In Vivo Kinetics of Radiolabeled Monoclonal Anti-CEA Antibodies in Animal Models

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Studies were performed to determine the effect of the radiolabel and circulating carcinoembryonic antigen (CEA) on the pharmacodynamics of monoclonal anti-CEA antibodies (MoAbs). The studies were performed in normal BALB/c mice and in nude mice bearing human colon tumors. Three different tumors were used, each of which produced CEA levels characteristic of that particular tumor's secretory rate. The CEJ-326 MoAb labeled with either ^{111}In or ^{125}I was used in all studies. Circulating CEA induced the removal of ^{125}I and ^{111}In MoAbs from the vascular compartment. Liver concentrations of ^{111}In increased and ^{125}I levels decreased as the CEA secretory rate of the tumor rose. This indicates that circulating CEA complexes form in the vascular compartment which, in an animal model, are removed by the liver and spleen. This results in decreased tumor uptake of the labeled MoAb. The iodinated MoAb complexes are dehalogenated while the ^{111}In is retained by the liver. This dehalogenation may account for the relatively low liver activity observed in radioimmunoimaging with intact radioiodinated anti-CEA MoAbs, provided the CEA complexes are similarly removed from the vascular compartment by the human liver.

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A variety of tumors have been shown to produce antigens (*1–4*). Some of these antigens remain bound to the cells (*1,2*). Others, such as carcinoembryonic antigen (CEA) and prostate specific antigen (*3,4*) leave the tumor and circulate in the blood. Polyclonal and monoclonal antibodies (MoAb) have been raised against both bound and secreted antigen, radiolabeled and used to detect cancer in patients.

When circulating antigen is present, a complicating variable is added to the radioimmunoimaging equation, namely, intravascular formation of antigen:antibody complexes. As the tumor burden of the host increases, the absolute levels of CEA in the serum rises (*5*). Theoretically, immune complex formation should also rise following administration of anti-CEA antibodies and, in fact, immune complexes of CEA have been shown to form in patients with high serum CEA levels. Such complexes should be removed by the liver (*6*), and to a lesser extent, by other organs. It is frequently denied, however, that such complexes alter the pharmacokinetics of the antibody or radioimmunodetection of the tumor (*7*).

The use of radioiodine as the tracer could add to the complexity of interpretation of in vivo data if the antigen circulates. In vivo dehalogenation has been described multiple times and is probably variable in magnitude depending on the antibody, its metabolic breakdown, and the method of iodination (*8–11*). The use of the indium-111 (^{111}In) label (*12*) confers greater stability on the antibody-radionuclide complex (*13*) and is probably less likely than iodine to leave a tissue once metabolism of the MoAb has

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occurred. The use of ^{111}In as the tracer could, therefore, be a useful adjunct for studies of circulating antigen.

Using the ^{111}In and iodine-125 (^{125}I) labels as a tool, we wish to (a) establish in a preliminary study the rate of egress of ^{125}I and ^{111}In from a CEA producing tumor; (b) establish the effect of circulating CEA on the pharmacodynamics of ^{111}In-labeled anti-CEA MoAb in the absence of the tumor; (c) determine the effect of circulating CEA, produced by a human tumor in a nude mouse model, on the pharmacokinetics of ^{125}I- and ^{111}In-labeled anti-CEA MoAb.

MATERIALS AND METHODS

Antibodies

The anti-CEA MoAb used in this experiment was a murine IgG_1 raised against a CEA producing colon tumor, and designated CEJ-326. It was developed by the standard hybridoma technique and purified from ascites fluid by DEAE chromatography. It has an affinity of $>10^9$ mole per liter for CEA (*14*), and is ~70% immunoreactive as measured by the double antibody technique (*15*).

