Pharmacokinetic Studies of Mouse Monoclonal Antibodies to a Rat Colon Carcinoma: I. Comparison of Biodistribution in Normal Rats, Syngeneic Tumor-Bearing Rats, or Tumor-Bearing Nude Mice

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The pharmacokinetics of two iodine-131- (^{131}I) labeled murine anti-rat colon carcinoma monoclonal antibodies (D3 and E4) were compared in normal Sprague Dawley rats, syngeneic BDIX rats, or nude mice bearing that tumor. Results of antibody uptake after i.v. administration were analyzed in terms of accumulation and localization indices for normal tissues and tumor. Statistically significant differences between rat and mouse tissue biodistribution were found. D3, which reacts in vitro with the tumor and several normal rat tissues, cleared quickly from the blood of rats and was specifically targeted to several normal tissues, notably the lung. Virtually no targeting to the tumor was observed. Nude mice, however, showed a slower blood clearance and specific antibody targeting only in the tumor. Similar results were seen after injection of another antibody, E4, which is tumor-specific in vitro. Data suggest that studies on the xenogeneic nude mouse model may not necessarily be relevant to the choice of monoclonal antibodies for clinical diagnostic imaging or therapy.

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The use of radionuclide-labeled antibodies to localize tumors in vivo was reported 30 yr ago by Pressman (*1*). Since then, several investigators have used polyclonal antibodies raised in a variety of species as diagnostic or therapeutic agents in oncology (*2–5*). With the advent of monoclonal antibodies (MAbs), radioimmunodetection of tumors has been more widely implemented clinically, with scattered reports of promising initial results (*6–10*). Most of the experimental data which support clinical use, including therapeutic trials, are based on in vitro reactivity and biodistribution studies in nude mice engrafted with tumor (*11–13*).

This paper compares the pharmacokinetics of mouse MAbs against rat colon carcinoma in normal rats, nude mice, or rats bearing a syngeneic colon carcinoma. These studies address the lack of information on the relevance of pharmacokinetics in a xenogeneic model to those of syngeneic tumors. The differences observed in these studies carry important implications for understanding the basis for successful clinical use of murine MAbs for imaging and therapy of human cancers.

MATERIALS AND METHODS

Animals

BDIX rats were purchased from Centre National de la Recherche Scientifique (Orleans, France) and were housed and bred at the National Institutes of Health (NIH) animal facility. Animals were 3–6 mo old when used. Sprague Dawley rats and nude mice (6–12 wk old) were obtained from NIH. Animals were provided ad libitum with NIH rat and mouse ration (NIH-07) and tap water and kept in individual cages. Three days before injection of radiolabeled antibodies, animals were provided with 2% potassium iodide enriched water, in order to saturate their thyroid with cold iodine.

Cell Lines

Rat colon adenocarcinoma DHD K12 TRb, (Trypsin-resistant "TR"), was generously provided by F. Martin (Dijon, France) (*14*). Cells were cultured in RPMI 1640 (Gibco Inc., Grand Island, NY) and were enriched with 10% fetal bovine serum (Hyclone, Logan, UT) and 1% Penicillin-Streptomycin (Gibco). To engraft animals, and to maintain cells in culture, cells were detached from flasks by a 1-min incubation with a solution of EDTA disodium salt (1 mg/ml, Sigma, St. Louis, MO) followed by 3 min of incubation with 1 mg/ml trypsin

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(Sigma) in Ca^{2+}-Mg^{2+}-free Hank's balanced salt solution (Gibco).

Monoclonal Antibodies

Murine monoclonal anti-colon carcinoma antibodies used in this study have been described previously (*15*). E4 reacts histochemically with TR tumor but not with normal tissues; D3 demonstrates cross-reactivity between TR tumor and a variety of normal rat tissues, including colon and lung. The isotype-matched IgG_{2a} mouse MAb, 17.1A, (kindly provided by Centocor Inc., Malvern, PA) was used as a control. 17.1A reacts with a tumor-associated antigen of human colon carcinoma and does not react with the rat tumor or with normal rat or mouse tissues. Monoclonal antibodies were radiolabeled with iodine-131 (^{131}I) (D3 and E4) or ^{125}I (17.1A) by using 1,3,4,6-Tetrachloro-3a,6a-diphenilglycouril (Sigma, St Louis, MO) as an oxidizing agent; following previously described procedures under conditions where no loss of immunoreactivity was incurred (*15*). The specific activity varied between 1–2 μCi/μg.

