Localization of Pulmonary Human Sarcoma Xenografts in Athymic Nude Mice with Indium-111-labeled Monoclonal Antibodies

John A. Greager, Tzu-Chieh Chao, Michael J. Blend, Robert W. Atcher, Otto A. Gansow, Martin W. Brechbiel, and Tapas K. Das Gupta

Division of Surgical Oncology, Department of Surgery, University of Illinois College of Medicine at Chicago; Cook County Hospital; the West Side Veterans Administration Hospital; the Hektoen Institute for Medical Research; Division of Nuclear Medicine, Michael Reese Hospital, Chicago, Illinois; Argonne National Laboratory, Argonne, Illinois; and National Institutes of Health, Bethesda, Maryland

In order to study localization of metastatic tumors with a radiolabeled monoclonal antibody, a pulmonary metastases model was devised in athymic mice. Metastatic pulmonary sarcoma colonies were verified by histological examination. A murine monoclonal antibody (MAb 19-24) directed against a human sarcoma antigen was labeled with indium-111 (^{111}In) by use of the linker 1-(p-isothiocyanatobenzyl)-diethylenetriaminepentaacetic acid (SCN-Bz-DTPA). MAb P3 was similarly labeled as a negative control. In the group given MAb 19-24, the percent injected dose per gram lung tissue bearing tumor colonies (30.1%, 29.6%, and 27.7% on Days 1, 2, and 3, respectively) was significantly ($p < 0.05$) higher than in those receiving MAb P3. Hepatic activities of both ^{111}In-MAb 19-24 and ^{111}In-MAb P3 were low. The lungs with tumor colonies demonstrated clearest images on Day 3. The specific binding of ^{111}In-SCN-Bz-DTPA-labeled MAb 19-24 to pulmonary xenografts without appreciable liver uptake indicates that it may be useful in the clinical localization of pulmonic metastatic lesions.

J Nucl Med 1990; 31:1378–1383

Soft-tissue sarcomas generally disseminate hematogeneously and commonly metastasize to the lungs. The lungs are involved in over 80% of the patients who develop metastases from sarcomas and they are the only site with metastases in 70% of the patients (*1,2*). Despite improvements in the local control of soft tissue sarcomas, pulmonary metastases remain a challenging therapeutic problem and continue to be the primary cause of death in these patients. Approximately 25% of patients undergoing initial pulmonary resection achieve long-term survival. The major reason for this low survival is that detection of pulmonary metastases is often relatively late affording these patients a suboptimal therapeutic situation. Early detection with timely resection of the metastatic disease is essential for prevention of early patient death from soft-tissue sarcomas.

Conventional X-rays, whole-lung tomography, computed tomography (CT), and magnetic resonance imaging (MRI) are the most widely used methods to detect pulmonary metastases, but they are not always satisfactory for identifying early pulmonary metastases (*3*). Radiolabeled tumor specific antibodies can be used to localize and identify tumor deposits throughout the whole body. By utilizing hybridoma techniques originally developed by Kohler and Milstein (*4*) large amounts of monoclonal antibody (MAb) can be generated against a variety of tumor cell surface antigens. This capability provides the potential for a new era in the clinical imaging of tumor deposits. Radiolabeled MAbs have been used to successfully localize subcutaneous tumors in athymic nude mice (*5,6*), and they also have been applied in preliminary clinical studies to detect metastatic lesions in patients with melanoma (*7,8*), soft-tissue sarcomas (*9*), colon (*10*), and breast cancer (*11*). However, to our knowledge, localization of human sarcoma pulmonic xenografts in nude mice with ^{111}In-labeled MAb 19-24 has never been previously reported.

A murine monoclonal antibody (MAb 19-24) directed against a human soft-tissue sarcoma, malignant fibrous histiocytoma (MFH), was produced in our laboratory (*12*). MAb 19-24 reacts with a cell surface membrane antigen (a sarcoma associated antigen, known as p102) which has a molecular weight of 102 KD and is found in most human sarcomas at an average concentration of 300,000 sites per cell. Lower levels of p102 are discovered in some tumors other than sarcoma, and little or no antigen is present in normal tissues (*12*). We have detected subcutaneous sarcoma

Received Oct. 13, 1989; revision accepted Feb. 8, 1990.

