Annals of Nuclear Medicine Vol. 13, No. 6, 407–413, 1999

Tumor scintigraphy by the method for subtracting the initial image with technetium-99m labeled antibody

Yoshiharu KARUBE,* Kentaro KATSUNO,* Sanae ITO,* Kazuhisa MATSUNAGA,* Jiro TAKATA,* Masahide KUROKI,** Masaaki MURAKAMI** and Yuji MATSUOKA**

*Faculty of Pharmaceutical Sciences, Fukuoka University
**School of Medicine, Fukuoka University

The method for subtracting the initial image from the localization image was evaluated for radioimmunoscintigraphy of tumors with technetium-99m (Tc-99m) labeled antibodies. Monoclonal antibodies were parental mouse and mouse-human chimeric antibodies to carcinoembryonic antigen (CEA), designated F11-39 and ChF11-39, respectively, both of which have been found to discriminate CEA in tumor tissues from the CEA-related antigens. After reduction of the intrinsic disulfide bonds, these antibodies were labeled with Tc-99m. *In vivo* studies were performed on athymic nude mice bearing the human CEA-producing gastric carcinoma xenografts. Though biodistribution results showed selective and progressive accumulation of Tc-99m labeled antibodies at the tumor site, high radioactivity in blood was inappropriate for scintigraphic visualization of the tumors within a few hours. We examined the subtraction of the initial Tc-99m image from the Tc-99m localization image after a few hours. Subtracted images of the same count reflected the *in vivo* behavior of the Tc-99m radioactivity. The subtracted scintigrams revealed excellent tumor images with no significant extrarenal background. Visualization of the tumor site was dependent on antigen-specific binding and nonspecific exudation. These results demonstrate that a method of subtraction of the initial image may serve as a potentially useful diagnostic method for an abnormal site for agents with a low pharmacokinetic value.

Key words: technetium-99m, subtraction, monoclonal antibody, tumor imaging, radioimmunoscintigraphy

INTRODUCTION

SEVERAL RADIOLABELED monoclonal antibodies have been extensively investigated for detection of tumors in both humans[1,2] and animal models.[3,4] For specific tumor accumulations to be visible, radiolabeled antibodies must bind to cell surface antigens. If tumor cells are exposed to the lumen of a blood vessel, accumulation of antibodies is more rapid but still relatively slow because of the small fraction of the cardiac output received by the tumor.[5] A large proportion of the intravenous dose remains in the circulatory system and other normal tissues and may obscure specific tumor imaging.

Goldenberg et al.[1,6] introduced the subtraction method to reduce the nonspecific background effect of iodine-131 (I-131) labeled antibody persisting in normal tissues. The finding that injection of Tc-99m labeled human serum albumin and Tc-99m pertechnetate gives an image of normal tissues similar to that of I-131 labeled antibody is generally valid. After subtraction of the Tc-99m image from that of I-131, localization of antibody in the tumor is shown by the areas of residual activity. These results were soon confirmed by other groups.[7–12] This method often suffers from a number of disadvantages such as different distribution of I-131 and Tc-99m in the circulation and in the urinary tract, a halo effect due to different energies of I-131 and Tc-99m, the thyroid and mucosa uptake of iodide, and the gut excretion of iodide.

Technetium-99m has ideal physical properties for many

Received May 17, 1999, revision accepted September 2, 1999.

For reprint contact: Yoshiharu Karube, Ph.D., Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma 8–19–1, Jonan-ku, Fukuoka 814–0180, JAPAN.
E-mail: karube@fukuoka-u.ac.jp

clinical applications by virtue of its ready availability, low irradiation of the patients and low cost. Technetium-99m labeled antibodies have therefore been evaluated for radioimmunoscintigraphic studies of tumors.[13–18] Recently the advantages of using Fab′ and $F(ab')_2$ fragments instead of whole antibody were reported.[19–21] Despite its general use for routine nuclear medical studies, Tc-99m has not achieved widespread use as a radiolabel for monoclonal antibodies in immunoscintigraphic studies because of the certainty that its short half-life is inappropriate for antibodies with such low pharmacokinetics.