Radiopharmaceuticals

The ^{111}In used in these experiments was obtained commercially.* The antibodies were labeled with the [^{111}In]chloride by a modification of the bifunctional chelation technique described by Krejcarek and Tucker (*12*). In this technique diethylenetriaminepentaacetic acid (DTPA) is reacted with five equivalents of triethylamine and the reaction product lyophilized. It is then dissolved in dry acetonitrile. When this compound reacts with isobutylchloroformate, an anhydride of DPTA is formed. This anhydride then reacts with amine groups on the MoAb resulting in conjugation of the DPTA to the antibody. Large amounts of free DPTA are produced in this reaction which can be separated from the conjugated material by a Sephadex G-75 column.

In the labeling step, ^{111}In is buffered in 0.13*M* citrate to pH 4.0 and reacted for 80 min with the conjugated antibody which has been mixed with equimolar amounts of human serum albumin. This preparation is again purified on a Sephadex G-75 column. The fraction eluting with the void volume contains the [^{111}In]MoAb.

All of the iodinated antibody used in this study was prepared using the lactoperoxidase technique. The immunoreactivities of antibodies labeled by this method were identical with those achieved using the ^{111}In technique (70%).

Animal models

The BALB/c mice used in these experiments were obtained commercially. †The nude mice were obtained from a nude mouse facility‡ as were all of the tumors transplanted into the nude mice.§ All of the CEA-producing tumors were of colon carcinoma origin and implanted by the mince-trochar technique. The colon tumor models used in this series of studies all grew to 1g within 6 wk. The secretory rates of the T-379, T-84, and T-157 tumors were defined by the same technique used to study the T-380 CEA-secreting tumor (*16*). The three tumors secreted ~20, 44, and 1,000 ng of CEA/g of tumor/hr, respectively. Each had at least 50 μg of CEA/g of tumor. Three large T-379, T-84, and T-157 tumors produced serum CEA levels of 7.5, 115, and 4,700 ng/ml, respectively, when measured by the Abbott Kit.

All of the animals used in these experiments were fed food and water ad libitum.

Experiment 1—Change in radiopharmaceutical concentration in the tumor with time

The change in tumor concentration of [^{125}I]- and [^{111}In]-MoAb as a function of time was studied by administering the two radiopharmaceuticals simultaneously (1 μg, 5 and 15 μCi of each, respectively) to mice bearing T-379 tumors of nearly identical size (0.51–0.63 g). The mice were killed over a 120-hr time period by cervical dislocation. Multiple organs were taken, washed twice in water, blotted dry, and the solid tissues wet-weighed on an analytical balance followed by counting in a well counter along with a standard of the injected materials. The data were processed as %dose/g and %dose/organ. The total quantity of radioactivity in the blood was established by estimating the blood volume to be 7% of the weight of the mouse with the tumor present.

Immune complex experiments: Normal mice

Prior to doing experiments involving immune complex formation in tumor-bearing animals, it was necessary to perform experiments in normal mice to insure that the presence of the tumor itself did not alter the formation or pharmacokinetics of immune complexes. In the first experiment, aliquots of human serum containing 120 ng of CEA was administered i.v. by way of the tail vein to BALB/c mice. The serum was obtained from a colon cancer patient with CEA levels of 1,200 ng/ml as assayed by a double antibody assay. Human serum-derived CEA was previously shown to have a vascular half-time of 2.5 hr in normal BALB/c mice and to disappear in a single exponential manner (*16*). The injection of the serum was immediately followed by i.v. injection of 120 ng of [^{111}In]MoAb. A second group of BALB/c mice received 100 λ of the serum which had been previously incubated for two hr with 120 ng of [^{111}In]MoAb. A third group of mice received 120 ng of [^{111}In]anti-CEA MoAb as a control.