For reprints contact: John A. Greager, MD, Division of Surgical Oncology, Department of Surgery, University of Illinois College of Medicine, 840 South Wood St., Chicago, IL 60612.

xenografts in athymic nude mice (*5,6*) and in patients with metastatic or recurrent soft-tissue sarcomas (*9*) by using MAb 19-24 labeled with either iodine-125 (^{125}I) or ^{111}In. In this study, we intravenously injected the athymic nude mice with human sarcoma cells to develop an experimental pulmonary metastases model, used a new chelation method to label MAbs with ^{111}In, and employed this ^{111}In-labeled MAb to localize lung metastases.

MATERIALS AND METHODS

Tissues and Cell Lines

Crude plasma membrane fractions were prepared as described by Howard et al (*13*). The human fibrosarcoma cell line HT-1080 was obtained from the American Type Culture Collection (Rockville, MD). HT-1080 is a tumor cell line derived from the poorly differentiated fibrosarcoma of a 35-yr-old Caucasian man who died without having received chemotherapy or radiotherapy (*14*).

Monoclonal Antibodies

Mouse MAb 19-24, an IgG_1 (with kappa light chains), was produced following fusion of immune BALB/c mouse splenocytes (immunized with membrane from human malignant fibrous histiocytoma) with the nonproducer myeloma X63-Ag8.653 (*12*). The antibody reacts with a cell surface membrane antigen quantitatively that is expressed to a much greater extent in many sarcomas than in other types of tumors or normal tissues (*12*). Mouse MAb P3, a nonspecific IgG_1 (also with kappa light chains) is secreted by BALB/c mouse myeloma P3-X63-Ag8 cell line (*4*). This antibody served as a nonspecific control, as it was nonreactive in our standard assays.

Antibody Purification

MAb 19-24 was purified from mouse ascitic fluid by ammonium sulphate precipitation followed by affinity chromatography on protein A-Sepharose (Pharmacia, Piscataway, NJ) (*12*). Sterile, pyrogen free reagents were used throughout the procedure. MAb P3 was purified from ascitic fluid directly on protein A-Sepharose as described previously. Purified antibodies were stored at −70°C. SDS-PAGE analysis of the purified MAbs (19-24 and P3) showed no contaminating proteins (*12*).

MAb Conjugation

MAb 19-24 and P3 were conjugated to 1-[p-isothiocyanatobenzyl]-diethylenetriaminepentaacetic acid (SCN-Bz-DTPA) according to the technique of Brechbiel et al. (*15*). Briefly, the SCN-Bz-DTPA was reacted with the MAbs at an initial 3:1 ratio of chelate to antibody in 50 m*M* HEPES buffer, pH 8.5. After 24 hr of reaction, MAbs were separated from unreacted chelate by dialysis.

MAb Radiolabeling

Sixty microliters of ^{111}In-chloride (Amersham Corporation, Arlington Heights, IL) (2 mCi, 0.05 M HCl) were added to 26.4 μl of 2 M HCl. After 5 min, the solution was adjusted to pH 4.2 with 1 *M* sodium acetate. Next, 200 μl of conjugated MAb 19-24 or P3 (3 mg/ml, pH 6.0) were immediately added and allowed to react for 30 min before purification. The labeled complex was separated from free ^{111}In chloride with the use of a 1 × 10 cm column of Sephadex G-25 (medium grade) equilibrated overnight in PBS buffer. Collection of 0.5-ml aliquots showed that labeled complex consistently appeared in tubes 5 and 6 with ~80% labeling efficiency.

Testing for Pyrogenicity and Sterility

Immediately after production, random preparations were tested for pyrogens with the Limulus Amebocyte Lysate (LAL) test (M.A. Bioproducts, Walkerville, MD), and for microbial contamination with the Mycotrim culture system (Hana Media, Berkeley, CA). No contaminated preparations were detected.

