Preclinical Studies on the Pharmacokinetic Properties of Human Monoclonal Antibodies to Colorectal Cancer and Their Use for Detection of Tumors

Richard P. McCabe,1 Leona C. Peters, Martin V. Haspel, Nicholas Pomato, Jorge A. Carrasquillo, and Michael G. Hanna, Jr.

ABSTRACT

We studied the pharmacokinetic properties of two human monoclonal antibodies to colon carcinoma cells and their ability to detect tumors in nude mice bearing primary human colon carcinoma xenografts. The 16-88 and 28A32 monoclonal antibodies are immunoglobulin M class human antibodies produced by cell lines derived from peripheral blood lymphocytes from patients with colon carcinoma. The patients received an autologous tumor cell vaccine as part of an active specific immunotherapy protocol. The 125I-labeled antibodies were cleared from the circulation of non-tumor-bearing and tumor-bearing nude mice with a 6-8 h half-life. The half-life of the antibodies in tumor tissue was 48 to 72 h compared to 8 to 12 h for normal tissues. Tumor: normal tissue ratios were highest 4 to 7 days postinjection with tumor: blood ratios of 12:1 for 16-88 and 10:1 for 28A32 antibody. Experiments with a control human immunoglobulin M myeloma protein confirmed the specificity of the human monoclonal antibodies. Radioimmunoscintigraphic studies using nude mice bearing contralateral antibody-reactive and nonreactive colon tumor xenografts further confirmed that the antibodies specifically localized in tumor tissues. The antibody-reactive tumors were clearly visible by radioimmunoscintigraphy within 4 days of injection. These experiments, undertaken as a preliminary step to clinical trials, demonstrated for the first time that i.v. administered human immunoglobulin M monoclonal antibodies could be taken up by human colon tumor tissue and retained to a sufficient extent to easily permit tumor detection by external radioimmunoscintigraphy. These studies also demonstrated that the nude mouse colon tumor xenograft model is a useful in vivo system for comparison studies of human monoclonal antibodies as part of a selection process for clinical trials and for evaluating immunoconjugates containing these antibodies for relative pharmacokinetic properties and potential diagnostic or therapeutic efficacy.

INTRODUCTION

The ability to specifically concentrate diagnostic and therapeutic agents in tumor tissue may well be the most significant application of monoclonal antibody technology to health care. Monoclonal antibodies have been developed that recognize tumor-associated determinants on human tumor cells. In colon cancer and malignant melanoma, radionuclide monoclonal antibodies can be used with radioimmunoscintigraphy to detect clinical tumors that are not detectable by other methods (1, 2). However, before this technology can be generally applied, evidence that it is of substantial value in the management of cancer patients must be collected, further improvements in sensitivity must be made, and solutions to such significant problems as the immunogenicity of monoclonal antibodies of murine origin in most cancer patients must be found (3-5).

Human anti-mouse antibody responses develop in most patients after one administration of whole mouse immunoglobulin (3, 4) and in approximately 50% of patients after three or more injections of murine Fab fragments (5). Anti-mouse antibody alters the distribution of the monoclonal antibodies; most are deposited in the reticuloendothelial tissues (6) resulting in more rapid clearance, less tumor uptake, and possible damage to the liver and spleen, especially in instances where the antibody is conjugated to a toxin, drug, or radionuclide. Other responses such as immunocomplex-mediated serum sickness and manifestations of foreign protein toxicity (urticaria, fever, nausea, anaphylaxis) may also occur. Antibodies of human origin or murine antibodies in which the constant region domains have been replaced with human constant regions by means of recombinant genetic technology are less likely to elicit anti-immunoglobulin responses. Unlike murine antibodies, human antibodies can be potentially administered to patients for several months or years in large quantities to detect tumors, specifically concentrate toxic agents in tumor tissue, and monitor the results of therapy.

