Localization of $^{111}$In- and $^{125}$I-labeled Monoclonal Antibody in Guinea Pigs Bearing Line 10 Hepatocarcinoma Tumors


ABSTRACT

A murine monoclonal antibody (D3) with demonstrated specificity for the guinea pig line 10 hepatocarcinoma (L10) was radiolabeled with either $^{125}$I or $^{111}$In and used to image dermal tumors in vivo. In one set of experiments, L10 tumors were established middorsally in one group of animals, and the similarly derived, antigenically distinct line 1 tumor was established in another group of animals. In spite of background imaging of liver, kidney, and spleen, L10 tumors were visualized clearly. Incorporation of radiolabel was demonstrated to predominate in the L10 tumor. In a separate set of experiments, L10 and line 1 tumors were established in contralateral thighs in the same animals. L10 tumors were visualized clearly, and tissue uptake of radiolabel was demonstrated to reside predominantly in the L10 tumor.

INTRODUCTION

At the turn of the century, Paul Ehrlich first postulated the use of antitumor antibodies to selectively target toxic substances to tumor cells (6). However, it was not until 1948 that Pressman of antitumor antibodies to selectively target toxic substances to tumors in vivo. We have developed an animal tumor model which we believe is more directly analogous to the use of murine monoclonal antibodies in humans. Murine monoclonal antibodies were developed to the L10$^3$ of guinea pigs (3). This model provides a synergistic solid tumor with metastatic potential similar to solid tumors in humans, while retaining the use of xenogeneic monoclonal antibody. Immune responses in the guinea pig are in many ways analogous to those of humans, and guinea pigs are exquisitely sensitive to anaphylactic reactions, a consideration in cancer immunotherapy and immunodiagnosis utilizing murine monoclonal antibodies. In addition, nonspecific uptake of the xenogeneic mouse monoclonal antibody by the reticuloendothelial system may be similar in both guinea pigs and humans, allowing a more accurate assessment of the diagnostic imaging potential of radiolabeled mouse monoclonal antibodies in humans.

This paper describes radiolocalization and imaging studies using the murine D3 monoclonal antibody to the guinea pig L10 hepatocarcinoma. D3 was labeled with $^{111}$In or $^{125}$I and used to image dermal L10 and L1 tumors (L1 is a similarly derived regressor hepatocarcinoma of guinea pigs which is antigenically distinct from the regressor hepatocarcinoma L10).

MATERIALS AND METHODS

Tumor Model. The L10 hepatocarcinoma of strain 2 guinea pigs is a chemically induced, transplantable tumor. Following i.d. injection, it forms a localized tumor mass which is rejected within approximately 2 weeks, forming an open ulcerative wound. L1 was used as control tumor (3).

Guinea Pig Lines. Guinea pigs (500 to 600 g) were inoculated with either $10^6$ L10 or L1 viable tumor cells. Seven to 14 days later, 1-cm dermal tumors were present in all treated animals. At this time, animals were anesthetized and received from 100 to 270 Ci of labeled antibody i.v. Imaging began at 3 hr postinjection of radiolabeled antibody and at 24-hr intervals thereafter. In the first set of experiments, guinea pigs were given injections of either L10 or L1 tumors middorsally. In the derived monoclonal antibodies is also different from monoclonal antibodies in humans (allogeneic versus xenogeneic monoclonal antibody). We have developed an animal tumor model which we believe is more directly analogous to the use of murine monoclonal antibodies in humans. Murine monoclonal antibodies were developed to the L10$^3$ of guinea pigs (3).

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second set of experiments, guinea pigs were given injections of L10 in one thigh and L1 in the opposite thigh.

Iodination of D3. D3 was labeled with 125I by Boulton-Hunter’s reagents (monooiodinated, specific activity, 2200 Ci/mol; New England Nuclear, Boston, Mass.). Briefly, 1 mCi of Boulton-Hunter’s reagent was evaporated to dryness at room temperature under a nitrogen stream. D3 (100 μg/0.1 ml) in cold borate buffer, pH 8.3, was added to the reaction vial with occasional shaking. The reaction was allowed to proceed on ice for 5 hr. The reaction mixture was then passed through Sephadex G-75, and radioactive antibodies were collected from appropriate void volume fractions in phosphate-buffered saline. The specific activity of 125I-D3 was 3.0 μCi/μg protein. A second batch of 125I-D3 was obtained by a similar procedure, using 2 mCi of Boulton-Hunter’s reagent and 132 μg of antibody. The specific activity of this preparation was 6.5 μCi/μg. Both preparations of 125I-D3 contained no significant amounts of free 125I (<0.003%) as measured by dialysis and acid-precipitable counts.