To overcome the disadvantages of the short half-life of Tc-99m and the high radioactivity in blood, we examined the subtraction of the initial Tc-99m image from the Tc-99m image after a few hours. These subtracted images of the same count reflected the *in vivo* behavior of the Tc-99m labeled antibody. In this report, we evaluate the subtracted imaging characteristics of Tc-99m labeled parental mouse and mouse-human chimeric antibodies to carcinoembryonic antigen (CEA), designated F11-39 and ChF11-39, both of which have been produced.

MATERIALS AND METHODS

Monoclonal and chimeric antibodies

Parental mouse and mouse-human chimeric antibodies to carcinoembryonic antigen (CEA), designated F11-39 and ChF11-39, respectively, were used in this study. These antibodies have been found to recognize the protein epitopes present on domain B3 of the CEA molecule and to distinguish CEA in tumor tissues from CEA-related antigens. The purification, construction, expression and immunochemical properties of these antibodies have been described in detail previously.[22–25] A mouse IgG1(κ) myeloma protein, 11C-2A4-H2, a gift from Dr. Fujiwara (Osaka University, Osaka, Japan), and a human IgG1(κ) myeloma protein, BP078, purchased from a commercial source (The Binding Site, Birmingham, England), were used as controls.

Technetium-99m labeled monoclonal antibodies

Technetium-99m pertechnetate was eluted from a sterile Mo-99-Tc-99m shielded generator (Ultra-TechneKow, Dai-ichi Radioisotope Laboratories, Chiba, Japan) with isotonic saline.

The labeling procedure employed in this study was similar to the methods reported by Schwarz and Steinstraßer,[18] and Mather and Ellison.[15] Purified antibody (1.5 mg) was reduced by the reaction with a molar ratio of 2-mercaptoethanol to antibody of 10,000 at room temperature for 30 min. The reduced antibody was then separated by gel filtration on a mini-column prepacked with Sephadex G-25M (PD-10). This column was eluted with Dulbecco's PBS (−). For Tc-99m labeling, 50 μl of freshly prepared reducing solution constituting of stannous chloride (0.08 mg/ml), 1-hydroxyethane-1,1-diphosphonate (1.0 mg/ml) and ascorbic acid (0.4 mg/ml) was immediately added to the reduced antibody aliquot and mixed well. Stannous chloride was used as the reducing agent, 1-hydroxyethane-1,1-diphosphonate as the ligand to protect the binding of Tc-99m to the amino acid side chains, and ascorbic acid was the antioxidant. Technetium-99m pertechnetate (55.5 MBq) produced in a generator was immediately added to the reduced antibody mixture and incubated for 10 min.[26] Each Tc-99m labeled antibody was purified by gel filtration on a mini-column prepacked with Sephadex G-25M (PD-10). The labeling yields of these antibodies were greater than 95% when estimated by gel chromatography. The specific activities of these Tc-99m antibodies were about 50 MBq/mg.

Technetium-99m labeling of the mouse and human IgG1 (1.5 mg) was performed by the same method.

Stability of Tc-99m labeled monoclonal antibodies

Tc-99m labeled monoclonal antibody solutions were allowed to stand at room temperature for 10 hr and 24 hr. The stability of the Tc-99m F11-39 and ChF11-39 was assessed by gel chromatography on a PD-10 column.

The solution of Tc-99m F11-39 or ChF11-39 was also mixed for transchelation with such Tc-99m avid agents as cysteine and diethylenetriaminepentaacetic acid (DTPA). The mixing agent to a protein molar ratio of 500 : 1 was chosen. After a reaction time of 1 hr at room temperature, preparations were analyzed by thin layer chromatography.[26] The transchelation study was performed in triplicate.

Immunoreactivity of Tc-99m labeled monoclonal antibodies

Immunoreactivity of Tc-99m labeled antibodies was evaluated with the *in vitro* cell binding assay. These Tc-99m labeled antibodies (2 μg) were incubated with increasing numbers of MKN-45 cells (1×10^4 to 5×10^6) suspended in 1 ml of RPMI-1640 in 12- × 75-mm tubes for 1 hr at 37°C. The cells (2.5×10^5) in 1 ml of RPMI-1640 were also incubated with reducing concentrations of these Tc-99m labeled antibodies (2 μg to 2 ng) under the same conditions. The cells were centrifuged, and washed with PBS. After centrifugation, the radioactivity bound to cells and combined supernatant was counted in a well-type gamma counter (Packard COBRA II, Meridien, CT). The assay was performed in triplicate, and the percent radioactivity bound to cells was calculated.[26]