Pharmacokinetic Studies

For each experimental antibody (D3 or E4), 9 BDIX rats or 9 nude mice received subcutaneous injections of 10^7 or 5×10^6 TR cells, respectively. Ten days later, tumor had reached 170 ± 19 mg and 43 ± 12 mg (mean ± s.e.m.) for rats and mice, respectively. At this point, 50 μg (rat) or 10 μg (mouse) of either ^{131}I-E4 or ^{131}I-D3 and the same amount of ^{125}I-17.1A were injected (i.v.) into the anaesthetized animals. Male rats were injected in the dorsal vein of the penis, while females and nude mice were injected in a tail vein. One, 3, or 5 days after injection of labeled antibodies, three rats and three mice, each previously injected with experimental antibody and control, were anaesthetized and blood samples were drawn by cardiac puncture (rats) or eyebleeding (mice). Animals were killed by cervical dislocation, the organs were resected, washed in phosphate-buffered saline (PBS), and carefully blotted dry. Organs were weighed and the radioactivities of ^{131}I and ^{125}I determined in a gamma counter (1218 Compugamma, LKB, Bromma, Sweden). In the rat studies, one animal of the Day 5 group was placed in a metabolic cage and its urine recovered on Days 1, 3, and 5. The ^{131}I and ^{125}I radioactivities of a 1-ml aliquot were determined as described.

Calculations of the dose of radioactivity injected and the percent of the dose per gram of tissue were performed as follows: for both ^{131}I- and ^{125}I-labeled antibodies, a standard syringe was prepared together with the syringes to be used for injection. The amount of radioactivity used did not allow a direct determination of the dose injected by means of the gamma counter, therefore the syringes were counted in a gamma camera (Raytheon WB-1, Nuclear Diagnostic, Chicago, IL) before the injection. Simultaneously with the injection of the animals, the content of the standard syringe was transferred into 20 ml of PBS. All the empty syringes were counted in the gamma camera again and the difference between the full and empty values was the injected dose, as determined by the gamma camera. As the gamma counter was used to measure the radioactivity incorporated in the animal tissues, it was necessary to calculate the dose of radioactivity injected that the gamma counter would measure. To determine the factor that existed between the measurement of the gamma camera and the gamma counter, three aliquots (1 ml) of the 20-ml standard solution were counted with the tissues at each day studied. The average of these values was used to calculate the dose of radioactivity transferred into PBS, as determined by the gamma counter. This last value permitted the calculation of the relationship between the two counting devices and was used to calculate the injected dose for each animal, as determined by the gamma counter. The counting of the standard aliquots simultaneously with the tissues at each day studied also corrected for the radioactive decay.

Data Analysis

Results are expressed by calculating two different parameters from the counting data. First, the accumulation index (AI) is defined as the ratio between the percent of the injected dose per gram (%ID/g) found in each tissue and the dilution factor, equivalent to the theoretical value of %ID/g that would be found in all the tissues if the dose injected were identically distributed in the animal's entire body. For example, in a 100-g animal, 1% of the injected dose would ideally be found in each and every gram of tissue. Therefore, this factor is 100/body weight. For the mice used in this study with an average weight of 17 g, this value is 5.8; for the rats (average weight of 325 g) this value is 0.3. The AI allows comparison of the antibody uptake by the tissues of animals of different weights and corrects for the difference in volume of distribution in animals of different size. The AI is an indication of the avidity of a tissue for a given antibody: the AI is <1 in tissues in which antibody uptake is not significantly different from the uptake due to the dilution of the antibody through the body, considering that some antibody has been eliminated through the urine. An AI of 1 or greater in a given tissue means that the antibody, regardless of its antigen specificity, accumulates in that tissue.

The second parameter is the localization index (LI), first described by Moshakis (*16*) and defined as:

$$\frac{\dfrac{\%\ \text{dose/g relevant antibody (tissue)}}{\%\ \text{dose/g control antibody (tissue)}}}{\dfrac{\%\ \text{dose/g relevant antibody (blood)}}{\%\ \text{dose/g control antibody (blood)}}}.$$

This parameter is a measure of the specificity of the antibody localization. It is designed to correct for immunoglobulin uptake by organs which is not due to binding of the experimental antibody to its intended antigen. This is achieved by taking into account the "irrelevant" antibody. Since LI is calculated as a ratio of tissue:blood of two antibodies in the same animal, it is independent of the animal's weight.

