Radioimmunodetection of Small Human Tumor Xenografts in Spleen of Athymic Mice by Monoclonal Antibodies

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ABSTRACT

The ability of radiolabeled monoclonal antibodies to accumulate in and image small human tumors growing in the spleen of athymic mice was assessed. The antibodies B6.2 and B72.3, which reacted against human breast (Clouser) and colon (LS174T) tumor cells in vitro and in vivo, respectively, and the isotype matched anti-horseradish peroxidase antibody, which did not react with any of the three antibodies were used as additional controls. Radioiodinated "tumor specific" and nonspecific antibodies were injected i.v. into athymic mice bearing intrasplenic tumors and the mice were sacrificed at various times to assess the specificity of uptake of these antibodies into tumor and normal host tissues.

The accumulation of B6.2 in the Clouser tumor was maximal at 24 h as indicated by a localization index (specific/nonspecific antibody in tumor divided by the same ratio in blood) of about 4.0. The uptake of B72.3 in LS174T tumor increased with time with a localization index of about 12.0 observed at 50 h postantibody injection. Localization indices for the control A375 tumors and for all normal mouse tissues, including the uninvolved portion of the tumor bearing spleen, were between 0.8 and 1.0, thus indicating no specific antibody accumulation. The relative blood flows of the Clouser and A375 tumors, as determined by the 82RbCl method, were similar. The results suggested that immunospecificity was a major factor in antibody localization in vivo.

Specific images of approximately 100 mg Clouser tumors with radiolabeled B6.2 and of LS174T tumor with radiolabeled B72.3 were seen by 24 h after antibody injection. Images of smaller (about 20 mg) LS174T tumors were seen by 48 h following B72.3 injection. The control antibody, anti-horseradish peroxidase, did not image either Clouser or LS174T tumor. Also the control tumor was not imaged with any of the three antibodies tested.

The data generated with this novel animal model support the concept of using radiolabeled monoclonal antibodies for detecting and possibly treating small metastatic visceral tumors in cancer patients.

INTRODUCTION

The ability to detect small deep-seated metastatic tumors using radiolabeled monoclonal antibodies has significant clinical importance (1–5). Most of the published data on tumor imaging by radiolabeled monoclonal antibodies in animal models have used relatively large superficial tumors (6–8). The pharmacokinetics of monoclonal antibodies in and imaging of small clinically relevant sites has rarely been demonstrated. Some recent studies have used the mouse subrenal capsule model for antibody localization and imaging studies (2, 9–12). In the present work, we have explored the spleen of the nude mouse as an alternate site for supporting human tumor growth. The spleen was chosen instead of the commonly used s.c. site on the mouse flank since it is located near well-perfused organs such as the kidneys and liver. Antibody accumulation by these organs could potentially interfere with specific tumor imaging by radiolabeled monoclonal antibodies and better reflects the clinical situation. Preliminary work has shown the feasibility of using the intrasplenic tumor model for antibody localization studies (9, 12).

Thus the pharmacokinetics of the monoclonal antibodies B6.2 and B72.3 in an intrasplenic human mammary carcinoma and a human colorectal carcinoma target tumor were compared. A human melanoma and a nonspecific antibody were included as controls. The ability of tumors and normal mouse tissues to accumulate antibodies was compared to their relative blood perfusion in order to further characterize this new tumor model.

MATERIALS AND METHODS

Tumors. A human melanoma tumor (A375), two human infiltrating duct cell carcinomas of the breast (Clouser and MCF-7), and a human colorectal carcinoma (LS174T) were used in the present work. The LS174T, MCF-7, and A375 tumors were maintained as tissue culture cell lines. Clouser was carried as a solid tumor in 4–5-week-old female athymic nu/nu mice of a BALB/c background (Charles River, Inc., Wilmington, MA). The LS174T tumor was obtained from the American Type Culture Collection (Rockville, MD). The MCF-7, A375, and Clouser tumors were obtained from the National Cancer Institute (13). Donor s.c. tumors were induced by injecting either 3 × 10⁶ A375 cells, 2 × 10⁶ LS174T cells, or Clouser slices [0.2 ml of a 20% (w/v) mince in RPMI 1640 medium; M. A. Bioproducts, Walkersville, MD] into the flanks of athymic mice. Intrasplenic tumors were initiated by implanting a 1-mm³ tumor piece from s.c. growths into the spleen bed towards the other visceral organs using a 16-gauge trocar. Imaging and biodistribution experiments were performed at 14–17 days after tumor implantation. Tumors of up to 100 mg grew entirely within the spleen, whereas tumors larger than 100 mg grew partly in the spleen and partially in the abdominal cavity.