Experimental Pulmonary Metastases in Athymic Nude Mice

A pulmonary sarcoma metastatic model was devised by using athymic NCr-nu/nu nude mice (N. C. I. Frederick Cancer Research Facility, Frederick, MD) injected intravenously through tail veins with various amounts of HT-1080 cells and then killed at intervals. Tissues were collected, weighed, and fixed in 2% paraformaldehyde for histological examinations to verify pulmonary metastases. The dosage of HT-1080 cells which produced pulmonary colonies most consistently was used as the model for study.

Eighteen male athymic NCr-nu/nu nude mice, 6 to 8 wk old and 20–22 g in weight, were randomly divided into two groups. One group of nine athymic mice bearing pulmonary human sarcoma xenografts was injected intraperitoneally with 10 μCi of ^{111}In-SCN-Bz-DTPA-MAb 19-24; the other group of nine athymic mice also with pulmonary xenografts was given 10 μCi of ^{111}In-SCN-Bz-DTPA-MAb P3 as a nonspecific antibody control. Three mice from each group were killed and imaged with a gamma camera at 1, 2, and 3 days after antibody administration. Following imaging, the animals were autopsied and their tissues and blood were weighed and then counted for radioactivity. The results are presented as percent injected dose per gram tissue (% ID/g); tissue-to-blood ratio (cpm/g in tissue divided by cpm/g in blood); and localization index (tissue-to-blood ratio for specific antibody divided by tissue-to-blood ratio for nonspecific antibody).

Imaging

Posterior views of recently killed mice were obtained with a standard large field of view (LFOV) gamma camera fitted with a pinhole collimator of 4 mm aperture. Symmetric energy windows (20%) were placed over the 173 and 247 keV photopeaks of ^{111}In. Imaging times were typically 10 min per view, although a preset protocol of 50,000 counts per image was followed.

Statistical Analysis

Significant differences between test values were determined with the use of Student's t-test.

RESULTS

Experimental Pulmonary Metastases

There were neither gross nor microscopic pulmonary metastases in the mice injected with fewer than 10^5 HT-1080 cells. Two out of the six mice injected with 10^5 cells developed pulmonary colonies, which were detected on Day 14. At an inoculation dosage of 10^6 cells,

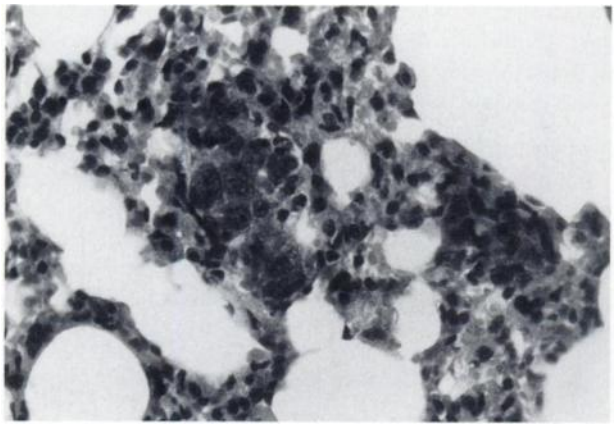

FIGURE 1
Colonies of different-sized, hyperchromatic sarcoma cells in the lungs of mouse 21 days after injection of 10^6 tumor cells. (400X, H and E stain).

all the mice had multiple micrometastases in the lungs which were evident on Day 7 after injection of tumor cells and the colonies increased in number and size with time, most evident on Day 21 (Fig. 1). There were no metastatic colonies in the liver, spleen, heart, and other tissues. Therefore, the mice i.v. injected with 10^6 cells were used to produce pulmonary metastases for localization studies.