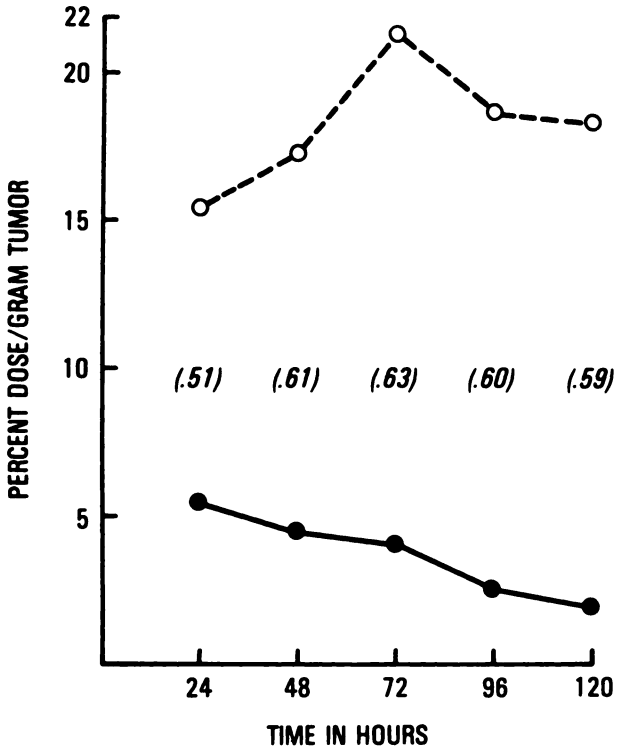

FIGURE 1
Effect of time on tumor uptake of [^{111}In]- and [^{125}I]MoAb when tumor size and secretory rate are constant. Tumor concentrations of [^{111}In]- and [^{125}I]MoAb in tumors of similar size observed over period of 120 hr. Note steady egress of ^{125}I from tumor while ^{111}In radiopharmaceutical peaks at 72 hr then decreases slightly. ( ) Tumor wt. in g; (○—○) [^{111}In]MoAb; (●—●) [^{125}I]MoAb; T-379 tumor; Sec rate < 20 ng/ g/hr

In a second BALB/c mouse experiment, 100 ng of [^{111}In]MoAb was administered i.v. by way of the tail vein followed by five successive tail vein injections of serum containing 100 ng of CEA. The injections were delivered hourly. A second group of mice received 100 ng of [^{111}In]-MoAb as a control. All mice were killed 24 hr after the radiopharmaceutical administration. Multiple organs were taken, and processed as previously described.

Tumor-bearing mice

The effect of endogenously produced CEA on the kinetics of [^{111}In]anti-CEA MoAb was studied by administering 1 μg of MoAb (0.5 μg [^{111}In]MoAb and 0.5 μg [^{125}I] MoAb) containing 1–10 μCi of tracer to nude mice bearing either the T-379, T-84, or T-157 tumors. The tumors varied in their secretory rates, and therefore in the absolute levels of circulating CEA. A range of tumor burden occurred for each model which further varied with serum CEA levels since the quantity of CEA produced is directly proportioned to tumor mass (*16*). Following the injection of the radiopharmaceuticals, the mice were housed in cages in which the bedding could be collected. The feces was separated from the urine-soaked bedding, and the excretion of ^{111}In and ^{125}I in urine and feces calculated. The mice were killed at 48 hr, multiple organs taken, and processed as previously described. Cross-channel corrections were used in the counting procedure.

RESULTS

Figure 1 illustrates the concentrations of ^{111}In and ^{125}I in the T-379 tumor over a time period ranging from 4 to 120 hr. Peak tumor levels of ^{125}I are achieved at (or before) 24 hr postadministration with a steady decrease in the count rate through 120 hr. Indium-111 levels peak at 72 hr followed by only a slight decrease in ^{111}In counts over the later time points.

