Tumor Size: Effect on Monoclonal Antibody Uptake in Tumor Models

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Studies were performed to determine the effect of tumor size on the incorporation of radiolabeled monoclonal antitumor antibodies (MoAbs) into human tumors growing in nude mice. The colon tumors ranged in size from 0.03–1.6 g, the melanoma from 0.1 to 6.7 g, and the lymphoma from 0.06 to 10.2 g. Indium-111 was primarily used as the radiolabel, however, both ^{125}I and ^{111}In were used as tracers for the MoAb in one experiment. The per g radiopharmaceutical uptake by tumors was inversely proportional to tumor size when tumor specific MoAb was administered. This finding was independent of the radiolabel and was demonstrable when the mice bore two tumors of differing size. When the MoAb was not specific for the tumor, the data were less well defined and a statistically significant correlation with size did not occur. These data are strong evidence for a decrease in per g uptake of labeled tumor specific antibodies as tumors increase in size.

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The pharmacokinetics and tumor uptake of radiolabeled antibodies are controversial. For example, tumor growth was reported by Baldwin and Pimm to be accompanied by a linear uptake of iodine-125- (^{125}I) labeled monoclonal antibody (MoAb) in a murine-human tumor system (*1*). The antigen in that model has not been detected in the circulation. Menard et al. (*2*), however, reported that enlargement of tumors was accompanied by a measurable decrease in tumor concentration of iodine-131 (^{131}I) MoAb. Here the model was a murine lymphoma which produced a circulating antigen. Epenetos et al. (*3*) reported that nonspecific antibody uptake varied directly with the tumor size but was diminished by necrosis. Finally, Moshakis et al. (*4*) reported an inverse relationship between tumor size and MoAb uptake.

To add to the difficulty in interpreting the literature, the studies indicated above were performed with radioiodinated antibodies. This tracer-antibody linkage is known to be unstable in vivo (*5–7*), and could have had an effect on the experiments.

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Recently, we have reported a stable ^{111}In-labeled MoAb preparation ([^{111}In] MoAb), and proved it to have pharmacokinetics nearly identical to endogenously labeled MoAb (*8*). Using this as a tool, we wish to address the question of how tumor size effects the tumor concentration of radiolabeled MoAbs in the nude mouse model.

MATERIALS AND METHODS

Antibodies

The anti-carcinoembryonic antigen (CEA) MoAb is a murine IgG_1 raised against a CEA producing colon tumor (*8*) 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/l for CEA, and is ~70% immunoreactive as measured by a double antibody tandem method (*9*). This same MoAb was endogenously labeled with selenium-75 (^{75}Se) using a technique described earlier (*8*). Its affinity and immunoreactivity were the same as the ^{111}In-labeled MoAb.

The 96.5 anti-melanoma MoAb is a murine IgG_{2a} which targets a 97 kdalton glycoprotein on the surface of the melanoma cell (*10*). Its affinity is $>10^9$ mole/l, and the immunoreactivity of various preparations has ranged from 35–55%.

Immunoreactivity was measured by determining the maximum percentage of radiolabeled antibody which could bind to a solid phase antigen. Basically, the antigen from a SKMEL cell line is immobilized on a nitrocellulose disk. All nonspecific binding sites on the disk are blocked and 1 Ng of radiolabeled MoAb is incubated with the disk at 4°C, overnight. The disk is then counted, washed with PBS-Tween 20, then recounted and the percent binding calculated.

The 9.2.27 anti-melanoma MoAb is an IgG_{2a} which targets a 200-240 kdalton glycoprotein on the surface of the melanoma cell (*11*). It has an immunoreactivity of 80% as measured by the method described for 96.5.

The T-101 MoAb used with the lymphoma model is an IgG_{2a} and was derived by immunizing mice with the 8402 T-cell leukemia cell line, followed by hybridization in the standard manner. The T-101 MoAb targets a 65 kdalton antigen (*12*) on the surface of the T cell. T-101 is 90% immunoreactive as determined by the double antibody assay. Its affinity is $\sim 10^9$ mole/l.

The PSA-399 MoAb is an IgG_1 derived by immunizing mice with purified prostate specific antigen followed by hybridization in the standard manner. Although PSA-399 has been proven to circulate, it is not specific for any antigen on the lymphoma tumor used in this series of experiments and represents a nonspecific control.