Results of 125I-D3 contained no significant amounts of free 125I, and 132 μg of antibody. The specific activity of this preparation was 6.5 μCi/μg. Both preparations of 125I-D3 contained no significant amounts of free 125I (<0.003%) as measured by dialysis and acid-precipitable counts.

Sixty-seven % of the antibody of human IgG was biologically active (as determined by binding of labeled antibody to L10 cells).

111In Labeling. D3 was further purified on a DEAE-Sephatrel (Pharmacia Fine Chemicals, Piscataway, N. J.) ion-exchange column equilibrated in 20 mM Tris, pH 7.3. The immunoglobulin was applied to the column in 20 mM Tris, pH 7.3, and eluted with a 20 to 180 mM Tris, pH 7.3, gradient. Antibody-containing fractions were found in a range characterized by conductivity of 5.4 to 7.4 mmho and pooled. Purified antibody was reacted with diethylenetriaminepentaacetic acid using methods described by Krejcarek and Tucker (11). The resulting antibody conjugate was concentrated from unbound chelating material using a Sephadex G-75 column. Antibody fractions eluting in the void volume were pooled.

In a typical iodination experiment, 100 μg of antibody were labeled with 1 μCi of 111In chloride by the method described previously by Mears et al. (15). Unbound iodine was separated from the antibody preparation using a Sephadex G-75 column. The 111In-antibody conjugate eluted in the void volume of the column with a specific activity of 2 μCi/μg. Sixty-seven % of the labeled antibody retained biological activity.

Imaging Methods. Scintigraphic images were obtained using a γ camera with a medium-energy parallel-hole collimator, collecting 100,000 counts per image. Guinea pigs were anesthetized by i.m. injection of ketamine and were viewed in the posterior projection at 3, 26, 72, 96, and 148 hr after i.v. injection of radiolabeled antibody. Additional views in both lateral projections were obtained at 96 and 148 hr, 50,000 counts each. Images were recorded from the 35-keV photo peak of 125I and for the 172- and 247-keV photo peaks of 111In using a 20% window for each energy peak. Data were recorded on photographic film and by a Hewlett-Packard 21MX scintigraphic data analyzer using a 64- x 64-pixel image size.

Image Analysis Methods. Computer-assisted image analysis was performed by manually selecting regions of interest (pixel groups) for each image to represent the tumor and a neighboring background area. The number of counts per pixel in each region was integrated for successive images of L10 and L1 tumor-bearing animals, and corrections were made for variable counting times. Values are expressed in cpm/pixel. A single animal representing each tumor-antibody system was used for variable counting times. Values are expressed in cpm/pixel.

Analysis of Tissue Counts. Following imaging, guinea pigs were sacrificed by suffocation in CO2 and immediately dissected. Samples of liver, kidney, spleen, sternum, L10 and L1 tumors, lung, and blood were obtained. One-half ml of blood was allowed to clot for direct γ counting. Other tissue samples were dissected free of fat and connective tissue, minced with scalp and scissors, homogenized on ice for 30 sec (Polytron; Brinkmann Instruments, Inc., Westbury, N. Y.), and washed 3 times in phosphate-buffered saline by centrifugation. Following the final centrifugation, supernatant fluids were aspirated, and tissue samples were weighed and counted in an LKB γ counter (LKB Instruments, Rockville, Md.). Counts were expressed as cpm/g of wet tissue.
Monoclonal Antibody Radiolocalization of LW Tumor labeled D3. However, the location of tumors near the liver, spleen, and kidneys made difficult the accurate definition of tumors because of high-background radioactivity in these organs. However, by rotating the camera and obtaining views from different angles, it was possible to define these tumor images (Fig. 1). This was less of a problem in animals treated with 125I-labeled antibody, as the lower energy levels of 125I and the more rapid clearance of nonspecifically bound isotope made liver, spleen, and kidney imaging less problematic (Fig. 2).