Radioiodinated Monoclonal Antibodies. The murine monoclonal antibodies B6.2 and B72.3 were obtained from Dr. J. Schlom of the National Cancer Institute. The B6.2 antibody binds to Clouser, MCF-7, and LS174T tumor cells, whereas B72.3 antibody binds to only the LS174T tumor cells. Methods for generating these antibodies and the radiolabeling procedure with Na¹³¹I or Na¹²⁵I by the iodogen method have been published previously (13). The anti-HRP² monoclonal antibody, prepared by the Immunology Research Department of DuPont, served as a control antibody for the present work. All three antibodies were subclass matched IgG1s.

Assay of Immunoreactivity of the Radioiodinated Antibodies Using Live Cells. Labeled antibody (2 ng) was added to either LS174T, MCF-

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² The abbreviations used are: anti-HRP, anti-horseradish peroxidase; ID, injected dose; LI, localization index.
7, or A375 tumor cell suspensions and incubated at 37°C for 2 h. The cell number ranged from \(3 \times 10^3\) to \(2 \times 10^7\) cells/0.1 ml RPMI medium containing 1% bovine serum albumin. At the end of the incubation period, the cells were washed twice with RPMI in microtubes using a Beckman B high speed centrifuge. The amount of antibody bound to the cells was determined by counting the cell pellets in a gamma scintillation counter.

Tumor Imaging and Pharmacokinetics of Antibodies. For imaging studies, groups of four to six athymic mice bearing intrasplenic tumors were given injections via the tail vein of 25–50 \(\mu\)Ci \(^{131}I\)-B6.2 (specific activity, 23.3 \(\mu\)Ci/\(\mu\)g), \(^{131}I\)-B72.3 (specific activity, 26.35 \(\mu\)Ci/\(\mu\)g), or \(^{125}I\)-anti-HRP (specific activity, 42.95 \(\mu\)Ci/\(\mu\)g). Gamma camera images were obtained using a Med-X-modified Pho/Gamma HP Camera (Nuclear-Chicago) fitted with a 4-mm pinhole collimator. Nembutal (0.83 mg pentobarbitone sodium i.p.; Abbott Laboratories, North Chicago, IL) anesthetized mice were positioned with the dorsal surface facing the camera, and each image was formed by the accumulation of 100,000 counts.

Biodistributions were performed on mice given injections of 4–6 \(\mu\)Ci of \(^{131}I\)-labeled specific antibody (either B6.2 or B72.3 for Clouser and LS174T tumors, respectively) and about an equal amount of \(^{125}I\)-labeled nonspecific antibody anti-HRP. Groups of four to six mice were sacrificed by cervical dislocation at the indicated times and radioiodine content of various tissues was determined by using a gamma counter. The results were expressed as ID of antibody per g wet weight of tissue. The LI was calculated using the formula (14)

\[
LI = \frac{\text{Specific/nonspecific antibody in tumor}}{\text{Specific/nonspecific antibody in blood}}
\]

**Blood Flow Measurement.** The relative blood perfusion was estimated indirectly by fractional distribution of \(^{86}Rb\)Cl (specific activity, 5.23 mCi/mg; DuPont/NEN Products, North Billerica, MA) (15). This isotope when injected i.v. becomes distributed between tissues in a concentration proportional to the tissue fraction of the cardiac output (16). A miniature cadmium-telluride probe (1 × 1-mm CdTe crystal; Radiation Monitoring Devices, Watertown, MA) placed over the tumor growing in spleen or kidney or on the hind thigh muscle was used to determine the kinetics of \(^{86}Rb\) accumulation in the various tissue. These studies showed that the \(^{86}Rb\) content of the organs reached a plateau level about 2 min after i.v. injection. Therefore mice were sacrificed 2 min after injection of 75 \(\mu\)Ci \(^{86}Rb\) and the relative blood perfusion values were calculated as percentage of ID \(^{86}Rb\)/g tissues. \(^{86}Rb\) content of the tissues was determined by counting the tissues in a gamma scintillation counter.