Human monoclonal antibodies to human tumor tissues have been developed (7-11). Murine antitumor antibodies with genetically engineered human constant domains have also been described (12, 13). In 1985 we reported the development of 20 human monoclonal antibodies from the peripheral blood lymphocytes of colorectal cancer patients (7) participating in an active specific immunotherapy trial for treatment of residual disease (14). Two human IgM monoclonal antibodies (16-18 and 28A32) were selected from the original panel for further development based on their specificity, cell surface reactivity, and avidity. The cell lines selected were stable and able to produce gram quantities of pharmaceutical grade antibody. These antibodies are currently being used in a phase I tumor detection and escalating-dose therapy trial in colon cancer patients. The objectives of the trial are to evaluate the potential efficacy of the human antibodies as diagnostic and therapeutic agents and also to assess the pharmacokinetics of tissue clearance, their ability to detect tumor, and the immunogenic properties of allogenic radiolabeled human antibody in colon cancer patients. A preliminary report of the results of this trial has been presented (15).

Experiments described in this paper were undertaken to evaluate the ability of human monoclonal antibodies to enter and be retained in human colon tumor tissues. They also allowed us to evaluate the nude mouse model as a tool for comparing human antibodies and for assessing the effects of modification of antibodies on their pharmacokinetic properties. The intended role for this model is to aid in developing concepts for clinical studies. Based, at least partially, on the successful tumor localization described in this paper the clinical tumor detection trial was begun. This model will be used to develop and evaluate additional applications of the human antibodies as conjugates with radiometals, toxins, or drugs and to select among the various approaches those most promising for clinical studies.

MATERIALS AND METHODS

Antibodies. Human monoclonal antibodies 16-88 and 28A32 were developed from the peripheral blood lymphocytes of colon carcinoma patients.
patients as described previously (7). The patients were participating in a clinical trial of active specific immunotherapy and were receiving injections of irradiated autologous tumor cells (14). Peripheral blood lymphocytes were obtained 1 week after the second of three weekly immunizations. Antibody 28A32 is the product of a human-mouse (NS-1) heterohybridoma and recognizes an antigen on the surface and in the cytoplasm of colon carcinoma cells. Antibody 16-88 is produced by a spontaneously transformed human lymphoblastoid cell line which expresses the Epstein-Barr nuclear antigen. It recognizes a predominantly cytoplasmic antigen and has an apparent avidity in the range of $5 \times 10^8$ M$^{-1}$ as measured with glutaraldehyde-fixed colon carcinoma cells or crude soluble antigen using a modification of the method described by Lindmo et al. (16). Both antibodies react with paraffin sections of colorectal tumors and have been tested extensively for reactivity and specificity (17).

The cell lines were propagated in hollow fiber culture cartridges and highly concentrated crude antibody supernatant fluids were obtained. The antibody was clarified by centrifugation to remove cell debris and was then concentrated by ammonium sulfate precipitation. The antibody was purified by sequential steps of gel filtration and high performance ion exchange chromatography. The purified antibody (~95%) was free of detectable endotoxin.

A control human myeloma IgM was purchased from Cappel Laboratories (Malvern, PA).

Radiolabelling. Iodo-Gen (Pierce Chemical Co., Rockford, IL) was used to label the antibodies with $^{125}$I according to the method of Fraker and Speck (18). The antibodies were diluted with 0.04 M PBS, pH 7.2, at concentrations of 1.0 to 6.5 mg/ml. In a typical reaction 40 $\mu$g Iodo-Gen was allowed to react with 2 mg antibody and 4 $mcI$ $^{125}$I for 10 min at room temperature. The labeled antibody was purified on a PD-10 column (Pharmacia, Piscataway, NJ) equilibrated with NMS-PBS. The labeled antibody was eluted from the column with NMS-PBS and diluted with NMS-PBS to give 100 $\mu$g antibody/ml. The specific activity was approximately 1.0 $mcI$/mg. Immunoactivity was at least 80% as determined by the method of Lindmo et al. (16).