Trichloroacetic acid precipitation

To ensure that the radioactivity found in the tissues was due to antibody and not to its catabolites or free iodine, several organs from one animal of each day studied were homogenized in a solution of 20% trichloroacetic acid in water. The homogenized organs were centrifuged at 3,000 g for 15 min and the precipitate and supernatant radioactivities were counted.

Statistical Analysis

Analysis of variance was performed using BRIGHT STAT-PAK (Bright Software, Rutgers, NJ). Bartlett's F-test was used

to determine the presence or absence of heterogeneity of variance and Duncan's multiple range test was used for statistical comparisons between groups. Significance was accepted at $p < 0.05$.

RESULTS

Pharmacokinetics of the Control Antibody 17.1A

Accumulation indices were calculated in mice and rats for 17.1A used as a control antibody (Table 1 and Figures 1 and 3). Nonspecific accumulation of murine MAb occurred in rat and mouse tissues. Radiolabeled 17.1A was found in rat lung, kidney, spleen, and liver, in addition to accumulating in tumor tissue. Nonspecific antibody retention in mice occurred essentially in the spleen and the tumor and, to some extent (Day 1 only), in skin, liver, and lung. Monoclonal antibody cleared from the blood at a slower rate in rats than in mice.

Pharmacokinetics of D3 Antibody

Accumulation and localization indices of the D3 antibody were calculated in tissues of normal Sprague Dawley rats, and BDIX rats or nude mice engrafted with the rat tumor. The biodistribution of D3 in normal Sprague Dawley and tumor-bearing BDIX rats was similar (data not shown). AI from several tissues were different when rats and nude mice were compared (Fig. 1). More D3 accumulated in mouse blood, skin, liver and spleen than in the respective rat tissues. Rat kidney (Day 1), heart (all days), and lung (all days) accumulation indices were significantly higher than in mice. The difference in lung was most striking, with values ranging from 58 (Day 1) to 20 (Day 5) in rat lung versus 0.85 (Day 1) and 0.25 (Day 5) in mice. The tumor accumulation indices differed between tumor-bearing BDIX rats and nude mice ($p < 0.05$). In BDIX rats, the index was low, as compared with other organs, and decreased with time (0.60, 0.32, and 0.30 on Days 1, 3 and 5, respectively). In contrast to observations in rats, in nude mice the tumor AIs were significantly greater than those of any other organ (3.69, 3.88, and 3.00 on Days 1, 3 and 5, respectively).

The differences in D3 biodistribution between BDIX rats and nude mice became more conspicuous when the localization indices were examined. With the exception of the liver, the LIs of all the rat organs (Fig. 2A) were greater than those of the tumor during the three days studied. In general, for all tissues including tumor, the value was >1, indicating a preferential specific uptake of D3 as compared to the control antibody, 17.1A, by the majority of rat tissues. In contrast, the tumor LI in nude mice (Fig. 2B) was the highest of all murine tissues. The LIs in normal tissues of nude mice were slightly less than one, indicating the absence of a preferential uptake of D3 over the control antibody in this model.

Statistical analysis indicated that all murine tissues, except skin and liver, showed significantly different LI from those of rat tissues on all days. In contrast to the difference between tumor and normal tissue in nude mice, at no time did rat tumor LI differ from rat tissue LI.

Pharmacokinetics of E4 Antibody

Accumulation and localization indices of E4 also were calculated in normal Sprague Dawley rats, and tumor-bearing BDIX rats or nude mice. In spite of preferential reactivity of E4 with the tumor and metastases by immunohistochemical analysis (*15*), high accumulation indices were found in the liver, stomach, and lung of BDIX rats, as well as in the tumor (Fig.