Tumor:background ratios were measured from computer-stored images on a pixel-to-pixel basis. Tumor:background ratios were above 2.0 in L10 tumor-bearing animals beginning 24 hr after injection of either 111In- or 125I-labeled antibody. Table 3 shows tumor:background ratios from 3 to 148 hr after treatment with 111In- or 125I-D3 antibody.

Tumor:background ratios reached their maximum at 24 hr in animals which received 111In-D3 and declined very slowly over the course of these experiments. L1 tumor:background ratios also remained relatively constant throughout this time period. However, L10:L1 ratios peaked at 24 hr at 8.5 and decreased slowly to 5.0 at 148 hr.

Table 3

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>L10:background ratio</th>
<th>L1:background ratio</th>
<th>L10:L1 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.46</td>
<td>1.21</td>
<td>2.9</td>
</tr>
<tr>
<td>24</td>
<td>2.33</td>
<td>1.17</td>
<td>8.5</td>
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<tr>
<td>72</td>
<td>2.33</td>
<td>1.32</td>
<td>7.9</td>
</tr>
<tr>
<td>96</td>
<td>2.23</td>
<td>1.35</td>
<td>5.3</td>
</tr>
<tr>
<td>148</td>
<td>2.15</td>
<td>1.26</td>
<td>5.0</td>
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</table>

Comparison of cpm/pixel was made from computer image of tumor compared to adjacent tissue or L1 tumor. The localization of tumors overlying major abdominal organs caused a higher background and thus lower tumor:background ratios in animals with abdominal tumors than in those with tumors on contralateral thighs.
L10 tumor than blood (411,000 cpm/g of L10, 203,000 cpm/g of blood) with minimal counts in the L1 tumor (7,600 cpm). Kidney and spleen both contained approximately 130,000 cpm/g of tissue, while liver contained fewer than 25,000 cpm/g of tissue.

DISCUSSION

Early localization studies using 125I-labeled conventional antisera demonstrated that radioactively tagged antibodies could image tumors in vivo with some specificity. However, the lack of specificity of these crude antisera made it difficult to define the utility of the technique. It was unclear whether nonspecific localization was due to lack of antisera specificity or problems associated with normal serum pooling of labeled antisera.

The advent of monoclonal antibodies made it possible to study these parameters with more accuracy than ever before. However, the fate of murine monoclonal antibodies in a murine host is likely to be different from murine monoclonal antibodies in a heterologous host. We developed the L10 tumor-D3 solid tumor model to investigate the utility of murine monoclonal antibodies in a heterologous host. We believe that this model more closely approximates the situation in humans, where murine monoclonal antibodies may be used for diagnosis and therapy. D3 is a true monoclonal antibody with specificity only for cell surface determinants in the guinea pig L10 tumor (3, 10).

Animals receiving 125I-D3 showed constantly increasing tumor-background ratios from 3 to 148 hr as blood, liver, spleen, and kidney levels of iodine continually decreased over time, while 125I-D3 remained bound to L10 tumors. L10:L1 ratios reached their peak with a value of 6.9 at 72 hr in animals receiving 125I-D3 as opposed to 24 hr in animals which received 111In-D3. However, absolute levels of radioactivity were much lower in animals which received 125I-D3 than in animals which received 111In-D3.

Guinea pigs bearing L10 and L1 tumors in the contralateral thighs received either 150 or 250 µCi of 125I-D3 antibody (specific activity, 6.5 µCi/µg). These animals were imaged at 72 hr, at which time L10 tumors were densely labeled and clearly visible, whereas L1 tumors showed minimal 125I accumulations which were shown subsequently to be due to blood-borne label in these ulcerating tumors (Fig. 3). Animals receiving 150 µCi of 125I-D3 antibody were sacrificed subsequently at 96 hr, and cpm/g of tissue were determined. L10 tumor contained 469,000 cpm/g of tissue; L1 tumor contained 21,000 cpm/g of tissue; liver, kidney, and spleen all contained fewer than 63,000 cpm/g of tissue; and blood contained 192,000 cpm/g of tissue (Table 4). Animals receiving 250 µCi of 125I-D3 antibody were sacrificed at 120 hr. These animals contained approximately 2 times more cpm/g of tissue than that contained in animals receiving 150 µCi of 125I-D3 antibody (Table 4).