Radiopharmaceuticals

The ^{111}In used in these experiments was obtained commercially.* The antibodies were labeled with the [^{111}In]chloride by the bifunctional chelation technique described by Krejcarek and Tucker (*13*) as modified by our group (*14*).

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%).

All statistical evaluations in this manuscript were performed using the two-tailed Student's t method.

Animal Models

The BALB/c mice used in these experiments were obtained commercially†. The nude mice were obtained from a nude mouse facility‡ athymic mouse facility§. The tumors transplanted into the nude mice were also obtained originally from a nude mouse facility§.

The CEA-producing tumor was of colon carcinoma origin, designated T-380, and implanted by the mincetrochar technique. The T-380 grew to 1 g within 3 wk and was, in general, less necrotic than the melanoma tumors to be described below. Previous studies have defined the rate at which it secretes CEA (*15*). The T-380 tumor has a CEA secretory rate of 14 ng/g of tumor/hr. It remains highly viable until it is large. The circulating levels of CEA remain near 0 in T-380 until the tumor exceeds lg (*15*). The CEA concentration in this tumor is ~ 20 μg/g, and is abundant in lesions as small as 100 mg.

The source of the melanoma tumor was a metastatic lesion from a patient. The presence of both the 97 kdalton antigen and 240 kdalton antigen was confirmed using the immunoperoxidase technique. This tumor grew at a rate which produced a l-g mass in ~2-3 wk. After a l-g size is reached, the growth rate is rapid with tumors of 2-5 g produced in another week. Necrosis is common in the tumor as it becomes large, and has frequently been seen in tumors <0.5 g.

The T-cell lymphoma model was established using the method of Ziegler et al. (*16*). Five-week-old nude mice were irradiated with 200 rad of total-body external beam radiation weekly for three consecutive weeks. One week after cessation of the radiation 1×10^7 MOLT-4 cells derived from a patient with T-cell lymphoma and bearing the T-65 antigen on their membrane surface, were administered subcutaneously together with HT-1080 fibrosarcoma cells. Tumors formed in 80% of the animals and achieved a noticeable tumor mass in 1 wk. Growth following this was rapid and some very large tumors were present within 3 wk. The tumor continued to express the T-65 antigen on histological staining. The fibrosarcoma cells disappeared from the tumor by the time it reached the size used in these experiments. It is characteristic of this tumor that despite its rapid growth, it remains mostly viable, even at large size.

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

Experiments

Experiment 1. The effect of tumor size on the uptake of endogenously and exogenously labeled anti-CEA in the CEA producing nude mouse—T-380 colon tumor model.

Nude mice bearing T-380 tumors ranging in weight from 0.47-1.2g were administered 1 μg of [^{111}In]MoAb (~10 μCi/μg) i.v. using a Hamilton syringe. All of the animals were killed 72-hr postinjection. Tumor tissue, liver, spleen, and blood were taken, and the solid tissues washed twice in water, blotted dry, wet-weighed on an analytical balance and counted in an auto gamma well counter. A standard of the injected material was also counted and used to quantitate the uptake of the radiopharmaceuticals. The data were processed as % uptake/g of T-380 tumor, and % uptake in the whole T-380 tumor.

The second study in this model utilized the CEJ-326 MoAb which had been endogenously labeled with ^{75}Se (*8*). The tumors varied in weight from 0.03 to 1.6 g. The mice received 0.4 μCi of ^{75}/Se-CEJ 326 associated with 1 μg of protein, i.v., and were killed at 24, 48, and 72 hr. The rest of the protocol was as indicated in the above study.

Experiment 2. A melanoma model was used for studies similar to the one above. It is different from the colon tumor model because it represents a tumor system in which two monoclonal antibodies were available that targeted two different noncirculating antigens on the cell surface of the tumor. Three studies were performed in the melanoma model.

In the first of these, 2 μg (~15 μCi) of 9.2.27 [^{111}In]MoAb were administered i.v. in 100 λ of solution, and the mice killed in groups of six at 4, 24, 48, 96, and 144 hr after injection. The tissues were processed as indicated for the T-380 experiment.

In the second melanoma study, ~l μg (1-10 μCi) each of ^{125}I and ^{111}In 96.5 MoAb was administered i.v. simultaneously (mixed in the same syringe) to six nude mice bearing the melanoma tumor. The mice were killed at 24 hr and the tissues processed as previously indicated with the exception that cross channel corrections were performed.