TABLE 1
17.1A Accumulation Indices in BDIX Rats or Nude Mice

	BDIX Rats			Nude Mice		
Organ	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Blood	5.5 ± 2.1	4.1 ± 1.6	3.4 ± 1.4	3.0 ± 1.8	2.5 ± 1.3	0.8 ± 0.9
Skin	0.7 ± 0.7	0.8 ± 0.1	0.9 ± 0.2	1.5 ± 0.4	0.7 ± 0.4	0.4 ± 0.4
Muscle	0.4 ± 0.4	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
Liver	1.0 ± 0.4	0.7 ± 0.2	0.6 ± 0.2	1.3 ± 0.6	0.7 ± 0.2	0.3 ± 0.1
Kidney	1.5 ± 0.6	1.0 ± 0.4	0.8 ± 0.5	1.0 ± 0.5	0.6 ± 0.3	0.2 ± 0.2
Colon	0.6 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.9 ± 0.5	0.5 ± 0.1	0.2 ± 0.1
Spleen	1.2 ± 0.4	0.7 ± 0.2	0.6 ± 0.1	2.9 ± 1.6	1.7 ± 0.9	0.8 ± 0.5
Stomach	0.7 ± 0.2	0.7 ± 0.7	0.3 ± 0.1	0.6 ± 0.3	0.3 ± 0.1	0.2 ± 0.2
Heart	1.2 ± 0.4	1.0 ± 0.4	0.8 ± 0.3	0.7 ± 0.3	0.5 ± 0.2	0.2 ± 0.2
Lung	1.9 ± 0.7	1.9 ± 0.9	1.4 ± 0.4	1.2 ± 0.6	0.8 ± 0.4	0.3 ± 0.3
Bone	0.2 ± 0.1	0.2 ± 0.4	0.2 ± 0.8	0.6 ± 0.3	0.5 ± 0.1	0.2 ± 0.1
Brain	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Tumor	1.5 ± 0.6	2.1 ± 0.5	1.7 ± 0.7	3.1 ± 1.5	3.0 ± 1.2	1.0 ± 0.5

± = standard deviation (n = 6).

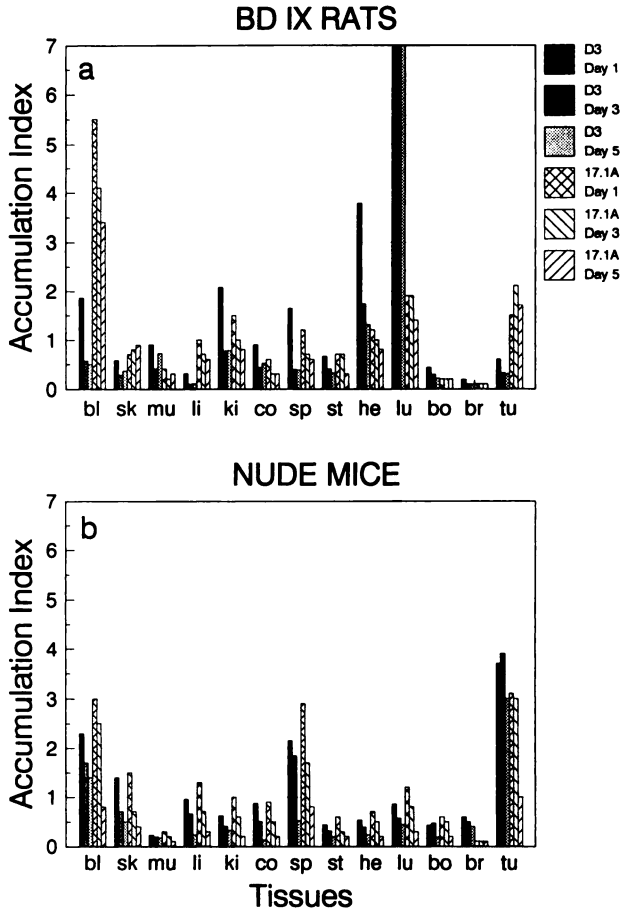

FIGURE 1
Comparison of D3 accumulation indices in tumor-bearing BDIX rats (A) and tumor-bearing nude mice (B). Bars represent the average of the values obtained with three animals injected with experimental antibody and six injected with control at each time point. The s.d. of the pooled AI data from all days was 0.67. Abbreviations: bl = blood; sk = skin; mu = muscle; li = liver; ki = kidney; co = colon; sp = spleen; st = stomach; he = heart; lu = lung; bo = bone; br = brain; and tu = tumor.