Xenografts. Human colon tumor xenografts were developed in male outbred 6- to 8-week-old BALB/c-nu/nu specific-pathogen-free virus-free mice from enzymatically dissociated human tumor cells. The tumor cells were prepared as described previously for preparation of the active specific immunotherapy vaccine (19). Briefly, the tumor was carefully fragmented, and the cells were dissociated in a solution of 0.14% collagenase (type 1, 200 units/mg; Sigma Chemical Company, St. Louis, MO) and 0.10% DNase (500 Kunitz units/mg; Sigma) in HBSS. Single-cell suspensions (1.5 $\times 10^7$ to $2.0 \times 10^7$ cells/ml) were frozen in a controlled-rate freezer at $-1^\circ$C/min to $-80^\circ$C. The cells were maintained in liquid N$_2$ until used to develop the xenografts (20). Tumors were developed from the frozen cell stocks after careful thawing to maximally preserve cell viability. The cells were washed in HBSS and $3 \times 10^4$ viable tumor cells in 0.1 ml HBSS were injected s.c. in the upper right dorsal quadrant. When tumors developed to approximately 1.0 cm in diameter they were removed, enzymatically dissociated, and reinjected into additional mice. This procedure was repeated until the tumors grew at a reproducible and predictable rate after which a bank of frozen tumor cells was maintained to control for passage number and physiological and histological deviation in subsequently developed tumors.

The THO and ATK xenografts were developed from tissues taken from patients with well-differentiated colon carcinomas. THO cells were derived from a local recurrence of the primary tumor, and ATK cells were from a primary tumor. The EPP xenograft cells were derived from a poorly differentiated primary colon carcinoma.

Experimental Design. Mice with colon tumor xenografts (passage $\leq 15$) 0.5 to 1.5 cm in diameter, depending upon the design of the experiment, were given injections in the tail vein of 0.5 ml of $^{125}$I-labeled monoclonal antibody in NMS-PBS. The concentration of antibody was 100 $\mu$g/ml unless otherwise stated. At various times from

1. The abbreviations used are: PBS, phosphate-buffered saline; NMS-PBS, 10% normal mouse serum in 0.01 M phosphate-buffered saline, pH 7.4; HBSS, Hanks' balanced salt solution; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin.

15 min to 8 days after injection, groups of three animals were sacrificed and tumors, spleens, livers, kidneys, bloods, and thigh muscles were removed. Tissues were weighed and fixed in 10% buffered formalin, and levels of $^{125}$I were determined with a $\gamma$-radiation spectrometer (LKB Instruments). Blood was allowed to clot at 4°C, and serum was removed for $^{125}$I counting. Results were expressed as cpm/g tissue or cpm/ml serum. Hematoxylin- and eosin-stained sections of the tumor tissues were examined for appropriate histology.

IgM ELISA. Extracts of cultured colon carcinoma cells (WiDr) at a protein concentration of 4 mg/ml in PBS were used to coat wells (0.1 ml) of 96-well polystyrene plates. The wells were coated overnight (4°C) and nonspecific binding sites were blocked with 1% BSA in PBS (60 min, 25°C). A standard curve of purified 16-88 antibody in 10% NMS-PBS was run with each assay. Samples of the standards or specimens of antigen diluted 1:10 (PBS), 1:100 (PBS), and 1:1,000 (PBS) were added to each well. After incubation overnight at 4°C the wells were washed with 1% BSA-PBS and 0.1 ml of peroxidase-conjugated goat anti-human IgM ($\mu$-specific) (KPL Laboratories, Gaithersburg, MD) was added. The peroxidase-conjugated antibody was diluted 1:800 in 1% BSA-PBS. The plates were incubated for 60 min at room temperature; the wells were washed with 1% BSA-PBS, and 0.2 ml 0.03% 2,2'-azino-di-[3-ethyl]-benzethizalone sulfonic acid containing 0.03% H$_2$O$_2$ was added for 10 min at room temperature. The reaction was stopped with 0.025 ml 0.8% NaF. The absorbance at 405 nm was determined and the concentrations of human IgM in the mouse serum samples were determined from the standard curve.

Radioimmunoscintigraphy. Two groups of three animals bearing contralateral THO (right) and EPP (left) xenografts were given injections of 100 $\mu$g (100 $mcI$) $^{125}$I-labeled human monoclonal antibody in the tail vein. One group received $^{125}$I-labeled 16-88 and the other received $^{125}$I-labeled 28A32. Animals from each group were sacrificed at 1, 4, and 8 days postinjection. Serum, tumor, liver, kidney, spleen, and thigh muscle were monitored for $^{125}$I, and the results were expressed as cpm/g tissue or cpm/ml serum.