In the third study, ten nude mice were transplanted with

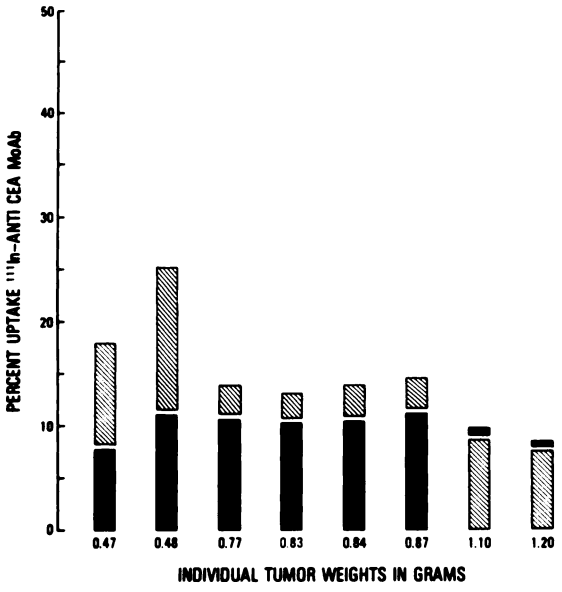

FIGURE 1
^{111}In anti-CEA antibody incorporation into T-380 tumor as function of tumor size. (▨) % injected dose/g of tumor; (■) % of dose in the entire tumor. Colon tumor T-380; Sec rate-14 mg CEA/g/hr; Sac time-72 hr postinj.; Quantity [^{111}In] MoAb admin.-1 μg; MoAb #-CEJ-326

both the melanoma and T-380 tumors, i.e., each mouse carried both tumors. The mice were divided into groups of six and four, and were administered i.v. 1 μg (8 μCi) of ^{111}In CEJ-326 and 1 μg (8 μCi) of ^{111}In 96.5 MoAb, respectively. All mice were killed at 72 hr after injection, and the tumors and other tissues processed as described.

Experiment 3. This experiment was performed in the lymphoma model. Fifteen nude mice bearing tumors that ranged in size from 0.06–10.2 g were injected with 2 μg (10 μCi) of ^{111}In-T101 MoAb i.v. by way of the tail vein. The mice were killed 48 hr after injection and the tissues processed as described above.

The second study in this model involved the i.v. (tail vein) administration of 1 μg (5 μCi) of ^{111}In-PSA-399 into 15 nude mice bearing lymphomas ranging in size from 0.1–9.8 g. This MoAb has no known affinity for the lymphoma and as such qualifies as a nonspecific IgG. The mice were killed at 48 hr and processed as described earlier.

RESULTS

Figure 1 shows the changes in the % dose/g, and the total tumor uptake of ^{111}In anti-CEA antibody into the T-380 tumor. The %dose/g of ^{111}In uptake in the smallest tumors is obviously higher than the absolute concentration of tracer in the tumor. As the tumors enlarge, the %dose/g of ^{111}In decreases.

Figure 2 shows the same general trend of uptake of ^{75}Se anti-CEA antibody as seen in Experiment 1 and for every time period. The average per g radiopharmaceutical uptake by all T-380 tumors <500 mg is over twice as great as the average uptake in those T-380 tumors >500 mg ($p < 0.001$).

Figure 3 indicates tumor acquisition of the 9.2.27 [^{111}In]MoAb is highest among the smallest tumors in all groups and at the majority of time points beyond 1 hr. There are exceptional data points. If, however, one compares the average uptake of the 15 smallest and 16 largest tumors (irrespective of time periods), the differences are significant at the $p < 0.001$ level.

Figure 4 indicates that the changes noted in Fig. 3 are not unique to the antibody or to the radiolabel. Incorporation of [^{125}I] and [^{111}In]MoAb 96.5 follows the same pattern as 9.2.27 even to the extent of the exceptions. The difference in % uptake/g between the smallest and largest tumors is a factor of ~2.