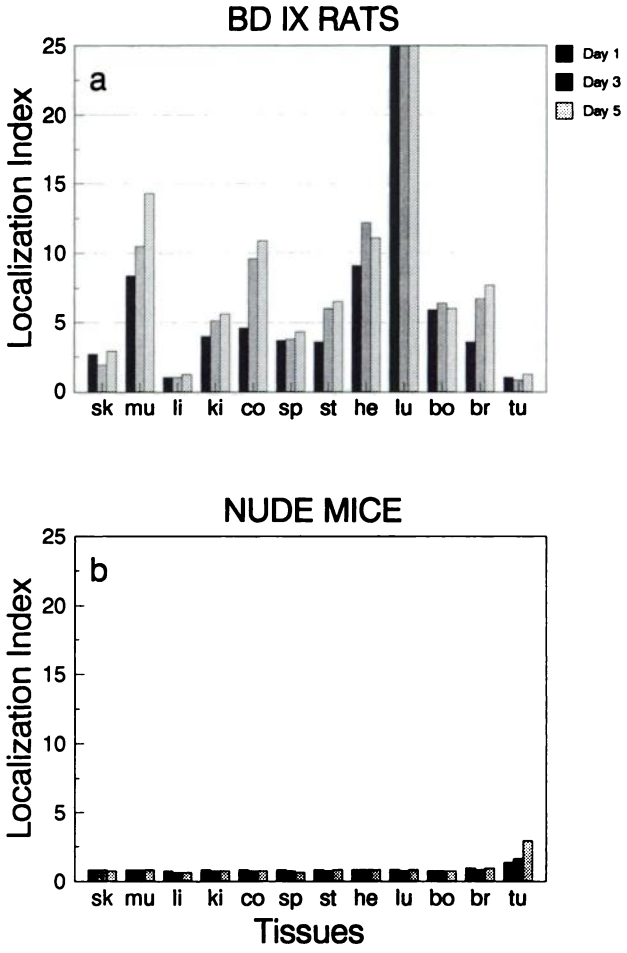

FIGURE 2
Comparison of D3 localization indices in tumor-bearing BDIX rats (A) and tumor-bearing nude mice (B). Bars represent the average of three individual values. The s.d. of the pooled AI data from all days was 1.19. The tissue abbreviations are explained in Figure 1.

3A). These organs from Sprague-Dawley rats also showed high AI values (data not shown).

Several differences in E4 biodistribution were observed between tumor-bearing BDIX rats and nude mice. Over the period studied, AI in nude mice (Fig. 3B) were greater than AI of BDIX rats in blood and spleen ($p < 0.05$), bone, and tumor, and smaller in liver ($p < 0.05$), heart, and lung ($p < 0.05$; Day 3, Day 5). In BDIX rats, the AI of the tumor was the highest value found on Day 1. However, on Days 3 and 5, the lung had greater AI values than the tumor. In contrast, tumor AI in nude mice were the highest throughout the entire period studied ($p < 0.05$).

As in the case of D3 antibody, the differences in the biodistribution of E4 between BDIX rats and nude mice become more prominent when localization indices were examined. In this case, however, only the liver on Days 1 and 3, the colon, stomach, and lung on Days 3 and 5, and the kidney on Day 5 showed greater LI than the tumor. LI values of all rat tissues were >1, indicating a specific uptake of E4 as compared to 17.1A. In nude mice (Fig. 4B), tumor LIs were higher than those of the other tissues on Days 1 and 3, but not on Day 5, where it dropped below 1, reflecting the absence of prolonged targeting of the antibody at the tumor site. For most mouse tissues, LIs were around one or slightly lower, indicating no discrimination between E4 and 17.1A uptake by these tissues. Statistical analysis identical to that applied to D3 showed that mouse and rat tissues differed significantly throughout the study. On Day 1, however, kidney and brain were not different. Bone, brain, kidney, and spleen were not found different on Day 3 and Day 5. Rat tumor LI differed from other rat organs only on Day 1 (except for liver). We conclude that, while greater tumor accumulation occurred using E4 than D3, in neither case was the nude mouse model predictive of the results in the syngeneic model.