Serial scintiphots were obtained with selected animals from each group at 1, 4, and 8 days postinjection. Before imaging, avertin was given by i.p. injection to ensure that the animals were anesthetized adequately. A Raytheon Anger gamma camera equipped with a pinhole collimator (aperture, 0.25 inch) was positioned at a standard distance above the dorsum of each animal. A 20% energy window was centered over the low-energy $\gamma$-ray and X-ray photopeak of $^{125}$I. Analog images were acquired onto X-ray film for a standard time of 400 s (day 1) or 600 s (days 4 and 8) (5,000 to 65,000 cpm). No electronic data subtraction was performed.

RESULTS

Non-Tumor-bearing Mice. To assess the effects of tumor xenografts on the pharmacokinetic properties of radiolabeled human IgM monoclonal antibodies in the nude mouse, we first determined the base-line pharmacokinetic properties of the antibodies in non-tumor-bearing nude mice. We found that in non-tumor bearing nude mice $^{125}$I-labeled 16-88 (50 $\mu$g, 50 $mcI$) was quickly (<15 min) distributed throughout the circulation and through the tissues of the liver and spleen (Table 1). Highest levels of labeled antibody in kidney and thigh muscle occurred 2 h after administration.

<table>
<thead>
<tr>
<th>Location of antibody</th>
<th>Injected dose/g tissue (%)</th>
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<tbody>
<tr>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>Serum</td>
<td>66.2</td>
</tr>
<tr>
<td>Spleen</td>
<td>5.1</td>
</tr>
<tr>
<td>Liver</td>
<td>18.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.2</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table 1 Injected dose of $^{125}$I-labeled 16-88 (50 $\mu$g, 50 $mcI$) remaining in the serum and tissues of non-tumor-bearing nude mice 15 min to 8 days postadministration
Clearance of $^{125}$I-labeled 16-88 from the circulation followed a biphasic pattern as shown in Table 2. Measured over 0–24 h, 0–48 h, or 24–48 h the apparent half-life of $^{125}$I-labeled 16-88 in serum was 7 to 8 h. The rapid component lasted 48 h and removed all but 1–2% of the injected dose. The slower component measured from 24 to 96 h or 48 to 96 h had an apparent half-life of 12 h and reduced the circulating radioactivity to 0.1% of the injected dose.

Clearance from spleen, liver, kidney, and thigh muscle showed an initial rapid clearance for the first 24 h with apparent half-life retention of $^{125}$I-labeled 16-88 of 8 h (spleen), 5 h (liver), 12 h (kidney), and 9 h (muscle). The slower clearances from 24 to 96 h were at rates of 19 h (spleen), 17 h (liver), 24 h (kidney), and 16 h (muscle). For both the rapid and slower clearance phases, the kidney was substantially slower in removing the radioactivity than were the other organs. Most significant, however, was our finding that the rapid clearance over the first 24 h reduced the radioactivity in these organs and tissues to 1.5% or less of the injected dose.

An ELISA for human IgM was used to monitor 16-88 antibody concentrations in mouse serum. Experiments using crude or purified antibody showed no difference in clearance rates from serum as determined by ELISA, demonstrating that our results were not influenced by denaturation of the antibody during purification. Furthermore, iodination of 16-88 did not affect the serum clearance rate as shown by the fact that radiolabeled and unlabeled 16-88 cleared identically when compared in separate groups of mice. In mice given injections of $^{125}$I-labeled 16-88, both the $^{125}$I label and 16-88 antibody cleared from the serum at the same rate.

There was no evidence of any increase in free $^{125}$I in the mouse serum since the proportion of serum $^{125}$I bound to acid-precipitable protein remained between 90 and 95% for at least 24 h, the period required to clear 93% of the injected dose. After 24 h there was too little remaining activity in the serum to determine the protein-bound fraction.

The pharmacokinetic properties of antibody 28A32 in the non-tumor-bearing nude mice were very similar to those of 16-88, also exhibiting a serum half-life of 6 to 8 h for the initial 24 h postinjection and initial tissue clearance rates of 5–12 h for liver, spleen, muscle, and kidney.

