, 24 h after i.p. administration of $^{111}$In-labeled 2B3M-DTPA IgM conjugates. Peritoneal tumors are of human colorectal carcinoma origin (SW620). 1, mediastinal lymph node; 2, tumor; 3, liver; 4, pancreas; 5, kidney.
considerable activity and was seen as a dark circle in the thoracic cavity. Otherwise, most of the injected activity appeared to be contained within the abdominal cavity.

**Biodistribution in Beagle Dogs.** Fig. 6 shows the biodistribution of $^{111}$In-labeled IgM in normal beagle dogs. The animals were euthanized 7 days after i.p. administration. The result obtained showed that the radioimmunoconjugates were retained within the peritoneal cavity. Transport out of the peritoneal cavity was slow. Less than 1% of the injected activity was recovered in blood or urine. Gamma camera images of beagle dogs obtained at 1, 4, 24, 48, 72, and 144 h showed similar distribution and retention of radioactivity. The only radioactivity observed outside the peritoneal cavity was in the anterior midline structure in the chest. At autopsy, the radioactive mediastinal structures were small (multiple nodules of approximately 0.3 g each) and confirmed to be mediastinal lymph nodes by histological analysis. The biological half-life of the radiolabeled IgM in mediastinum and peritoneal cavity was found to be 137 h.

**Discussion**

A number of earlier RIT studies have utilized i.p. administration of murine monoclonal IgG radiolabeled with $^{131}$I (10—12, 15, 16). Because murine mAbs may frequently induce HAMA formation in patients (6—8), human or humanized mAbs have been considered as replacements for the murine mAbs because they have the potential advantage of having low or absent immunogenicity (9). In considering an appropriate isotope for therapeutic applications, $^{90}$Y is probably a more effective therapeutic isotope than $^{131}$I (3). Unfortunately, most of the clinically utilized $^{90}$Y-labeled i.p.-administered mAbs appeared to be unstable *in vivo* or released a high level of radioactivity in the circulation and produced limited tumor responses (13, 15, 17, 22, 23).

This preclinical study demonstrates that i.p.-administered human monoclonal IgM conjugates labeled with $^{111}$In remain within the peritoneal cavity with early uptake in the tumor. The peritoneal membrane appears to form a strong barrier against the diffusion of the large IgM molecules into the circulation. The peritoneal residence time of the IgM is long enough to promote high uptake in tumor. Selective and prolonged retention at the tumor site may also be related to the high affinity of IgM for tumor antigens. The effective tumor half-life is approximately 40 h, long enough for significant tumor radiation if $^{90}$Y instead of $^{111}$In is utilized for the labeling of the IgM conjugates. The biological half-life of the immunoconjugate in the tumor is over 4 days. The large IgM molecule does not appear to penetrate deeply into the tumor (Fig. 5). Even with the long range of $^{90}$Y emissions, some parts of larger tumor deposits might be undertreated and indicate the need for fractionated RIT. Fractionation is possible with human IgM. Most normal tissues, including the liver, kidney, spleen, and pancreas, show only moderate uptake. This might be due to the filtering characteristics of these organs (liver, spleen, and kidney) or antigenic cross-reactivity (pancreas). Blood and femur uptake are expected to correlate with the hematological toxicity of radiolabeled antibodies. Both of these tissues showed very little radioactivity uptake in these studies.

In contrast, i.v. administration of radiolabeled human IgM showed very little tumor uptake and rapid accumulation of radioactivity in liver, spleen, kidney, and pancreas. The large size of this immunoglobulin makes it susceptible to aggregation in the circulation and removal by filtration in the organs listed (pancreas being the exception). The human origin of the IgM might also be a determinant of its short survival time in this allogeneic situation (*i.e.*, murine recipients). A pharmacokinetic comparison between human and mouse IgM after i.v. administration to nude mice remains to be performed.

Whole-body autoradiography of nude mice confirmed the biodis-
distribution data by showing high uptake around the tumor periphery. In addition, mediastinal lymph nodes showed high uptake. This observation suggests that the radioimmunoconjugate follows the same anatomical route as metastatic peritoneal carcinoma cells (1). Hence, it would be possible to detect and eradicate peritoneal tumor deposits as well as metastatic mediastinal disease; hematogenous metastases would not be reached by i.p.-administered radiolabeled IgM. The pharmacokinetics for both $^{111}$In and $^{90}$Y-labeled 2B3M-DTPA IgM conjugates in the nude mouse are similar, indicating that $^{111}$In signals can be used for the prediction of tumor and normal tissue dosimetry for subsequent $^{90}$Y-labeled IgM administration.

Biodistribution studies in normal beagle dogs confirmed the containment of IgM in the peritoneal cavity after i.p. administration. Approximately 75% of the administered radiolabeled IgM decayed in the peritoneal cavity. The majority of the remaining conjugate decayed in mediastinal lymph nodes. Less than 1% of the radioactivity was found in blood. The small amount of radioactivity found in the blood was associated with low molecular weight species (data not shown) and was presumably due to catabolism of the radiolabeled IgM. Thus, hematological side effects are not expected to limit the amount of radioactivity that can be administered i.p. The dose-limiting normal structure after the i.p. administration of $^{90}$Y-labeled IgM remains to be determined. The model provides an important advantage over the nude mouse model for radioimmunotoxology studies by having organs of anatomical dimensions closer to the ones encountered in human patients. The longer biological half-life of $^{111}$In-labeled IgM in the normal dog peritoneal cavity is a disadvantage in terms of normal organ toxicity. Future studies in human patients with i.p. $^{111}$In-labeled IgM will document whether the dog or the nude mouse is the better predictor for i.p. residence time of IgM in human patients.

The biodistribution and pharmacokinetic information obtained in this study supports the conduct of diagnostic studies with $^{111}$In-labeled human IgM conjugates in patients with peritoneal carcinomatosis. A study of $^{111}$In-labeled ACC63-2B12 in patients with epithelial ovarian carcinoma is approved at the M. D. Anderson Cancer Center. If favorable biodistribution information is obtained in human patients, a therapeutic study with $^{90}$Y-labeled IgM conjugates will be pursued.

Acknowledgments

We thank Dr. Sen Pathak for providing cytogenetic analysis of the SW620 cell line, Dr. David Yang and Shuisheng Wang for their technical assistance in autoradiography and gamma camera imaging studies, and Barbara Bickerstaff for preparation of this manuscript.

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