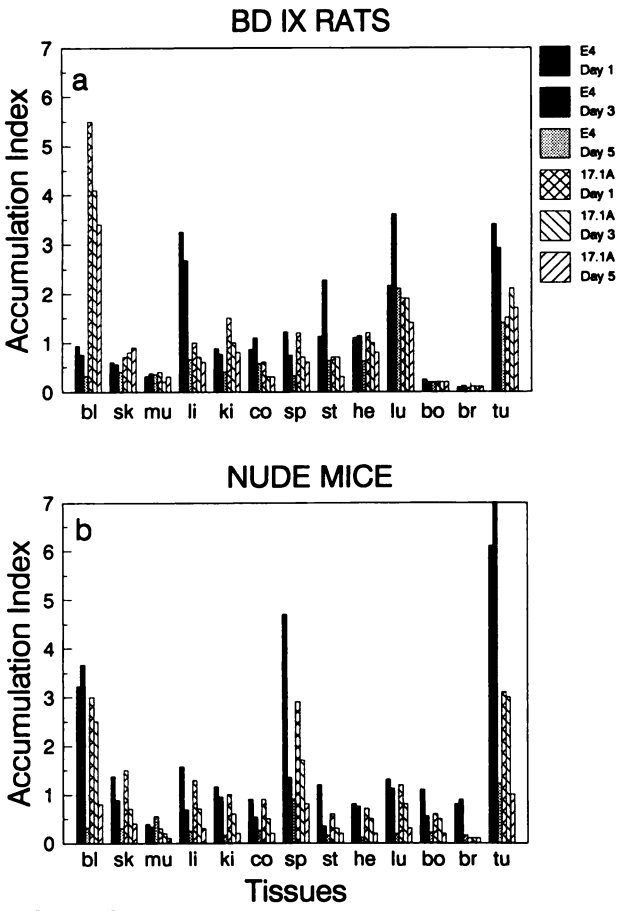

FIGURE 3
Comparison of E4 accumulation indices in tumor-bearing BDIX rats (A) and tumor-bearing nude mice (B). Bars represent the average of the values obtained with three animals injected with experimental antibody and six injected with control at each time point. The s.d. of the pooled AI data from all days was 0.97. The tissue abbreviations are explained in Figure 1.

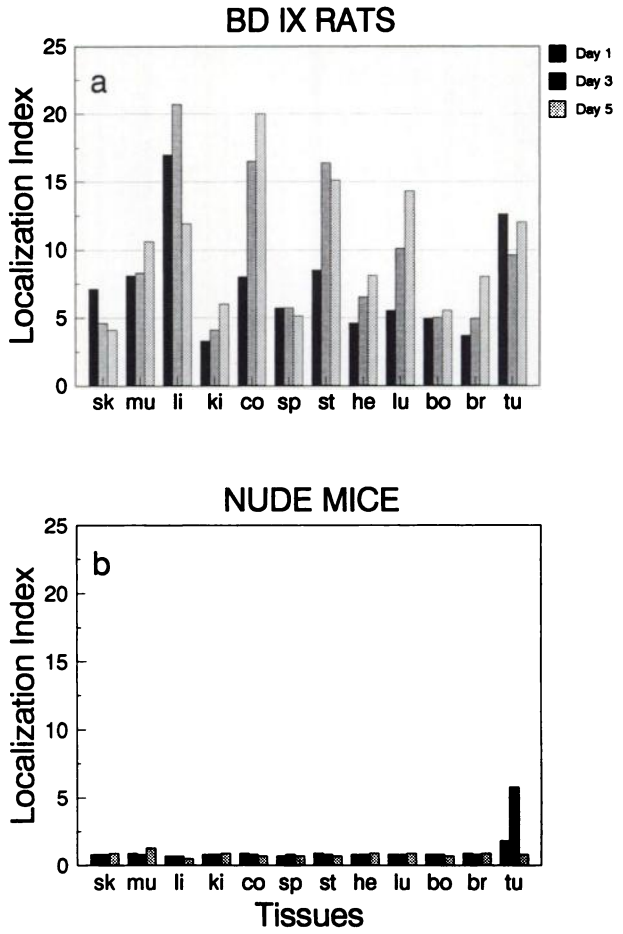

FIGURE 4
Comparison of E4 localization indices in tumor-bearing BDIX rats (A) and tumor-bearing nude mice (B). Data are the average of three individual values. The s.d. of the pooled AI data from all days was 1.47. The tissue abbreviations are explained in Figure 1.