Biodistribution Studies

Indium-111-SCN-Bz-DTPA-labeled MAbs were given to the mice on the 21st day after inoculation of 10^6 HT-1080 cells. In the group given ^{111}In-labeled MAb 19-24, the percent injected dose per gram of tissue (% ID/g) was markedly and significantly ($p < 0.05$) higher in the lungs bearing tumor colonies (30.1%, 29.6%, and 27.7% on Days 1, 2, and 3 respectively) than blood and the other normal tissues over time (Table 1). The %ID/g of lungs from mice given MAb 19-24 was significantly ($p < 0.05$) higher than % ID/g of lungs from mice given nonspecific MAb P3 at various time points. This finding suggests specific uptake of MAb 19-24 in the pulmonary tumor deposits. The uptake in the liver was low with a maximum value of 6.0% (ID/g) on Day 1, which decreased to 4.3% (ID/g) on Day 3. Radioactivity in the kidneys and spleen was similarly low and decreased slowly to 5.8% and 4.0% (ID/g) on Day 3 for kidneys and spleen, respectively. Maximum blood activity of 13.2% (ID/g) was seen on Day 1 which then decreased faster than found in liver, kidneys, and spleen to 4.9% on Day 2 and 7.0% on Day 3. The other tissues showed similar clearance curves. In contrast to the findings of indium-labeled specific antibody, ^{111}In-SCN-Bz-DTPA-MAb P3 failed to demonstrate specific uptake of MAb into pulmonic human sarcoma xenografts. The uptake of ^{111}In-labeled MAb P3 in blood, liver, kidneys, spleen, and other normal tissues was slightly higher than that found with ^{111}In-labeled MAb 19-24.

Tissue-to-blood ratios of radioactivity were calculated as cpm/g in tissue divided by cpm/g in blood (Table 2). For ^{111}In-labeled MAb 19-24, the lung-to-blood ratios were 2.75, 6.13, and 4.18 on Days 1, 2, and 3, respectively, reflecting more rapid clearance of ^{111}In-labeled MAb 19-24 from blood than from the lungs containing tumor colonies. At each time point, lung-to-blood ratios of labeled MAb 19-24 were significantly ($p < 0.05$) higher than those obtained with labeled MAb P3. The tissue-to-blood ratios obtained for the majority of mouse tissues other than lungs were less than 1.0 and the values for ^{111}In-labeled MAb 19-24 did not differ significantly ($p > 0.05$) from the corresponding values for labeled MAb P3.

The localization index, defined as tissue-to-blood ratio for MAb 19-24 divided by the corresponding ratio for MAb P3, was calculated for several major tissues. The lungs with tumor deposits had higher localization

TABLE 1
Tissue Uptake of ^{111}In-labeled MAbs 19–24 and P3 in Tumor-Bearing Mice (% ID/g)*

Day	MAb	Blood	Lung (tumor)	Liver	Spleen	Kidney	Stomach	Bone	Muscle	Skin
1	19–24	13.2 (8.0–18.6)	30.1 (19.1–36.1)	6.0 (3.8–7.4)	4.8 (3.0–5.7)	7.1 (5.8–8.4)	0.7 (0.5–1.2)	3.3 (2.3–4.8)	1.6 (1.2–1.8)	7.1 (5.3–8.2)
	P3	16.1 (15.0–16.6)	11.0 (9.4–12.7)	6.9 (6.2–7.5)	5.3 (4.8–5.8)	8.3 (7.8–9.2)	0.9 (0.7–1.2)	4.1 (3.7–4.4)	1.7 (1.5–1.8)	7.3 (7.2–7.4)
2	19–24	4.9 (3.3–6.0)	29.6 (22.1–34.5)	4.4 (3.7–5.0)	4.5 (3.4–5.4)	6.5 (5.9–7.4)	0.5 (0.4–0.7)	2.5 (1.9–2.9)	0.8 (0.6–0.9)	4.1 (3.7–4.4)
	P3	12.3 (11.3–13.4)	10.1 (9.5–10.4)	6.8 (5.7–7.7)	5.8 (4.6–7.1)	10.6 (8.2–12.9)	0.8 (0.7–0.9)	3.8 (2.9–4.8)	1.3 (1.2–1.4)	6.3 (5.8–6.9)
3	19–24	7.0 (5.4–8.7)	27.7 (27.0–28.5)	4.5 (3.7–5.4)	4.0 (3.3–4.7)	5.8 (4.8–6.8)	0.7 (0.5–0.8)	2.6 (2.4–2.9)	0.9 (0.7–1.1)	4.0 (3.1–4.9)
	P3	10.0 (9.7–10.4)	13.1 (9.5–15.0)	5.2 (4.8–5.6)	5.2 (5.1–5.4)	8.0 (7.8–8.3)	1.0 (0.9–1.0)	3.5 (2.8–4.0)	1.1 (1.0–1.2)	4.8 (4.7–5.1)

* Mean value from three animals and range in parentheses.