**RESULTS**

**Binding of Antibodies to Tumor Cells in Vitro.** Chart 1 shows the in vitro immunoreactivity of radioiodinated B6.2 and B72.3 with either MCF-7, LS174T, or A375 tumor cells. The binding of either B6.2 or B72.3 (2 ng protein of each antibody) with MCF-7 and LS174T target tumor cells, respectively, increased with increasing cell number. Maximal binding of B6.2 with the MCF-7 tumor cells was 90% under the experimental conditions used. Since the B6.2 localized similarly in s.c. MCF-7 and Clouser tumors in athymic mice, the MCF-7 cell line was routinely used to assess the immunoreactivity of radioiodinated B6.2 prior to animal injection. Approximately 30% of the added B72.3 bound to \(2 \times 10^7\) LS174T tumor cells. No significant binding of either B6.2 or B72.3 was observed with the antigen negative A375 tumor cells (Chart 1). From 3 to 5% of the control antibody, anti-HRP, bound nonspecifically to either MCF-7, LS174T, or A375 tumor cells (2 \(\times 10^7\); data not shown).

**Pharmacokinetics of Antibodies and Localization Index.** Blood clearances of B72.3 or B6.2 and anti-HRP (\(\omega\)HRP) from blood (A), liver (B), kidneys (C), and uninvolved spleen (D) of nude mice bearing intrasplenic human tumors. Radioiodinated antibodies were injected i.v. at 14–17 days after tumor implant and tissues (wet weight) from five mice at each time point were analyzed for \(^{131}I\) and \(^{125}I\) activity. Either \(^{131}I\)-B72.3 or \(^{131}I\)-B6.2 was injected into mice bearing either intrasplenic LS174T or Clouser tumors, respectively. \(^{125}I\)-anti-HRP was used as nonspecific antibody control in each experiment. The blood clearances of either \(^{131}I\)-B72.3 or \(^{131}I\)-B6.2 (common line) shown by * were from nude mice bearing control A375 intrasplenic tumors. •, blood clearance of \(^{131}I\)-B6.2 in Clouser tumor bearing mice; □, blood clearance of \(^{131}I\)-B6.2 in Clouser tumor bearing mice; ▲, blood clearance of \(^{125}I\)-anti-HRP in either LS174T or Clouser tumor bearing mice with similar results.

\[\text{LI} = \frac{\text{Specific/nonspecific antibody in tumor}}{\text{Specific/nonspecific antibody in blood}}\]

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\(^{8}\) S. A. Shah and P. L. Jones, unpublished data.
Uptake of all three antibodies by liver, kidneys, or normal spleen was similar at all times examined, i.e., 7–10% ID/g at 5.5 h and about 3–7% ID/g at 24–75 h following antibody injection (Chart 2, B–D).

The accumulation of $^{131}$I-B72.3 in LS174T tumor decreased with the increasing tumor mass (Chart 3). This relationship was observed for LS174T tumors of varying size within the same group of mice examined at 24, 48, and 75 h following antibody injection. No such relationship was observed for the accumulation of either B6.2 in Clouser (Chart 3B) or B72.3 uptake by the A375 tumor (Chart 3C).

Specific accumulation of B72.3 in LS174T tumor was about 22.9 ± 8.5% ID/g at 5.5 h and about 36.2 ± 12.5% ID/g at 75 h following antibody injection (Chart 4). The relatively large SE observed may be the reflection of the range of tumor sizes (20–990 mg) studied. Accumulation of the nonspecific antibody anti-HRP in LS174T tumor was about 8% ID/g throughout the 75-h study period. The LI for the LS174T tumor increased from 3.3 ± 1.2 at 5.5 h to 12.6 ± 3.0 at 50 h and then decreased to 8.9 ± 1.0 at 75 h following antibody injection. The normal part of the tumor-bearing spleen and all other normal organs in these mice had LI’s of approximately 1 (Chart 4B).

Specific accumulation of B6.2 in Clouser tumor increased from 11.8 ± 3.2% ID/g at 5 h to 21.9 ± 4.5% ID/g at both 24 and 48 h after antibody injection. The B6.2 content of Clouser tumor decreased to 16.5 ± 3.5% ID/g by 72 h (Chart 4A). Accumulation of the nonspecific antibody anti-HRP in Clouser tumor remained at 6% ID/g throughout the study. The LIs in Clouser tumor increased from 1.7 ± 0.2 at 5 h after antibody injection to about 4.0 ± 0.4. The LIs for normal organs including the normal part of the tumor bearing spleen remained approximately 1 at all times examined (Chart 4B).

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The accumulation of $^{131}$I-B72.3 in the tumor-bearing spleen remained approximately 1 at all times examined, i.e., 7–10% ID/g at 5.5 h and about 3–7% ID/g at 24–75 h following antibody injection (Chart 2, B–D).