Figure 2 demonstrates that exogenous CEA administered within a few minutes of [^{111}In]anti-CEA MoAb results in a fall in blood levels, and a rise in liver and spleen concentrations of ^{111}In. When the ^{111}In anti-CEA MoAb is incubated prior to its administration with human serum containing CEA, the results are even more dramatic than when the antigen and [^{111}In]anti-CEA MoAb are injected separately. When multiple administrations of CEA occur following a single injection of [^{111}In]anti-CEA MoAb, the overall decrease in blood levels, and rise in liver and spleen concentrations of ^{111}In exceed those of the single injection experiments and approximate the changes induced by preincubation of the radiopharmaceutical.

Figure 3 shows the results of administering [^{111}In]anti-CEA MoAb to mice bearing a T-379 tumor. Only very small amounts of CEA are produced when the tumors are less than a gram in size. With tumors as large as 2 g, some

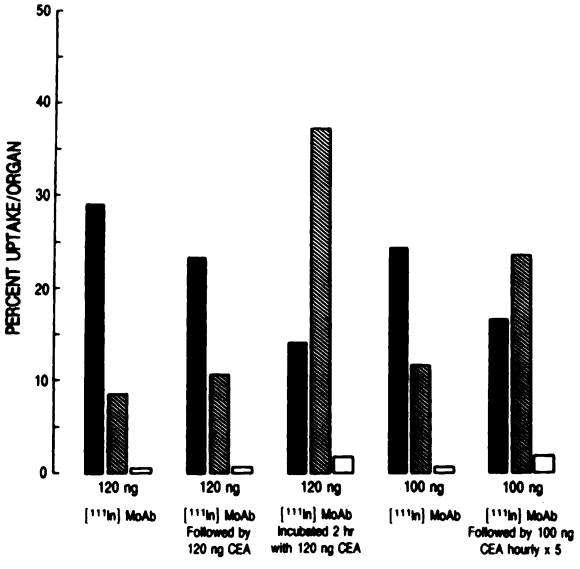

FIGURE 2
Administration of exogenous CEA to BALB/c mice followed by [^{111}In]anti-CEA MoAb. Compare this with distribution of in vitro formed [^{111}In]anti-CEA complexes. (■) Blood; (▨) Liver; (□) Spleen; Sac time–24 hr

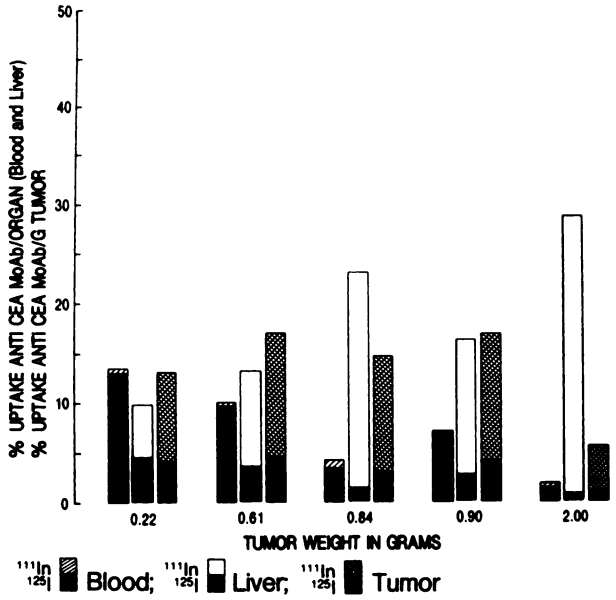

FIGURE 3
Effect of tumor size and secretory rate on [^{111}In]- and [^{125}I] MoAb distribution. Simultaneous administration of ^{125}I and [^{111}In]anti-CEA antibody in tumor model which produces moderate amounts of CEA. Note divergence of ^{125}I and ^{111}In in all tissues. Colon tumor–T-379; Sec rate–20 ng/g/hr; Sac time–48 hr; Quant.–[^{111}In]- and [^{125}I] MoAb admin. 1μg; MoAb-CEJ-326; Urinary ex^{125}In–43% (group)

CEA will be secreted. Blood levels of ^{111}In and ^{125}I dropped progressively and synchronously as the tumors enlarged. The liver concentrations of the two isotopes diverge, however, in the largest tumors with the ^{111}In liver concentration rising, and the ^{125}I concentration decreasing. The concentration of the two isotopes in the tumor is not affected until the 2-g level is reached at which point the count rate decreases for both to about the same extent. Forty-three percent of the ^{125}I, and 5% of the ^{111}In were excreted in the urine. Fecal excretion of ^{125}I was 5% compared with 7% for the ^{111}In.