TABLE 2
Tissue-to-Blood Ratios After Injection of ^{111}In-labeled MAbs*

Day	MAb	Lung (tumor)	Liver	Spleen	Kidney	Stomach	Bone	Muscle	Skin
1	19-24	2.75 (1.03–4.53)	0.47 (0.40–0.53)	0.37 (0.31–0.44)	0.57 (0.45–0.72)	0.07 (0.03–0.15)	0.25 (0.21–0.29)	0.13 (0.09–0.15)	0.56 (0.44–0.66)
	P3	0.68 (0.63–0.76)	0.43 (0.41–0.45)	0.33 (0.32–0.35)	0.52 (0.47–0.61)	0.05 (0.04–0.08)	0.25 (0.25–0.26)	0.11 (0.09–0.12)	0.46 (0.43–0.49)
2	19-24	6.13 (5.77–6.66)	0.93 (0.83–1.13)	0.94 (0.79–1.04)	1.42 (0.98–1.93)	0.11 (0.08–0.12)	0.51 (0.47–0.59)	0.16 (0.14–0.19)	0.88 (0.69–1.21)
	P3	0.82 (0.77–0.92)	0.56 (0.47–0.68)	0.48 (0.34–0.63)	0.86 (0.73–1.07)	0.07 (0.06–0.07)	0.31 (0.24–0.36)	0.11 (0.10–0.12)	0.52 (0.46–0.57)
3	19-24	4.18 (3.11–5.25)	0.65 (0.62–0.68)	0.58 (0.54–0.61)	0.84 (0.79–0.89)	0.09 (0.09–0.09)	0.39 (0.34–0.44)	0.13 (0.13–0.14)	0.57 (0.56–0.58)
	P3	1.31 (0.97–1.51)	0.52 (0.46–0.58)	0.52 (0.49–0.55)	0.81 (0.75–0.85)	0.10 (0.09–0.10)	0.35 (0.27–0.41)	0.11 (0.10–0.13)	0.48 (0.45–0.53)

* Mean value from three animals and range in parentheses.

indices than the other tissues, with a maximum value of 7.4 on Day 2 (4.0 on Day 1, and 5.4 on Day 3).

Imaging Results

Gamma camera images were obtained after killing animals bearing HT-1080 fibrosarcoma pulmonary xenografts prior to autopsies and determination of previously described biodistribution studies. In all of the animals injected with ^{111}In-SCN-Bz-DTPA-MAb 19-24, the pulmonic xenograft images became increasingly distinct with time as the background activity decreased. The clearest images were seen on Day 3 and correlated well with the subsequent biodistribution studies of the tissues. Infusion of ^{111}In-SCN-Bz-DTPA-MAb P3 did not give distinct images of pulmonic xenografts as seen with MAb 19-24. Image quality using MAb P3 did not improve over time and background activity was higher than with MAb 19-24.

DISCUSSION

Soft-tissue sarcomas commonly metastasize hematogeneously to the lungs. Dissemination of tumor cells is a complex, multistep process in which both host factors and peculiar biologic properties of the metastasizing cells play fundamental roles (*16,17*). To establish metastases, tumor cells should invade the surrounding tissues, penetrate into blood vessels and/or lymphatic vessels, survive in the circulation, be arrested in the capillary bed of distant organs, extravasate into organ parenchyma, and proliferate to form metastatic tumors. Experimental metastases refer to tumor colonies produced after i.v. injection of tumor cells. Although intravascular injection of tumor cells bypasses the early steps of penetration of malignant cells into the vessels, all the subsequent steps in the metastatic process must occur before metastases can be established.