Trichloroacetic Acid Precipitation

Table 2 shows the results of trichloroacetic acid precipitation of several rat and mouse tissues one to five days after the injection of the radiolabeled antibodies. The amount of precipitable radioactivity in the tissues was >90% (lower limit of 95% confidence interval of the mean of 96.8 ± 3.4 of all determinations). This indicates that no catabolites or free iodine was being measured. In the urine, however, the amount of precipitable radioactivity was very small, indicating that the radioactive antibody was eliminated after its catabolism. The only exception was seen in the urine recovered on Day 1 of one BDIX rat injected with E4 and 17.1A, which showed 16.8% precipitable radioactivity for E4 and 15.6% for 17.1A. These results indicate that a considerable amount of antibody is eliminated by urine without total degradation. In addition, somewhat <90% of iodine radioactivity was occasionally found in the stomach at Day 1, probably reflecting metabolism of free iodine through the gastric mucosa.

DISCUSSION

The pharmacokinetic studies reported here point up important considerations for the use of xenogeneic MAbs in a syngeneic host-tumor system. Murine monoclonal antibodies may accumulate in normal tissues, irrespective of their binding to specific antigen. Antibody distribution into various organs may differ between species as a function of a variety of factors, including: body mass, blood flow to various organs, as well as specific antigen targeting.

Two anti-rat colon carcinoma antibodies, each raised against the same tumor cell line, localized identically in rat tumors transplanted in nude mice. When injected into rats bearing the tumor to which the antibodies had been raised, these same two antibodies were taken up by a variety of normal tissues (in slightly different fashion from one another), as well as by the tumor. Similar targeting to normal tissues was also seen in another rat strain, indicating that this binding of xeno-

TABLE 2
Percentage of TCA-Precipitable Radioactivity in Rat or Mouse Tissues After Injection of Iodine-Labeled Monoclonal Antibodies

		D3			E4		17.1A	
Tissue	Normal	BDIX	Nude	Normal	BDIX	Nude	BDIX	Nude
Blood	98.0 ± 1.5	98.7 ± 0.2	97.4 ± 1.5	88.4 ± 4.8	95.2 ± 3.6	99.3 ± 0.3	98.7 ± 1.1	99.1 ± 0.6
Muscle	ND	99.1 ± 0.6	ND	ND	ND	ND	98.6 ± 0.8	ND
Liver	92.4 ± 5.2	95.8 ± 1.3	98.3 ± 0.6	99.2 ± 0.1	99.1 ± 0.2	98.5 ± 0.1	97.8 ± 2.1	98.5 ± 0.1
Kidney	98.2 ± 1.8	98.3 ± 0.2	96.3 ± 2.8	96.3 ± 1.2	94.9 ± 3.0	97.4 ± 1.4	97.0 ± 2.3	97.0 ± 0.6
Colon	97.3 ± 1.8	97.7 ± 1.2	97.9 ± 0.3	98.5 ± 1.5	96.3 ± 2.8	98.3 ± 0.3	99.4 ± 4.3	98.4 ± 0.1
Spleen	ND	97.6 ± 0.6	99.1 ± 0.1	ND	ND	98.7 ± 0.4	98.2 ± 0.3	99.1 ± 0.1
Stomach	93.1 ± 7.7	90.4 ± 1.5	90.9 ± 3.1	97.8 ± 2.5	91.8 ± 5.8	90.4 ± 6.1	89.2 ± 6.7	91.5 ± 1.6
Lung	99.8 ± 0.1	99.8 ± 0.5	96.9 ± 2.3	99.2 ± 0.8	98.4 ± 1.5	98.0 ± 1.1	95.6 ± 3.3	97.9 ± 0.1
Brain	98.0 ± 2.1	ND	ND	99.0 ± 1.0	ND	ND	ND	ND
Urine	4.0 ± 1.3	3.3 ± 0.8	ND	4.8 ± 0.2	9.8 ± 6.0	ND	8.2 ± 6.4	ND
Tumor	—	93.2 ± 0.4	97.0 ± 1.4	—	ND	ND	98.4 ± 0.5	98.5 ± 0.3

ND = not determined.
± = standard deviation.

geneic monoclonal antibodies may be a general phenomenon not limited to this particular strain or model.

The differences found between rat and mouse pharmacokinetics and targeting of the antibodies may be due in part to the expression of tumor or cross-reactive antigens on the normal rat tissues to which the antibodies are able to bind. As a consequence, antibodies are directed to different tissues, depending on antigen expression, accessibility, or tissue distribution of the antigens. In nude mice, the only tissue expressing the antigens to which D3 and E4 react is the transplanted (xenogeneic) tumor; the distribution of both antibodies in normal mouse tissues is identical, and only tumor is specifically targeted. However, these results in nude mice do not allow one to conclude that murine MAbs similar to D3 and E4 have a tumor specificity appropriate for use in rats or, moreover, in a less evolutionarily related syngeneic system.