### Table 4

<table>
<thead>
<tr>
<th>Radioactivity per g of tissue in animals bearing L10 and L1 tumors on contralateral thighs</th>
<th>cpm bound as % of input</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals receiving 150 µCi 125I-D3 analyzed at 96 hr</td>
<td></td>
</tr>
<tr>
<td>L10</td>
<td>469,000</td>
</tr>
<tr>
<td>L1</td>
<td>21,000</td>
</tr>
<tr>
<td>Liver</td>
<td>40,000</td>
</tr>
<tr>
<td>Spleen</td>
<td>39,000</td>
</tr>
<tr>
<td>Kidney</td>
<td>83,000</td>
</tr>
<tr>
<td>Lung</td>
<td>52,000</td>
</tr>
<tr>
<td>Blood</td>
<td>192,000</td>
</tr>
<tr>
<td>Animals receiving 250 µCi 125I-D3 analyzed at 120 hr</td>
<td></td>
</tr>
<tr>
<td>L10</td>
<td>411,000</td>
</tr>
<tr>
<td>L1</td>
<td>7,700</td>
</tr>
<tr>
<td>Liver</td>
<td>23,000</td>
</tr>
<tr>
<td>Spleen</td>
<td>128,000</td>
</tr>
<tr>
<td>Kidney</td>
<td>140,000</td>
</tr>
<tr>
<td>Lung</td>
<td>63,000</td>
</tr>
<tr>
<td>Blood</td>
<td>405,000</td>
</tr>
</tbody>
</table>

* Cpm bound/g of tissue determined from homogenized, washed tissue.
In was conjugated to D3 by a metal chelation technique (11, 15) to investigate this approach. Although tumors imaged well, 111In accumulation within liver, kidney, and spleen required views from several angles to clearly identify tumors in adjacent locations. Unlike 125I, 111In levels remained nearly constant in these normal tissues throughout the time course of our experiments. 111In localization was maximal, as determined by tumor:background ratios, at 24 hr and remained constant thereafter, whereas 125I tumor:background ratios continued to increase throughout the course of our experiments as iodine was cleared from the host.

Scheinberg et al. (22) used 111In-metal-chelated antibodies conjugated to Rauscher murine erythroleukemia virus M, 70,000 glycoprotein and successfully imaged erythroleukemic spleen but not normal tissues. It is not known if the reduced background labeling of liver and kidney in this study was due to better conjugation of 111In to the antibody or the use of an allogenic host. However, the former is probable as 111In-labeled monoclonal antibody to carcinoembryonic antigen, used in a nude mouse-human colon tumor xenogeneic model, produced similar results to our own with high levels of label in liver, kidney, and spleen (23).

Clearly, tumors can be imaged with radiolabeled monoclonal antibodies; however, further technical development will be necessary before these techniques will become important tools in clinical medicine. Computer subtraction techniques, using a second iodine isotope conjugated to an indifferent antibody, can be used to obtain usable images earlier than can be obtained with a single iodine-labeled antibody. However, specificity is not enhanced over what can be obtained using an iodine-labeled monoclonal antibody and imaging at later times. 125I can be used to deliver a therapeutic radiation dosage as well as imaging tumors (8).

111In has theoretical advantages over 125I because of its higher energy emissions, thus reducing photon attenuation and making imaging of deep visceral tumors less time consuming. In addition, more of the initial inoculum remains specifically bound to the tumor, further enhancing imaging. However, nonspecific localization was a problem in our studies and resulted in less than optimal images. However, rotating the camera (Fig. 1) clearly separated tumor from background, and by 24 hr, tumor:background ratios consistently exceeded 2:1. Thus, even deliberate placement of tumors over areas of high background did not eliminate our ability to adequately visualize these tumors. We are currently evaluating modified chelation techniques which bind 111In and antibody more tightly and which may limit liver, kidney, and spleen 111In accumulation. Attempts at blocking uptake by the liver are also being investigated and may be clinically useful.

Although the biochemistry of radiolabeling monoclonal antibodies needs further development, it is clear that such antibodies can be used to image tumors in heterogenous hosts. Radiotherapy using conjugated monoclonal antibodies will require careful selection of monoclonal antibody and radioisotope. By choosing isotopes of appropriate energy and emission characteristics, it may be possible to effectively treat tumors in which only a portion of the cells express the antigenic determinant recognized by the monoclonal antibody, while adjacent tumor cells might be destroyed by radiation from antibodies bound to neighboring cells. This might prove helpful with regard to heterogeneity of antigen expression.

REFERENCES

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