Athymic nude mice serve as a useful animal model to study human tumor in vivo. Xenografted human tumors grown in athymic nude mice maintain their karyotype, histologic appearance, and most biochemical characteristics (*18*). Subcutaneous human tumor xenografts in athymic nude mice are often used as a model for in vivo tumor studies. Spontaneous metastases do not occur ordinarily in adult athymic nude mice with subcutaneous xenografts (*19,20*). The low frequency of metastases in athymic mice has been attributed to high natural killer (NK) cell activity in adult nude mice. Young (≤3 wk), immunologically immature mice with low NK activity have been suggested to be best to allow growth of human tumor xenografts (*20, 21*). However, metastases have recently been reported in adult athymic mice with i.v. injection of human melanoma and human colorectal carcinoma cells (*22–24*). In the present study, i.v. injection of 10^6 human sarcoma cells into adult athymic nude mice produced pulmonic metastases in all animals; this provides a useful animal model for localization studies of human pulmonary metastases.

Currently, two major methods are used for the exogenous radiolabeling of MAbs: (1) iodination and (2) bifunctional chelation techniques for attaching metals to protein molecules (*25*). In vivo dehalogenation with rapid clearance of the tracer from various organ systems as well as from the tumor is a major problem when utilizing antibodies labeled with iodine isotopes. In contrast, ^{111}In released from catabolized antibody seems to be retained in most normal tissues and in the tumor tissue. Mixed anhydrides and cyclic anhydrides of DTPA conjugates are most commonly used to chelate ^{111}In to label MAb. There is, however, a major drawback with these chelates in the high nonspecific uptake of the metal by the liver.

A new chelation method using isothiocyanate (SCN-

Bz-DTPA) has been utilized with good uptake of radiolabeled MAb in subcutaneous tumors, improved specificity, decreased liver uptake (6-8% ID/g) and minimal background activity 72 hr after injection of ^{111}In-labeled MAbs (*6,26*). The reason for decreased hepatic uptake of ^{111}In conjugated with SCN-Bz-DTPA is not clearly understood, but it has been suggested that MAb-SCN-Bz-DTPA complex forms a very stable bond with ^{111}In in vivo thereby diminishing transferrin transport of indium to the liver (*26*).

The reported uptake of radioactivity (expressed as % ID/g) of ^{111}In-SCN-Bz-DTPA-MAbs present in tumor tissues 72 hr after injection is 30.0%-52.1% (*6,26*). In this study, uptake of specific ^{111}In-labeled MAb 19-24 in the lungs with sarcoma xenografts was significantly higher than in the other tissues at the various time points (Table 1). Lung-to-blood ratios of ^{111}In-MAb 19-24 were significantly higher than those of nonspecific MAb P3. This data suggests that ^{111}In-SCN-Bz-DTPA-labeled MAb 19-24 retained adequate immunologic activity and specifically localized human soft-tissue sarcoma pulmonic xenografts in the nude mice.

The clearance curves of both MAbs were similar (Fig. 2). The blood levels of ^{111}In-labeled MAb 19-24 were lower than those of ^{111}In-labeled MAb P3. The relatively lower blood activity of ^{111}In-labeled specific MAb should theoretically further improve tumor imaging. Uptake of ^{111}In-labeled antibodies using either mixed anhydrides or cyclic anhydrides of DTPA conjugates has been reported high in the kidney, spleen, and liver (*27,28*). Such high normal organ radioactivity creates high background activity and thus diminishes imaging quality. ^{111}In-SCN-Bz-DTPA-labeled MAb shows relatively low uptake into kidney, spleen, and liver in the present study (Table 1). The data agree with the observations reported by Blend et al. (*6*) and Esteban et al. (*26*) using the same conjugation technique in a subcutaneous tumor xenograft model. Therefore, because this new indium labeling technique decreases background activity, the relative labeled MAb localization and imaging quality was appreciably facilitated.

Although use of this experimental pulmonary human sarcoma metastases will require further study, our present results are encouraging. We have demonstrated that ^{111}In-SCN-Bz-DTPA-labeled MAb can be used to successfully localize pulmonary xenografts, with significantly decreased background activity in this animal model. Hopefully, information using this model and labeling technique might help develop new, innovative approaches appropriate for clinical detection of pulmonic sarcoma metastases.