Biodistribution in athymic nude mice

A human CEA-expressing gastric carcinoma cell line, MKN-45, was provided by the Japanese Cancer Research Resources Bank (Tokyo, Japan). The MKN-45 cells used as target cells for the development of tumor xenografts in athymic nude mice were grown in a RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum. Male athymic nude mice (Clea Japan Tokyo, Japan) with a BALB/c background were obtained at approximately 4

weeks of age. The mice were inoculated subcutaneously with MKN-45 cells (1×10^7 cells per animal) in the right foreleg because of hind legs being located near the bladder. At 2–3 weeks after the inoculation, the sizes of the MKN-45 tumors were from 0.5 to 1.5 cm in diameter. Each mouse received 0.10 m*l* (1 MBq, 17 μg) of antibody labeled with Tc-99m through the tail vein. The mice (4/group) were killed after blood collection from the heart at 3 min and 3 hr postinjection. The organs, blood, some muscle and the tumor were removed, weighed, and the radioactivity was counted in a well-type gamma counter. The radioactivity of all organs, blood, and urine was also counted in images in a gamma camera (Technicare Co.) to measure the radioactivity of the excreted urine and the total radioactivity. The correction of each count in two measurements was performed by coincidence with the blood radioactivity. The percentages of the injected dose per organ (ID/organ) or gram (ID/g) were determined by the ratio of tissue radioactivity to total radioactivity. Statistical analysis was performed by Student's t-test for unpaired data.

Imaging in athymic nude mice

The name of the gamma camera was SIGMA 420 (Technicare Co.). The camera type was a 37 tube array of 50.8 mm bialkali PMTs coupled to a 33.6 cm diameter by 0.63 cm thick NaI (Tl) Scintillation crystal by means of patient applied for Ohio-Nuclear electronic and optical techniques for ultra high resolution. Its field of view is a hexagon that is 24.8 cm across the flats (i.e., the inscribed circle diameter is 24.8 cm and the circumscribed circle is 28.6 cm in diameter). The name of the computer was Vip-450 (Ohio-Nuclear) and its software was Vip-450F V5.0 System.

Athymic nude mice bearing MKN-45 xenografts were injected through the tail vein with 0.20 m*l* (3 MBq, 50 μg) of antibody labeled with Tc-99m for imaging studies. Images of four mice were obtained at a predetermined time with a gamma camera (SIGMA 420) equipped with a high resolution parallel hole collimator in the fixed position of the mice and collected with a digital computer (Vip-450). The total count in each scintigram frame was 5×10^4. The subtraction of vascular activity with the initial image of the Tc-99m labeled antibody to enhance the tumor contrast was performed by computer techniques. The scintigrams at 3 min after injection were taken as the initial images.

RESULTS

Stability of Tc-99m labeled monoclonal antibodies

During the thin layer chromatography, all Tc-99m labeled antibodies in neutral saline solution after 10 hr remained at origin in the acetonitrile : water (7 : 3) solvent system on silica gel. No radioactivity was observed at the front position. After 1 hr incubation with diethylenetriaminepentaacetic acid (DTPA) solution, the peak of Tc-99m DTPA (Rf value = 0.5) was not observed. During 1 hr incubation with cysteine solution, the peak of Tc-99m cystinate (Rf value = 0.75) was present. These TLC results showed that about 8% of Tc-99m F11-39 and about 20% of Tc-99m ChF11-39 were removed by cysteine.

Table 1 Biodistribution data of Tc-99m F11-39 and mouse IgG1 in athymic nude mice bearing MKN-45 xenografts*