Tumor Xenograft-bearing Mice. No remarkable difference in the kinetics of clearance of $^{125}$I-labeled monoclonal antibody from the circulation and normal tissues was seen when mice bearing human colon tumor xenografts and control mice were compared. The clearance curves from the tumor-bearing mice were very similar to those of non-tumor-bearing mice (Fig. 1).

The pattern of antibody accumulation and clearance from the tumor xenograft tissue was markedly different from that of the normal mouse tissues. Accumulation of antibody in tumor tissues reached a peak 2 h after injection but antibody cleared much more slowly from tumor tissue than from the normal tissues (Fig. 1). The half-life of $^{125}$I-labeled 16-88 and 28A32 in tumor tissue was 48 to 72 h compared to 8 to 12 h for the normal tissues. Using indirect peroxidase staining of tumor and normal mouse tissues for the detection of human IgM, we determined that antibody was retained by tumors, and there was no detectable antibody in normal livers 2 days postinjection. This experiment confirmed that antibody was retained in the tumor and was cleared from the normal tissues eliminating the possibility that differences in rates of clearance were related entirely to differences in the metabolism of the $^{125}$I radiolabel.

The retention of 16-88 and 28A32 in the tumor was antibody specific. Fig. 2 shows the activity in tumor and normal tissues up to 7 days after injection of $^{125}$I-labeled 16-88, 28A32, or a control human myeloma IgM. Only 16-88 and 28A32 were retained in tumor tissues, the control IgM cleared from tumor tissues as rapidly as from normal tissues. When the data shown in Fig. 2 are expressed as a percentage of the injected dose, comparisons between specific and nonspecific antibody accumulation in the tumor xenografts can be made. At 4 and 8 days postadministration the differences in tumor uptake between the control IgM and both 16-88 and 28A32 are significant to at least the 95% confidence limit ($P < 0.05$) as determined by the standard Student's $t$ test. Also, the difference between tumor

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**Table 2** Clearance of $^{125}$I-labeled 16-88 from serum and tissues of non-tumor-bearing nude mice

<table>
<thead>
<tr>
<th>Half-life (h)</th>
<th>Serum</th>
<th>Spleen</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time peak to 24 h $^a$</td>
<td>7</td>
<td>8</td>
<td>5</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>24 h to 96 h $^a$</td>
<td>12</td>
<td>19</td>
<td>17</td>
<td>24</td>
<td>16</td>
</tr>
</tbody>
</table>

$^a$ Animals received 50 µg, 50 µCi $^{125}$I-labeled 16-88 i.v. Serum and tissues were collected from 15 min to 96 h postinjection.

$^b$ Rapid phase of serum and tissue clearance was measured from the time of peak activity (cpm/g) to 24 h. Peak activity occurred at 15 min for serum, spleen, and liver and at 2 h for kidney and skeletal muscle.
and serum levels of human antibody on days 4 and 8 postadministration are significantly (P < 0.05) different for mice given injections of 16-88 and 28A32 but not for those that received the control IgM.

Tumor:normal tissue ratios for 16-88 and 28A32 reached a peak 4 to 7 days after injection (Table 3). No peak of IgM retention was seen, and only twice were the tumor:normal ratios for the control IgM greater than 1.0, substantiating the tumor specificity of these antibodies. In this experiment, tumor:normal ratios greater than 1.0 were seen for 16-88 and 28A32 as early as 3 days after administration. The peak tumor:serum ratios of 7.2:1 for 16-88 occurred at 4 days and 5.8:1 for 28A32 occurred at 8 days. Both ratios were quite sufficient for detecting tumor and compared quite favorably with results obtained with murine antibodies in murine model systems. The tumor:serum ratios were expressed on the basis of the ratios of cpm/g tumor:cpm/ml serum. The tumor:serum ratio was nearly twice the tumor:serum ratio since the cellular elements of the blood excluded the radiolabeled antibody.