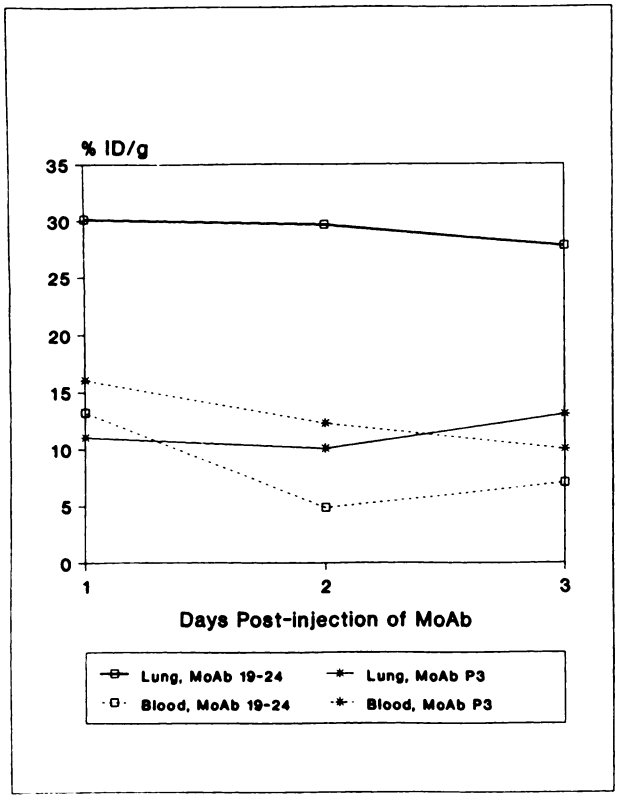

FIGURE 2
Percent injected dose per gram of lungs with HT-1080 fibrosarcoma xenografts and blood in athymic nude mice injected with ^{111}In-MAb 19-24 and ^{111}In-MAb P3.

ACKNOWLEDGMENT

This study is supported by the American Cancer Society (Illinois Division) Grant #88-53.

REFERENCES

1. Vezeridis MP, Moore R, and Karakousis CP. Metastatic patterns in soft-tissue sarcomas. *Arch Surg* 1983; 118:915–918.
2. Huth JF, Holmes EC, Vernon SE, Callery CD, Ramming KP, Morton DL. Pulmonary resection for metastatic sarcoma. *Am J Surg* 1980; 140:9–16.
3. Peuchot M, Libshitz HI. Pulmonary metastatic disease: radiologic-surgical correlation. *Radiology* 1987; 164:719–722.
4. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of prefused specificity. *Nature* 1975; 256:495–497.
5. Brown JM, Greager JA, Pavel DG, Das Gupta TK. Localization of radiolabeled monoclonal antibody in a human soft tissue sarcoma xenograft. *J Natl Cancer Inst* 1985; 75:637–644.
6. Blend MJ, Greager JA, Atcher RW, et al. Improved sarcoma imaging and reduced hepatic activity with indium-111-SCN-Bz-DTPA linked to MoAB 19-24. *J Nucl Med* 1988; 29:1810–1816.
7. Murray JL, Rosenblum MG, Sobol RE, et al. Radioimmunoimaging in malignant melanoma with In-111 labeled monoclonal antibody 96.5. *Cancer Res* 1985; 45:2376–2381.
8. Kirkwood JM, Neumann RD, Zoghbi SS, et al. Scintigraphic detection of metastatic melanomas using indium 111/DTPA conjugated anti-gp 240 antibody (ZME-018). *J Clin Oncol* 1987; 5:1247–1255.
9. Greager JA, Brown JM, Pavel DG, Garcia JL, Blend MJ, Das Gupta TK. Localization of human sarcoma with radiolabeled monoclonal antibody: a preliminary study. *Cancer Immunol*