The uptake of either B6.2 or B72.3 by the control A375 tumor decreased from about 5% ID/g at 5.5 h to about 2% ID/g 72 h after injection. The LIs for both the A375 tumor and normal organs were approximately 1 (Chart 4B).

Tumor Blood Flow and Antibody Localization. Despite equivalent perfusion of intrasplenic Clouser and A375 tumors, Clouser showed a 4-fold greater accumulation of B6.2 compared to the A375 tumor (Table 1). Blood flow to the normal part of tumor bearing mouse spleen was about 2.7 times greater than that to the Clouser tumor itself and yet no specific antibody accumulation was seen in normal spleen. The relative blood flows of well perfused organs such as lung and kidneys were 5 and 12 times more, respectively, than in the Clouser tumor. No specific B6.2 accumulation was observed in these organs. The blood perfusion of skin and liver was equal to that of Clouser tumor and no specific antibody localization was observed in these tissues. These results suggest that the B6.2 accumulation in intrasplenic Clouser tumor must be due to the immunospecificity of the antibody for this tumor since the isotype matched nonspecific antibody anti-HRP also showed no significant accumulation.

Tumor Imaging. Excellent images of the Clouser tumor were observed.
seen in six of seven mice at either 24 or 48 h after \(^{131}\text{I}-\text{B6.2}\) injection. The mouse that did not image was subsequently found to have had no tumor growing in the spleen. The weight range of Clouser tumors studied was between 104 and 510 mg. The control antibody anti-HRP did not image Clouser tumors of similar size. Fig. 1 shows whole body images of 104–141-mg Clouser tumors 24 h after injecting either \(^{131}\text{I}-\text{B6.2}\) or \(^{125}\text{I}-\text{anti-HRP}\).

Twelve mice, six each with either LS174T or A375 intrasplenic tumors, were imaged for up to 5 days after injecting either \(^{131}\text{I}-\text{B72.3}\) or \(^{125}\text{I}-\text{anti-HRP}\). At 24 h post-antibody injection, mice with LS174T spleen tumors of about 300 mg gave outstanding images with \(^{131}\text{I}-\text{B72.3}\). These mice showed little or no background activity. An additional mouse which had a 22-mg tumor in the spleen started to show localization at 48 h post-antibody injection (Fig. 2) and then showed a dramatic tumor specific image at 120 h. The control antibody did not image either intrasplenic LS174T or A375 tumors weighing up to 300 mg. The control A375 tumor failed to image with B72.3 at any of the time points studied (Fig. 3). There was no sequestering of the antibody by the A375 tumor.

**DISCUSSION**

Most of the published work on tumor detection with radiolabeled antibody have dealt with relatively large s.c. tumors that do not resemble the visceral sites and sizes of clinical metastases. This is the first detailed report demonstrating the feasibility of localizing small human breast and colon tumor xenografts by the use of monoclonal antibodies. The tumor size is an important parameter to consider while developing animal models that represent clinically relevant tumors. The subrenal and the more recent intrasplenic models have used tumors growing in the subrenal capsule for the evaluation of therapeutic (20) and radiodiagnostic (2, 9–12) agents. The subrenal and the more recent intrasplenic models described here may better reflect clinical conditions since they are located in the visceral sites and have much greater perfusion rates compared to the s.c. tumor growths commonly used for imaging studies (12).

The ultimate tumor uptake of antibody will largely depend on the immunospecificity of the antibody under study, although the initial delivery of antibody to the tumor would be expected to be blood flow dependent. The blood flow of both Clouser and A375 tumors was similar. The normal part of the tumor bearing spleen (with about 2.5 times greater blood flow than the tumor) showed no specific uptake of B6.2. Other highly perfused organs, such as kidneys and lungs, showed no specific accumulation of B6.2 (or of B72.3 in the case of LS174T tumor bearing mice). Therefore the differences observed in antibody uptake between antigen positive and antigen negative tumors, as well as normal tissues, reported here were not related to blood flow but were due to the immunospecificity of the antibodies.

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\(^4\) Unpublished work.

REFERENCES


Fig. 2. Images of 22-mg intrasplenic LS174T tumor at 24 (left), 48 (middle), and 120 (right) h after i.v. injection of $^{131}$I-B72.3. Other experimental details were as for Fig. 1.

Fig. 3. Images of an 80-mg intrasplenic control A375 tumor at 24 (left), 48 (middle), and 72 (right) h after i.v. injection of $^{131}$I-B72.3. The faint tumor image at 72 h was due to blood pool activity. Other details were as for Fig. 1.
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