Figure 4 shows the results of the same experiment in mice bearing the T-84 tumor which has a secretory rate of ~ three times that of the T-379. Blood levels of ^{125} and ^{111}In no longer change progressively with tumor size (and presumably serum CEA levels); however, the quantity of ^{125}I in the blood is only half that of ^{111}In. Liver concentration of ^{111}In exceeds 30% of the injected dose even with tumors as small as 0.6 g. This does not change greatly in nude mice with larger tumors. The ^{125}I concentrations in the liver never exceed 5% of the dose, and it is as low as 0.5% of the injected dose in the largest tumors. Uptake of both tracers in the tumor is higher when the tumors are small, with ^{111}In exceeding ^{125}I. The larger tumors concentrate less of the radiopharmaceutical on both a per gram and absolute basis. In the 1.2 g tumors, the ^{125}I and ^{111}In levels are approximately the same. Urine and feces excretion of ^{125}I are 49 and 6%, respectively; compared with 5 and 6% for ^{111}In, respectively.

Figure 5 shows the above experiment repeated in a tumor model known to produce extraordinary serum CEA levels. Again, the most striking finding is the high ^{111}In and low ^{125}I concentrations in the liver. The mouse with the largest tumor actually has over 60% of the injected dose of the ^{111}In in the liver with 1.6% of the dose of ^{125}I. Urine and fecal excretion for ^{125}I are 59 and 9%, respectively as opposed to 8 and 5% for ^{111}In.

The tracer in the blood shows a divergence between the ^{111}In and the ^{125}I blood levels. At all tumor weights the concentration of ^{111}In in the tumor far exceeds that of ^{125}I. Both decrease progressively as the tumors enlarge. Overall, the tumor uptake of both radiopharmaceuticals in the T-157 tumors is less than noted for the T-379 or T-84 tumors of comparable size.

DISCUSSION

These data are compatible with long held concepts of classical immunology. They also indicate that unrecognized problems may exist in the anti-CEA radioimmunoimaging data published in the literature (*7*).

There is little doubt that radioiodine does leave the tumor when radioiodine is the label for the MoAb. The loss is slow but inexorable in tumors of nearly identical

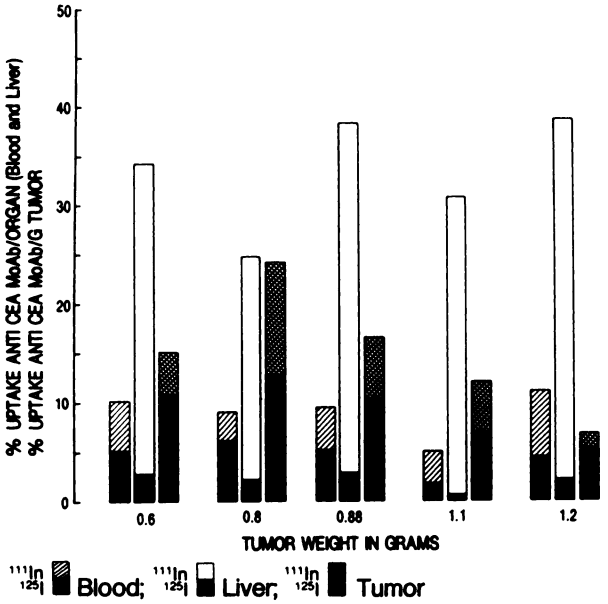

FIGURE 4
Effect of tumor size and secretory rate on [^{111}In]- and [^{125}I] MoAb distribution. Administration of [^{125}I] and [^{111}In]anti-CEA MoAbs into mice bearing tumor with low CEA secretory rate. Blood levels of both isotopes fall synchronously with divergence of ^{125}I and ^{111}In in liver and tumor as serum concentration of CEA rises. Colon tumor T-84; sec rate–44 ng/g/hr; Sac time–48 hr postinjection; Quant. [^{111}In]- and [^{125}I]MoAb admin.–1μg; MoAb 3-CEJ-326; Urinary ex–^{125}I–49% (group)