Figure 5 compares specific vs. nonspecific MoAb uptake into colon and melanoma tumors implanted in the same animals. In all of the animals, melanoma tumors were three to four times larger than the colon tumors and by far the more necrotic of the two. The nonspecific uptake of the ^{111}In 96.5 antibody is nearly as great in the small viable colon tumors as it is in the larger melanoma tumors for which it is specific. When ^{111}In-CEJ-326 is injected, the uptake in the colon tumor for which it is specific is nearly three times that of the nonspecific uptake by the larger melanoma. The specific uptake of ^{111}In-CEJ-326 in the colon tumor, however, is less than twice the nonspecific uptake of ^{111}In-96.5 in the small colon tumors.

Figure 6 shows the uptake of the tumor specific ^{111}In-T-101 MoAb in the lymphoma model. The graph clearly indicates a decrease in per g tracer uptake in the tumors as they enlarge. Some variability is noted in the pattern but the overall trend is obvious. When uptake in tumors <0.5 g is compared with that of tumors >0.5 g, the difference is significant at the $p < 0.001$ level.

Figure 7 shows the uptake of nonspecific ^{111}In-PSA-399 in the lymphoma model. The concentration per g of tumor is less than that of ^{111}In-T-101 and the trend is less obvious. If one

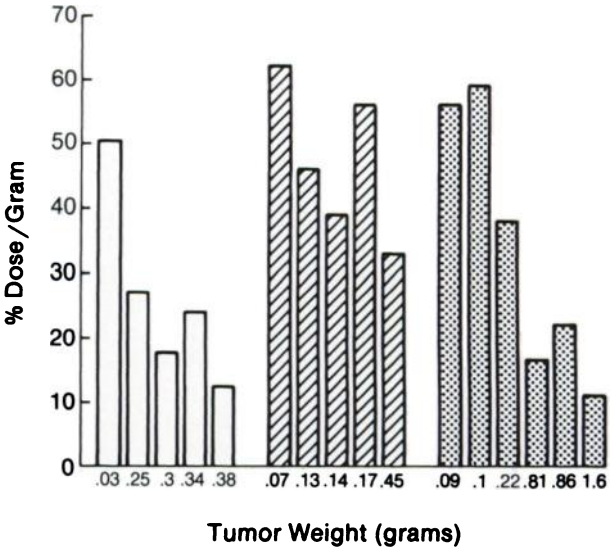

FIGURE 2
Uptake of ^{75}Se endogenously labeled anti-CEA antibody into T-380 tumor. At every time period, small tumors appear to acquire tracer in greater concentration than large tumors. (□) 24 hr; (▨) 48 hr; (▩) 72 hr

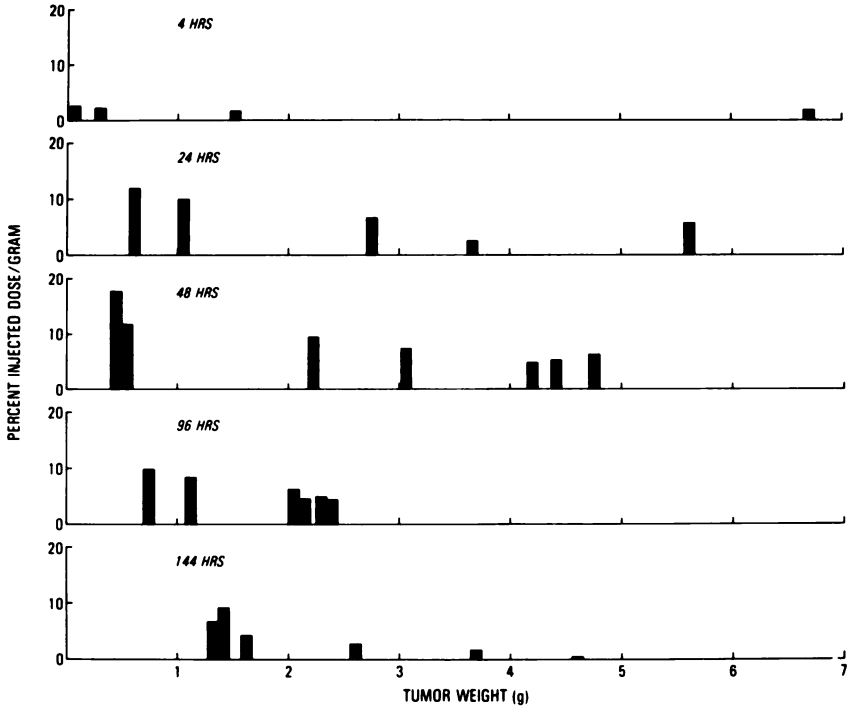

FIGURE 3
Uptake of the 9.2.27 ^{111}In anti-melanoma antibody vs. tumor weight at multiple time periods. All time periods show greater uptake of radiopharmaceutical in small tumors than in large tumors

leaves out the uptake in the two tumors with the highest per g uptake (both <0.5 g in size), the difference in uptake of tumors <0.5 g and >0.5 g fails to reach $p < 0.05$ significance levels. There does, however, appear to be an overall decrease in uptake as the tumor enlarges.