Immunother 1986; 23:148–154.
10. Moldofsky PJ, Sears HF, Mulhern CB, Jr, et al. Detection of metastatic tumor in normal-sized retroperitoneal lymph nodes by monoclonal antibody imaging. *N Engl J Med* 1984; 311:106–107.
11. Cote RJ, Rosen PP, Hakes TB, et al. Monoclonal antibodies detect occult breast carcinoma metastases in the bone marrow of patients with early stage disease. *Am J Surg Pathol* 1988; 12:333–340.
12. Brown JM. Detection of a human sarcoma-associated antigen with monoclonal antibodies. *Cancer Res* 1983; 43:2113–2120.
13. Howard FD, Ledbetter JA, Mehdi SQ, Herzenberg LA. A rapid method for the detection of antibodies to cell surface antigens: a solid phase radioimmunoassay using cell membranes. *J Immunol Methods* 1980; 38:75–84.
14. Rasheed S, Nelson-Rees WA, Toth EM, Arnstein P, Gardner MB. Characterization of a newly derived human sarcoma cell line (HT-1080). *Cancer* 1974; 33:1027–1033.
15. Brechbiel MW, Ganscow OA, Atcher RW, et al. Synthesis of 1-(p-isothiocyanatobenzyl) derivatives of DTPA and EDTA. Antibody labeling and tumor imaging studies. *Inorg Chem* 1986; 25:2772–2781.
16. Mantovani A, Giavazzi R, Alessandri G, et al. Metastatic potential of tumor cells from spontaneous metastases. In: Hellman K, Hilgard P, Eccles S, eds. *Metastases—clinical and experimental aspects*. The Hague: Martinus Nijhoff; 1980:215–221.
17. Poste G, Fidler IJ. The pathogenesis of cancer metastasis. *Nature* 1980; 283:139–145.
18. Sharkey FE, Fogh J. Consideration on the use of nude mice for cancer research. *Cancer Metastasis Rev* 1984; 3:341–360.
19. Sharkey FE, Fogh J. Metastasis of human tumors in athymic nude mice. *Int J Cancer* 1979; 24:733–738.
20. Fidler IJ. Rationale and methods for the use of nude mice to study the biology and therapy of human cancer metastasis. *Cancer Metastasis Rev* 1986; 5:29–49.
21. Hanna N. Role of natural killer cells in control of cancer metastasis. *Cancer Metastasis Rev* 1982; 1:45–64.
22. Kerbel RS, Man MS, Dexter D. A model for human cancer metastasis of a tumor pigmented melanoma and derived variant sublines in nude mice. *J Natl Cancer Inst* 1984; 72:93–108.
23. Fodstad Ø, Aamdal S, McMenamin M, Nesland JM, Pihl A. A new experimental metastasis model in athymic nude mice, the human malignant melanoma LOX. *Int J Cancer* 1988; 41:442–449.
24. Giavazzi R, Campbell DE, Jessup JM, Fidler IJ. Metastatic behavior of tumor cells isolated from primary and metastatic human colorectal carcinomas implanted into different sites in nude mice. *Cancer Res* 1986; 46:1828–1933.
25. Bradwell AR. The use of radiolabeled antibodies for the localization of tumors. In: Hancock BW, Ward AM, eds. *Immunological aspects of cancer*. Boston: Martinus Nijhoff Publishing; 1985:107–123.
26. Esteban JM, Schlom J, Gansow OA, et al. New method for the chelation of indium-111 to monoclonal antibodies: biodistribution and imaging of athymic mice bearing human colon carcinoma xenografts. *J Nucl Med* 1987; 28:861–870.
27. Stern P, Hagan P, Halpern S, et al. The effect of the radiolabel on the kinetics of monoclonal anti-CEA in a nude mouse-human colon tumor model. In: Mitchell MS, Oettgen HF, eds. *Hybridomas in cancer diagnosis and treatment*. New York: Raven Press; 1982:245–253.
28. Khaw BA, Strauss W, Cahill SL, Soule HR, Edington T, Cooney J. Sequential imaging of indium-111-labeled monoclonal antibody in human mammary tumors hosted in nude mice. *J Nucl Med* 1984; 25:592–603.