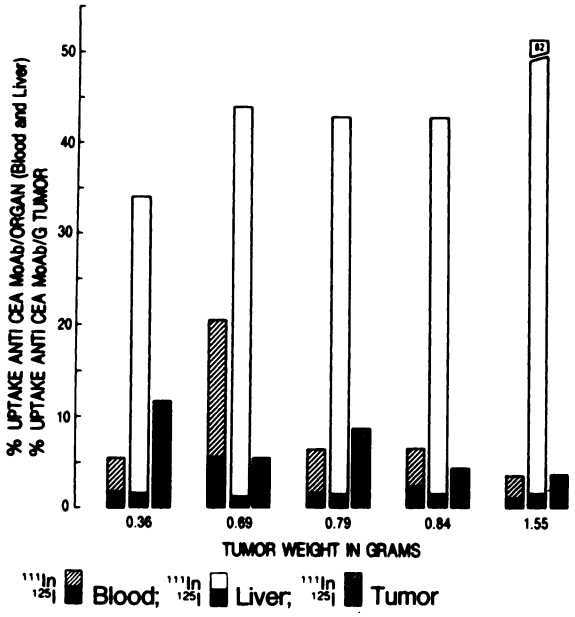

FIGURE 5
Effect of tumor size and secretory rate on [^{111}In]- and [^{125}I] MoAb distribution. Distribution of [^{125}I] and [^{111}In]anti-CEA antibody in tumor model system that produces extraordinary amounts of CEA. Compare with Figs. 3 and 4. Colon tumor T-157; Sec rate - 1,000 ng/g/hr; Sac time–48 hr postinjection; Quant. [^{111}In]- and [^{125}I] MoAb admin.–1μg; Urinary ex. ^{125}I–59% (group)

size. The process of dehalogenation appears to be minimal during the first 8 hr following injection, even in the liver, an organ that we have found to be one of the most aggressive tissues in this regard (*9*). By 24 hr, however, dehalogenation is well underway as indicated by the difference in the ^{111}In and ^{125}I concentrations in Fig. 1. Dehalogenation has been seen to vary with the iodination technique (*8–11*), and could well be different from one antibody to the next, depending on the steric arrangement of the tyrosine groups on the molecules and whether or not the MoAb is internalized by cells. The dehalogenation of immune complexes may be faster than that which occurs with uncomplexed iodinated MoAb as indicated in Figs. 3, 4, and 5, possibly because they are internalized by various hepatic cells. Assuming that the concentrations of ^{111}In are a correct indicator of the amount of labeled antibody and labeled antigen: antibody complex acquired by the liver in 48 hr, massive dehalogenation (from whatever cause) would have had to taken place to produce the ^{125}I liver levels that were seen in the T-157 tumor model. In normal BALB/c mouse studies in which both ^{111}In and ^{125}I MoAb were administered simultaneously (data not shown) the 48-hr liver data indicated that ^{125}I levels were ~50% those of the ^{111}In concentration. This is also the case in the animal bearing the very small tumor shown in Fig. 3. This further suggests that the dehalogenation process in the liver occurs more quickly if immune complexes form than in their absence.

The presence of the tumor itself, other than as a source of circulating antigen appears to have no effect on the liver acquisition of the antigen:antibody complex as indicated in Fig. 2. The complexes are removed by both the liver and the spleen with a concomitant decrease in blood values. It should be remembered that these changes have taken place in 24 hr indicating that the complexes are being removed at a fairly rapid rate.