DISCUSSION

The data from these experiments support some of the basic tenents of tumor physiology. The per g uptake of tumor specific [^{111}In]MoAb in tumors decreased as the tumors enlarged. This was independent of the antibody class, type of tumors, target antigen or mobility of the antigen. Indeed, if one compares the average uptake of all of the tumors <0.5 g in size (regardless of antibody or tumor model) with those >0.5 g, the difference is significant at $p < 0.001$. This represents studies in over 100 animals. These data are in keeping with the work of Menard et al. (*2*), and Epenetos et al. (*3*) and contrary to the work of Baldwin and Pimm (*1*). In defense of Baldwin and Pimm, the uptake of [^{111}In]MoAb was not always inversely related to tumor size, and it is possible that the difference in our data and that of the British group was partly a matter of tumor necrosis. The degree of necrosis and the tumor size at which it occurs varies in our experience with the tumor type selected for transplant, and in fact, with each individual tumor. This is also true of the concentrations of antigen on the cells. The trend toward a decrease in uptake per g with enlarging size is definite, however, when many tumors and time points are observed.

Another reason for the discrepancy in our data and those of Baldwin and Pimm lies in the fact that these investigators report whole tumor rather than uptake/g. When their data are replotted on a per g basis, there is a definite trend toward lower uptake in larger tumors. This is in keeping with the decrease in blood flow that occurs with tumor enlargement (*17*). In fact, in some

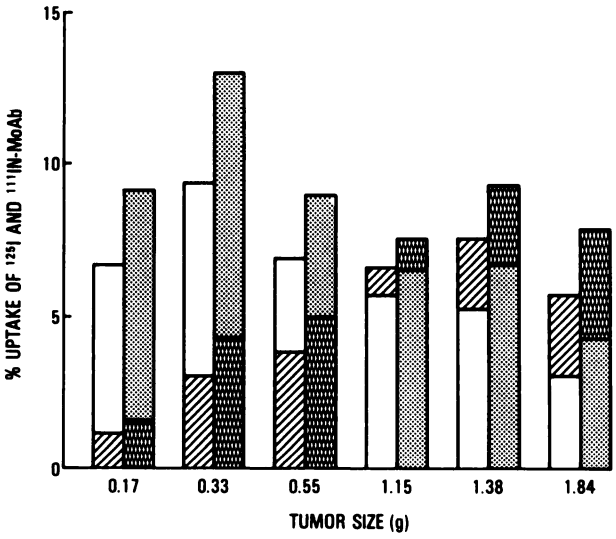

FIGURE 4
Incorporation of ^{125}I and ^{111}In 96.5 MoAb vs. tumor size in melanoma model. Kill time 24-hr postinjection. Note: Both radionuclides parallel each other regarding concentration in tumor. (□) % injected dose/g-^{125}I; (▨) % injected dose whole tumor-^{125}I; (▩) % injected dose/g-^{111}In; (▣) % injected dose whole tumor-^{111}In