Organ	3 min	3 hr
Salivary glands		
F11-39	0.53 ± 0.10	0.30 ± 0.05
mouse IgG1	0.51 ± 0.07	0.25 ± 0.06
Spleen		
F11-39	0.55 ± 0.17	0.23 ± 0.05
mouse IgG1	0.49 ± 0.10	0.25 ± 0.15
Stomach		
F11-39	0.54 ± 0.08	0.33 ± 0.05
mouse IgG1	0.56 ± 0.10	0.41 ± 0.07
Intestine		
F11-39	2.80 ± 0.41	3.82 ± 0.39
mouse IgG1	2.64 ± 0.35	4.93 ± 0.35
Liver		
F11-39	10.76 ± 1.01	8.81 ± 0.92
mouse IgG1	11.75 ± 0.87	11.92 ± 0.88
Kidneys		
F11-39	3.54 ± 0.51	3.36 ± 0.43
mouse IgG1	3.65 ± 0.31	3.39 ± 0.51
Bladder and Urine		
F11-39	2.71 ± 1.19	8.30 ± 1.13
mouse IgG1	3.05 ± 0.78	8.78 ± 1.23
Muscle 1 g		
F11-39	0.90 ± 0.15	1.19 ± 0.15
mouse IgG1	0.91 ± 0.13	1.03 ± 0.16
Tumor 1 g		
F11-39	0.57 ± 0.11	4.52 ± 0.51
mouse IgG1	0.58 ± 0.11	1.08 ± 0.24
(significance)	NS	$p < 0.01$
Blood 1 g		
F11-39	42.45 ± 2.01	32.41 ± 1.87
mouse IgG1	41.65 ± 1.86	33.07 ± 1.56

*Values are percent injected dose, mean ± s.d. for four mice at 3 min and 3 hr after injection.

Immunoreactivity of Tc-99m labeled monoclonal antibodies

The percentage of radioactivity bound to 2.5×10^5 cells of MKN-45 tumor cells expressing CEA antigen was about 22% for 0.02 μg of Tc-99m F11-39 and Tc-99m ChF11-39, and about 12% for 2 μg of Tc-99m F11-39 and Tc-99m ChF11-39, whereas no significant binding was observed with Tc-99m mouse IgG1 or Tc-99m human IgG1.

Biodistribution study

The biodistribution of Tc-99m F11-39, Tc-99m mouse IgG1, Tc-99m ChF11-39, and Tc-99m human IgG1 was

Table 2 Biodistribution data of Tc-99m ChF11-39 and Tc-99m human IgG1 in athymic nude mice bearing MKN-45 xenografts*

Organ	3 min	3 hr
Salivary glands		
ChF11-39	0.40 ± 0.06	0.30 ± 0.03
human IgG1	0.39 ± 0.05	0.27 ± 0.06
Spleen		
ChF11-39	0.31 ± 0.09	0.25 ± 0.07
human IgG1	0.29 ± 0.08	0.21 ± 0.10
Stomach		
ChF11-39	0.39 ± 0.10	0.42 ± 0.11
human IgG1	0.40 ± 0.12	0.39 ± 0.06
Intestine		
ChF11-39	2.13 ± 0.34	4.25 ± 0.55
human IgG1	2.21 ± 0.54	5.02 ± 0.54
Liver		
ChF11-39	8.69 ± 1.52	7.12 ± 0.98
human IgG1	8.39 ± 0.73	6.90 ± 0.43
Kidneys		
ChF11-39	3.14 ± 0.71	3.21 ± 0.61
human IgG1	3.18 ± 0.31	3.26 ± 0.53
Bladder and Urine		
ChF11-39	3.15 ± 1.12	26.02 ± 2.23
human IgG1	3.60 ± 1.28	25.51 ± 2.13
Muscle 1 g		
ChF11-39	0.91 ± 0.36	1.12 ± 0.26
human IgG1	0.94 ± 0.28	1.20 ± 0.09
Tumor 1 g		
ChF11-39	0.61 ± 0.11	2.40 ± 0.24
human IgG1	0.63 ± 0.15	1.18 ± 0.17
(significance)	NS	$p < 0.01$
Blood 1 g		
ChF11-39	43.40 ± 2.37	24.52 ± 3.57
human IgG1	45.82 ± 2.18	23.68 ± 3.03

*Values are percent injected dose, mean ± s.d. for four mice at 3 min and 3 hr after injection.