The tumor:normal tissue ratios (cpm/g) were not affected by the quantity of antibody administered over the range of 10 μg to 160 μg, by the specific activity of the radiolabel (0.5 μCi/μg to 2.0 μCi/μg), or by the size of the tumor xenograft (0.5 to 3.0 g). However, the total activity in the tumor did increase with tumor size and the amount of radiolabeled antibody administered. Optimal detection of 125I-labeled antibody occurred with 1.0 to 2.0 g tumor and 50 to 100 μCi of radiolabeled antibody. For the experiments described in Table 3 and Figs. 1 and 2 the average tumor size was 0.56 g with the standard deviation of 0.34 g reflecting the growth of the tumor during the 8-day study period. There was no significant difference in tumor size among animals receiving human antibodies 16-88 or 28A32 or the control human IgM. Optimal detection of 125I-labeled antibody occurred with 1.0 to 2.0 g tumor and 50 to 100 μCi of radiolabeled antibody.

Localization of 16-88 and 28A32 was seen with several tumor xenografts as well as with the THO tumor used in most of these experiments. Both 16-88 and 28A32 reacted better with more highly differentiated colon tumors in immunohistochemical studies. To further establish the specificity of the human antibody localization, we performed several experiments with a poorly differentiated colon tumor xenograft (EPP) which reacted poorly with 16-88 and 28A32 as determined by indirect peroxidase staining. Fig. 3 shows the tumor:normal tissue ratios 4 days after mice bearing the THO or EPP xenograft were given injections of antibody. Results with 16-88 in mice bearing the 16-88-reactive ATK colon tumor xenograft are also presented. As predicted by the indirect peroxidase staining results, there was little localization of 16-88 or 28A32 in the poorly reactive EPP xenograft and excellent retention of antibody by the strongly reactive THO and ATK xenografts. The low ratios with the EPP xenograft were the result of lack of retention of the antibody in the tumor and not to poor access of the antibody, as shown by the finding that approximately the same amount of 125I-labeled 16-88 (2%) was taken up by both the EPP and THO tumors. However, the labeled antibody was cleared from the EPP tumor at the same rate as from normal tissues, whereas clearance from the THO xenograft was much slower.

Results of radioimmunoscintigraphy with 125I-labeled 16-88 and 28A32 in mice bearing THO xenografts (right side) and contralateral EPP xenografts are shown in Fig. 4. With both 16-88 and 28A32 the reactive THO xenograft was easily seen by day 4. At 8 days, although the THO xenografts were still obvious, the thyroid was the only detectable normal tissue. The EPP xenograft was poorly visible at day 8 in the mice given injections of 16-88 and was not detected at any time in the mice given injections of 28A32.

**DISCUSSION**

This study showed that human IgM monoclonal antibodies can localize selectively and be retained by reactive primary human tumor xenografts on nude mice. The retention of antibody in the tumors together with the rapid clearance of the labeled antibody from normal tissues allowed us to detect tumors by external radioimmunoscintigraphy 2 to 4 days after injection of the antibody. Ratios of the amount of activity in serum to that in tumor adjusted to an activity per ml of serum or per g of tumor gave values typically in the range of 6:1 to 8:1 or more with both human IgM antibodies. These values are comparable to tumor: blood ratios of 10:1 to 14:1, which are considered very good when achieved with a murine antibody in a syngeneic system and are consistent with a very strong externally detected tumor image (21-23). The 7- to 8-h circulating half-life of the human antibody was not surprising since earlier studies showed that various murine IgM monoclonal antibodies had 4 to 18 h circulating half-lives in tumor-bearing nude mice (22-24) and since the human antibody, as a foreign substance.

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**Table 3 Ratio of the amount of 125I-labeled antibody per g tumor to the amount of 125I-labeled antibody per g of normal tissue or per ml serum as determined at 3, 4, 7, and 14 days after administration of 50 μg (approximately 50 μCi) 125I-labeled antibody 16-88, 28A32, or control IgM**