Circulating antigen induces a definite decrease in both [^{111}In]- and [^{125}I]MoAb acquisition by tumor in all the models studied. The tumor concentrations, however, are always highest for the ^{111}In by a factor of 2–5. The decrease in tumor uptake of the tracers was most dramatic in the T-157 tumor which produced very high serum CEA values.

The tumor/blood ratios are similar overall for ^{111}In and ^{125}I. This could account for the fact that liver metastases are sometimes seen in patients when [^{131}I]MoAb is used as the radiopharmaceutical, but are rarely seen with [^{111}In]MoAb (*17*). It might also account for the observations in the literature stating that elevated serum CEA does not constitute a problem in imaging. Our mouse data indicate that elevated serum CEA should result in the formation of MoAb:CEA complexes. The liver levels of radioisotope should rise as these immune complexes form and are cleared by this organ. One possible reason that this is not observed when radioiodine is used as the label for the MoAb is that rapid hepatic dehalogenation may occur with egress of the radioiodine from the liver. Such radioiodine would be rapidly excreted by the kidney. In the case of ^{111}In, the problems of complex formation are obvious with the liver becoming very radioactive since the metal ion leaves the tissue slowly.

The interpretation of these mouse data vis a vis the distribution and handling of CEA immune complexes in humans is very difficult. Our work (Hagan PL, et al., data unpublished) in humans using a [^{111}In]anti-CEA MoAb has so far indicated that immune complexes are indeed formed, and in cases where the serum levels of CEA are very high, as much as 100% of the serum radioactivity can exist as immune complex. To our surprise, they were not acquired by the liver, but continued to circulate. In one of the cases the primary lesion was not detected. Of further interest is the fact that when antimouse antibodies have formed following the administration of an IgG MoAb, a second administration of [^{111}In]MoAb complexed with the antimouse antibody and the radioactivity was immediately removed by the liver. The CEA immune complexes formed with MoAbs are theoretically much smaller than can be formed with polyclonal antibodies (the antimouse antibody complexes would be polyclonal) and small complexes may circulate longer than large complexes in the human. This may not be the case in the mouse. In every species, however, one would expect CEA:MoAb complexes to form if CEA was in the vascu-

lar compartment. This in turn could reduce tumor uptake by making it more difficult for the larger molecule to cross the tumor capillary (*18*) or by binding the variable regions, thus making them inaccessible for interaction with CEA at the surface of the tumor cell. Data in the nude mouse-T-380 colon tumor model indicates a dramatic decrease in tumor uptake of [^{111}In]anti-CEA if the [^{111}In]anti-CEA MoAb is administered as preformed immune complexes (Hagen PL, unpublished data).

In conclusion, this set of animal experiments indicates that circulating CEA has a dramatic effect on the kinetics of anti-CEA MoAb with the complexed radiopharmaceutical being rapidly removed by the mouse liver. The ^{125}I label is quickly removed under these circumstances making it appear that little of the complexes are removed by the liver, while the ^{111}In label indicates that the opposite is true. Dehalogenation of the MoAb occurs in other tissues as well, with a decrease in tumor concentration of ^{125}I being a steady process. These data indicate again the ease with which the radioiodine label may be lost from a tissue and further indicates the difficulties in interpretation of the tracer technique under those circumstances.

The animal data suggest that radioimmunoimaging with intact antibodies may not work optimally when the antigen circulates, especially if it does so in high concentrations. Even if the complex is not removed by the human liver with the same efficiency as it is in the mouse, the tumor uptake of the complex may be retarded with a resultant decrease in tumor acquisition of the radiopharmaceutical. Systems where the antigen does not circulate may ultimately prove to be the most appropriate for radioimmunoimaging and radioimmunotherapy.

FOOTNOTES

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