evaluated in tumor bearing mice at 3 min and 3 hr postinjection. The percentages of the injected dose per gram (ID/g) or organ (ID/organ) determined in the tumor and in all major organs are shown in Table 1 and Table 2. The uptakes in the tumor increased with time from 0.57 ± 0.11% ID/g at 3 min to 4.52 ± 0.51% ID/g at 3 hr for Tc-99m F11-39. Technetium-99m F11-39 showed significantly higher tumor uptake than Tc-99m mouse IgG1 at 3 hr. The uptakes in the tumor increased with time from 0.61 ± 0.11% ID/g at 3 min to 2.40 ± 0.24% ID/g at 3 h for Tc-99m ChF11-39. Technetium-99m ChF11-39 also showed significantly higher tumor uptake than Tc-99m human IgG1 at 3 hr. The blood clearance of Tc-99m ChF11-39 was faster than that of Tc-99m F11-39, and the blood retentions were 32.41 ± 1.87% ID/g at 3 hr for Tc-99m F11-39 and 24.52 ± 3.57% ID/g at 3 hr for Tc-99m ChF11-39. The lower tumor activity of Tc-99m ChF11-39 than of Tc-99m F11-39 may be due to the excretion of Tc-99m ChF11-39 from the circulation. Though the tumor : blood ratios increased with time for Tc-99m F11-39 and Tc-99m ChF11-39, high radioactivity in the blood was inappropriate for scintigraphic visualization of the tumors within a few hours. Differences in uptake in other normal tissue could be accounted for mostly by the difference in blood clearance. The uptakes in the tumor slightly increased with time for Tc-99m mouse IgG1 and Tc-99m human IgG1 due to blood flow or leakage of blood vessels. But these tumor uptakes were low. Hepatic uptake was relatively low for Tc-99m ChF11-39 and Tc-99m human IgG1. The cumulative urinary excretion was about 8% at 3 hr for Tc-99m F11-39 and about 26% at 3 hr for Tc-99m ChF11-39. The thin layer chromatography of urine activity showed another peak compared with the Tc-99m antibody peak, indicating that ligand exchange had occurred. The chromatographic peak in the urine analysis had the same Rf value as the peak, due to Tc-99m cystinate (Rf value = 0.75).

Imaging study

Scintigraphic studies were also performed on four athymic nude mice bearing the MKN-45 xenografts after an intravenous injection of purified Tc-99m antibody solution. Sequential scintigrams were obtained at 3 min, 1 hr, 2 hr, 3 hr, 4 hr and 5 hr with a gamma camera. Figures 1 and 2 show scintigrams of mice bearing MKN-45 xenografts after administration of Tc-99m F11-39 and Tc-99m ChF11-39. In both antibodies, the heart and liver were visualized soon after injection. The image of the tumor was vaguely recognized on the scintigram at about 1 hr after injection. Higher urinary excretion was observed for Tc-99m ChF11-39 than for Tc-99m F11-39 (Fig. 2 B). Imaging results were in accordance with the biodistribution data.

Computer techniques for subtraction of background activity by means of the initial image of Tc-99m labeled antibody resulted in high enhancement of tumor contrast. The scintigrams at 3 min after injection were taken as the initial images. Subtracted scintigrams of athymic nude mice bearing MKN-45 xenografts showed a transplanted tumor and bladder clearly visible at 1 hr with both Tc-99m labeled antibodies. Tumor images and bladder images increased with time after administration of the antibody. The imaging results showed selective and progressive accumulation of Tc-99m antibodies at the tumor site (Figs. 1 C and 2 C). The image of the tumor was visualized clearly by omission of urine activity or a decrease in the subtraction factor (Fig. 1 D). The tumor image at 4 hr was also visualized more clearly by subtraction (Fig. 2 D).

The distribution data for Tc-99m mouse IgG1 and Tc-99m human IgG1 in MKN-45 tumor tissue were significantly lower than those for Tc-99m F11-39 and Tc-99m ChF11-39. The subtracted images of Tc-99m labeled control IgG1 in MKN-45 tumor tissue were obscured at 1 hr, but were slightly visualized at 3 hr.