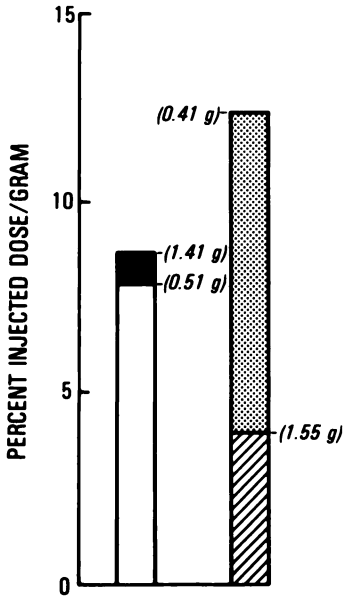

FIGURE 5
Uptake of ^{111}In-96.5 and ^{111}In anti-CEA in mice bearing both melanoma and colon tumors. Melanoma tumors were three to four times larger than colon tumors. Nonspecific uptake of 96.5 antibody is nearly as great in small colon tumors as in larger melanoma tumors for which it is specific. Conversely, specific uptake of the anti-CEA antibody in colon tumor is markedly higher than nonspecific uptake of this antibody by larger melanoma tumor. (■) Specific ^{111}In-96.5 uptake by melanoma; (□) Nonspecific ^{111}In-96.5 uptake by colon tumor; (▨) Nonspecific ^{111}In-96.5 uptake by colon tumor; (⊞) specific ^{111}In anti-CEA uptake by colon tumor; ( ) Average weight of tumors in group

tumor models a small tumor may concentrate nearly as much antibody in a nonspecific manner as a large tumor can on a specific basis (see Fig. 4). It is also true that absolute amounts of radiopharmaceutical in a large tumor can be greater than in a small tumor, even

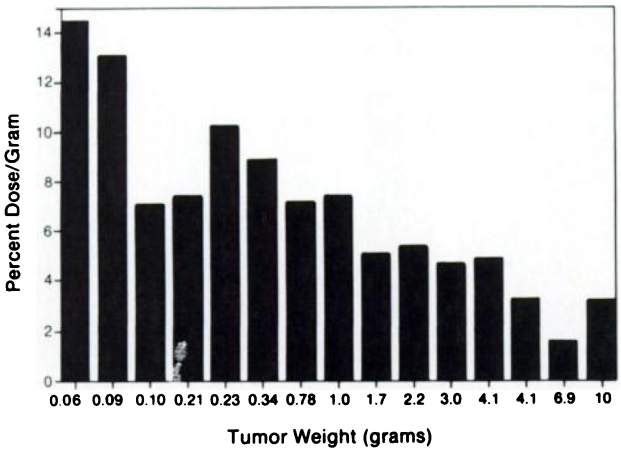

FIGURE 6
^{111}In-T-101 MoAb in lymphoma tumor. Radiopharmaceutical is specific for antigen on tumor

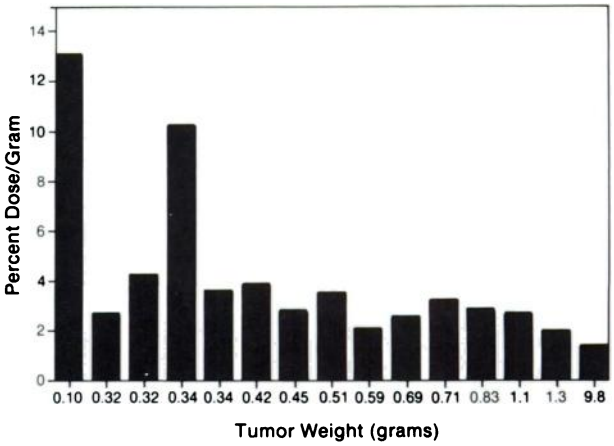

FIGURE 7
^{111}In-PSA-399 in lymphoma model. Radiopharmaceutical does not have specificity for antigen on tumor. Correlation of uptake and tumor size is less apparent than in specific antibody study

though the percent/g uptake by the small tumor far exceeds that of the large tumor. Indeed, in tumors of 5 g or greater, 10–20% of the dose of [^{111}In]MoAb has been observed in the tumor. This exceeds the quantity in tumors of 0.5 g in size even though the smaller tumor contains much greater tracer uptake on a per g basis.

Obviously, the interpretation of an experiment such as those depicted could be altered by the stability of the radiopharmaceutical. If the radiolabel is lost from the antibody it will not be detected if it is excreted and not fixed in the tissues. This was probably the case in the experiments of Baldwin and Pimm. The smaller, more viable tumors used in their study could logically be expected to induce events such as dehalogenation more rapidly than in larger tumors where the cells are hypoxic. By 24 hr, dehalogenation is well underway (*15*) as indicated by the difference in the ^{111}In and ^{125}I concentrations in Fig. 4. Dehalogenation has been seen to vary with the iodination technique (*18*), and could well be different from one antibody to the next, depending on the steric arrangement of the tyrosine groups on the molecules. If our own experiments had been performed using a radioiodine label, and the mice killed at 72 hr, the results might have indicated that tumor acquisition of the radiopharmaceutical did not vary as the tumors enlarged.