The LI is generally used to determine the specificity of antibody targeting in vivo (*16*); LI >1 indicates such specificity. Most rat tissues show localization indices >1 for both antibodies (the exceptions are tumor and liver in the case of D3). This may indicate real specific targeting of the antibodies to virtually all rat tissues. However, other factors may also account for LI values higher than 1. The LI actually corresponds to the division of tissue/blood ratio for specific antibody by the same ratio for the nonspecific antibody. The utility of this index is based on the assumption that the general distribution of an injected antibody is only dependent on its blood concentration. In our case, the presence of cross-reactive antigens in some rat tissues results in a decrease of D3 and E4 concentration in blood, unlike that which occurs for 17.1A. This decrease may happen more quickly than the decline in the concentration of these antibodies from the extravascular and extracellular space. As a consequence, differences in blood levels of specific and nonspecific antibodies will artificially increase LI values in some organs, even those binding very few tumor-directed antibody molecules. For example, brain tissue, which would not be expected to interact with injected D3 or E4, and did not retain these antibodies in excess of the nonspecific antibody in vivo (AI were similar), showed LI values clearly above 1 for both antibodies. It is likely that only for LI values much higher than those observed for the brain does the LI truly indicate specific targeting. In several rat tissues (muscle, colon, heart, lung for D3, and muscle, liver, colon, stomach, and lung for E4), the extremely elevated LIs clearly indicate a specific targeting of the respective antibodies to these tissues. Data in the nude mouse showed similar biodistribution of both specific and control antibodies in blood and other normal tissues, except the engrafted rat tumor. Therefore, LI values of around 1 were found for all normal tissues except tumor. In this case, use of LI >1 is meaningful and useful because no antigenic competition between tumor and other tissues occurs.

Previous studies (*15*) have shown that D3 antibody reacts strongly in vitro with a variety of normal rat tissues, including stomach, small intestine, and colon epitheliums; and, less strongly, with the bronchial epithelium and alveolar walls, bladder, myocardium, and skeletal muscle. Therefore, to a certain extent, the biodistribution data might not be unanticipated. E4, on the other hand, gave an intense histochemical reaction only with primary or metastatic tumor, although it also reacts faintly with stomach, small intestine, colon and lung. Other tissues are completely negative with respect to E4 binding. In this case, not dissimilar from a number of anti-colon carcinoma antibodies in investigational studies (proprietary information submitted to the

FDA in Investigation New Drug applications), the biodistribution data would not have been predicted. The comparison of these results with the biodistribution of D3 and E4 in vivo indicates that no clear correlation can be established between in vitro reactivity and in vivo targeting of these antibodies. For example, lung stains in vitro with D3, and takes up the antibody in vivo. However, differences between the intensity of in vitro staining and the degree of in vivo uptake were noted. Skeletal muscle and myocardium stained slightly for D3, but, after the lung, were the tissues most targeted. In the case of E4, liver, which was negative in vitro, retained as much antibody as did lung. Differences in vascularization resulting in enhanced accessibility of circulating antibodies to antigenic sites displayed on the surface of the tissue cells and metabolic fate of the antibodies may account for these differences, as suggested by Ballou (*17*). Experiments using $F(ab')_2$ fragments showed similar tissue distribution to that of the whole antibodies (data not shown), ruling out Fc receptor binding as an explanation.

Our results indicate that conclusions drawn from a xenogeneic model regarding the biodistribution of tumor-specific murine MAbs do not necessarily apply to syngeneic models, particularly when animals of differing size are compared. The situation found in our rat model may be more comparable to that of patients with colorectal carcinoma who have been injected with MAbs that have been selected by using the nude mouse model. Similarly, in patients with neuroblastomas or gliomas, Jones (*18*) has reported major differences with the findings in nude mice regarding quantitative antibody targeting, half-life, and blood values, as well as vascularization and accessibility. He concluded that the mouse xenograft model had actually very little similarity with the data obtained in humans. Our results also strongly support the idea that other animal models, more appropriate to each clinical situation and tumor type, are needed in order to obtain clinically pertinent information to the selection of monoclonal antibodies as candidates for imaging and therapy in humans.

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