<table>
<thead>
<tr>
<th></th>
<th>3 days</th>
<th>4 days</th>
<th>7 days</th>
<th>14 days</th>
</tr>
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<tbody>
<tr>
<td>16-88</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>1.1:1</td>
<td>7.2:1</td>
<td>5.2:1</td>
<td>1.9:1</td>
</tr>
<tr>
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<td>15.1:1</td>
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</tr>
<tr>
<td>Kidney</td>
<td>5.5:1</td>
<td>13.4:1</td>
<td>10.0:1</td>
<td>1.0:1</td>
</tr>
<tr>
<td>Tumor</td>
<td>1.0:1</td>
<td>0.7:1</td>
<td>0.3:1</td>
<td>0.2:1</td>
</tr>
<tr>
<td>% injected cpm</td>
<td>1.1 × 10^6</td>
<td>0.8 × 10^6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28A32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
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<td>4.4:1</td>
<td>5.8:1</td>
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<tr>
<td>Spleen</td>
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<tr>
<td>Liver</td>
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<td>7.3:1</td>
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<tr>
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<td>4.6:1</td>
<td>2.9:1</td>
<td>0.3:1</td>
</tr>
<tr>
<td>Tumor</td>
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<td>0.8:1</td>
<td>0.3:1</td>
<td>0.02:1</td>
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<td>% injected cpm</td>
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<tr>
<td>Serum</td>
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<td>0.8:1</td>
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<tr>
<td>Spleen</td>
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<tr>
<td>Liver</td>
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<td>0.6:1</td>
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<tr>
<td>Kidney</td>
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<tr>
<td>Tumor</td>
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<td>0.03:1</td>
</tr>
<tr>
<td>% injected cpm</td>
<td>0.4 × 10^6</td>
<td>0.6 × 10^6</td>
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in the mouse, would be expected to be cleared quickly. The situation in patients will be interesting since the circulating half-life of IgM antibody in humans is 5 days (25). The rapid clearance of human antibody from the mouse circulation could also have been the result of denaturation of the antibody, as indicated by the fact that we were able to increase the rate of clearance of human IgM in mice by heat denaturing the antibody. However, we confirmed the full functional integrity of the antibody used in each experiment and performed pharmacokinetics studies to show that purification and radiolabeling did not denature the antibody. For 16-88 we were able to assay the mouse serum for the cognate antigen (CTA 1). We found no evidence of the antigen in the mouse serum. Similarly, an ELISA for natural antibody reactive with the human IgM showed no evidence of anti-human antibody in the nude mouse. We conclude that for these two human monoclonal antibodies, 7 to 8 h is the circulating half-life in the mouse circulation. This finding probably cannot be generalized, however, because tests of another recently developed human antibody showed that it persists in the mouse circulation a much longer time (13 h serum half-life).

In colon cancer, if not in all cancers, there is little likelihood that radioimmunoscintigraphy with radiolabeled antibodies will ever be extensively used for detection of primary tumors. Current radiographic and endoscopic procedures are highly accurate and sensitive techniques for finding primary colon tumors. The contribution of radioimmunoscintigraphy to patient management will be in those areas where improvements are most needed: staging of tumors; monitoring disease progression; detecting recurrence; and, possibly, specific tumor therapy. Antibody will be most useful if it can be given frequently over a long period as the targeting component in several different therapeutic/diagnostic regimens. Diagnostic images developed early in the disease will be most useful when compared with images obtained later with the same antibodies as the disease progresses or as therapy reduces the amount of disease. Antibody-delivered therapeutic agents will be most effective if they can be administered over long periods and if antibody can continue to be used as the carrier as the need for new agents and new protocols develop. Thus it is of paramount importance that the patient does not develop an immune response to the antibodies. The advantages of human or human-mouse chimeric antibodies over murine antibodies are clear. Only allotypic and idiotypic determinants are potentially immunogenic with human monoclonal antibodies. Any immune response that does develop should be less intense than that elicited by murine antibody and should occur much later in the course of administration than the response of patients to murine IgG and IgM.

Allotypic and idiotypic responses can be managed by varying the specific human antibody used, e.g., using human antibodies of different idotype to the same antigen or human antibodies to other antigen(s) or with antibody to the same epitope derived from a different patient. Human anti-mouse antibody responses cannot be circumvented in this way and result in rapid removal of circulating antibody and its concentration in the spleen and liver, increasing the radioactive (or toxic drug) dose to this organ (6) and reducing the efficacy of tumor detection overall, especially in the liver. With murine Fab fragments the problem remains that at least 50% of patients will develop human antimouse antibody responses directed toward the common regions of the Fab fragment as well as anti-idotype responses after receiving murine monoclonal antibodies (3). Thus, monoclonal human antibodies or human-mouse chimeric antibody are the only appropriate alternatives if antibody-mediated tumor detection and therapy are to realize their full potential.

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REFERENCES

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