Finally, these data indicate that in any series of animal experiments, size of the tumor can dramatically vary the results of the study. Small tumors of similar size should be used whenever possible. In human studies it is possible that smaller lesions may acquire greater amounts of radiolabeled MoAb, making them more vulnerable to radioimmunotherapy.

In conclusion, tumor size appears to have a definite effect on the uptake of radiolabeled antibodies in the

nude mouse-human tumor model. Some exceptions are seen within each group and it is presumed that this is secondary to necrosis.

FOOTNOTES

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REFERENCES

1. Baldwin RW, Pimm MV: Antitumor monoclonal antibodies for radioimmunodetection of tumors in drug targeting. *Can Med Rev* 2:89-106, 1983
2. Menrad S, Miotti S, Tagliabue E, et al: Tumor radioimmunolocalization in the murine system using monoclonal antibodies. *Tumori* 69:185-190, 1983
3. Epenetos AA, Nimmon CC, Arklie J: Detection of human cancer in an animal model using radiolabeled tumor-associated monoclonal antibodies. *Br J Can* 46:1-8, 1982
4. Moshakis V, McIlhanney RAJ, Raghavan D, et al: Localization of human tumour xenographs after i.v. administration of radiolabeled monoclonal antibodies. *Br J Can* 44:91-99, 1981
5. Sullivan DC, Silva JS, Cox CE, et al: Localization of I-131-labeled goat and primate anti-carcinoembryonic antigen (CEA) antibodies in patients with cancer. *Invest Radiol* 17:350-355, 1982
6. Halpern SE, Stern PH, Hagan PL, et al: The labeling of monoclonal antibodies with 111-In. Technique and advantages compared to radioiodine labeling. In *Radioimmunoimaging and Radioimmunotherapy*, Burchiel S., Rhodes B., eds. New York, Elsevier North-Holland Biomedical Press, 1983
7. Stern P, Hagan P, Halpern S, et al: The effect of the radiolabel on the kinetics of the monoclonal anti-CEA in a nude mouse-colon tumor model. In *Hybridomas in Cancer Diagnosis and Treatment*, New York, Raven Press, 1982, pp 245-253
8. Halpern SE, Hagan PL, Garver PR, et al: Stability, characterization, and kinetics of 111In-labeled monoclonal antitumor antibodies in normal animals and nude mouse-human tumor models. *Can Res* 43:5347-5355, 1983
9. Sevier ED, David GS, Martines J, et al: Monoclonal antibodies in clinical encology. *Clin Chem* 27:1797-1806, 1981
10. Brown JP, Woodbury RG, Hart CE, et al: Quantitative analysis of melanoma-associated antigen p97 in normal and neoplastic tissues. *Proc Nat Acad Sci* 78:539-543, 1981
11. Morgan AC, Galloway DR, Reisfeld RA: Production and characterization of monoclonal antibody to a melanoma specific glycoprotein. *Hybridoma* 1:27-36, 1981
12. Shawler D, Royston I, Dillman RO: T-100 monoclonal antibody therapy of leukemia/lymphoma: Factors affecting *in vivo* binding to target cells. *Fed Proc*:1972, 1984
13. Krejcarek GE, Tucker KL: Covalent attachment of chelating groups to macromolecules. *Bio Chem Bio Phys Res Commun* 77:581-585, 1977
14. Hagan PL, Halpern SE, Chen A, et al: In vivo kinetics of radiolabeled monoclonal anti-CEA antibodies in animal models. *J Nucl Med:* in press
15. Martin KW, Halpern SE: Studies in carcinoembryonic antigen production, secretion and kinetics in BALB/c mice and a nude mouse-human tumor model. *Can Res* 44:5475-5481, 1984
16. Ziegler HW, Frizzera G, Bach FH: Successful transplantation of a human leukemia cell line. *J Nat Can Inst* 68:15-17, 1982
17. Cataland S, Cohen C, Sapirstein LA: Relationship between size and purfusion rate of transplanted tumors. *J Nat Can Inst* 29:389-391, 1962
18. Krohn KA, Sherman L, Welch NI: Studies of radioiodinated fibrinogen. 1. Physicochemico properties of ICL, chloramine-T and electrolytic reaction products. *Bio Chem Bio Phys Acta* 285:404-413, 1972