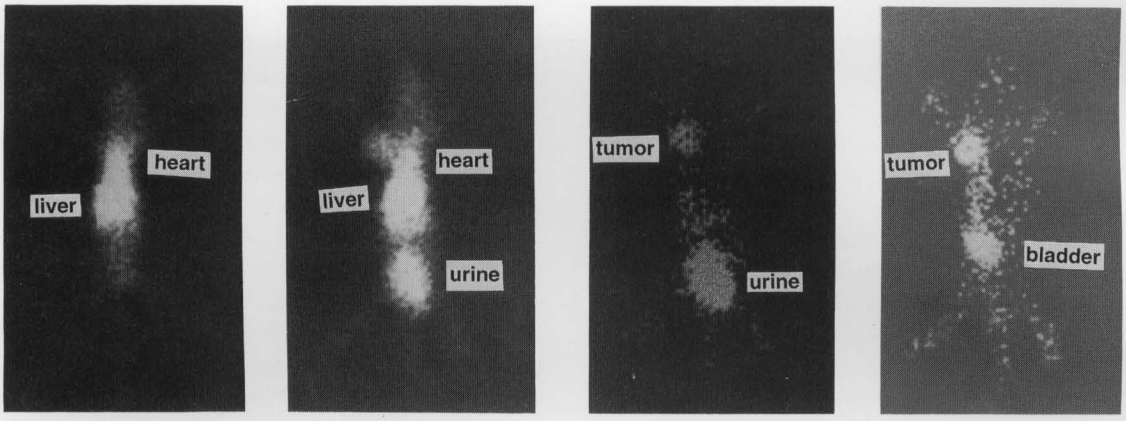

Fig. 1 Scintigrams in an athymic nude mouse bearing human CEA-producing tumor xenografts with Tc-99m F11-39. The tumor size was 1.0 cm in diameter. A: The initial (background) image at 3 min after injection of Tc-99m F11-39. B: The distribution image at 3 hr after injection of Tc-99m F11-39. C: The subtracted image of A from B after equalization of total counts. The subtraction factor was 1.0. D: The subtracted image of A after removal of urine activity from B. The subtraction factor was about 0.95. The subtracted images demonstrated that the transplanted tumor and bladder were clearly visible.

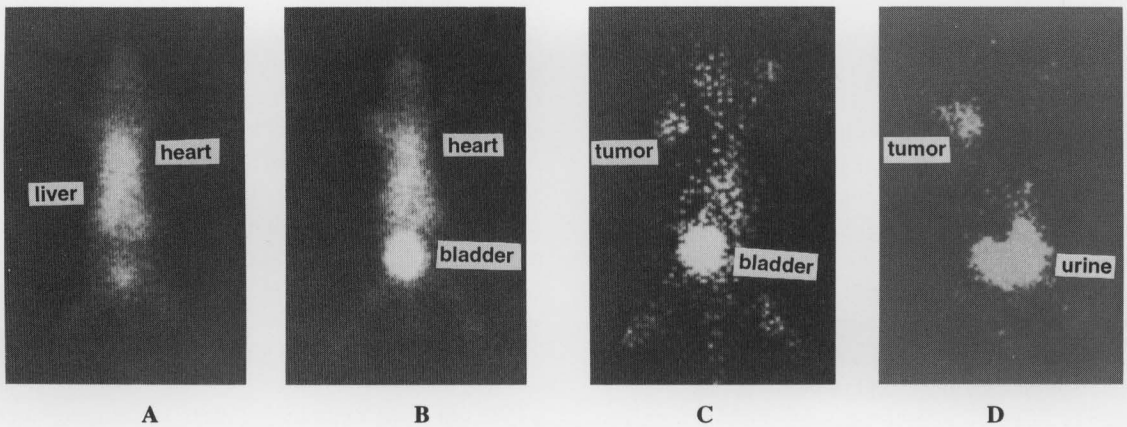

Fig. 2 Scintigrams in an athymic nude mouse bearing human CEA-producing tumor xenografts with Tc-99m ChF11-39. The tumor size was 0.8 cm in diameter. A: The initial (background) image at 3 min after injection of Tc-99m ChF11-39. B: The distribution image at 3 hr after injection of Tc-99m ChF11-39. C: The subtracted image of A from B after equalization of total counts. The subtraction factor was 1.0. D: The subtracted image of A from scintigram at 4 hr after equalization of total counts. The subtraction factor was 1.0. The subtracted images demonstrated that the transplanted tumor and bladder were clearly visible.

DISCUSSION

The objective of our study was to evaluate the subtraction of the initial Tc-99m image from the localization image of Tc-99m labeled antibody after a few hours as a potentially useful diagnostic method for various malignant tumors. For tumor immunoscintigraphy, it is necessary to prepare a superior monoclonal antibody and to label it with Tc-99m without the loss of immunoreactivity of the monoclonal antibody. F11-39 and ChF11-39 have been found to recognize the protein epitopes present on domain B3 of the CEA molecule and to discriminate CEA in tumor tissues from the CEA-related antigens.[23,25] Even though other factors such as the antibody delivery and the tumor biology may be involved in limiting tumor targeting, the antigenic content of tumors has a positive correlation with uptake of monoclonal antibodies *in vivo*.[5] After the Tc-99m labeling procedure, both Tc-99m F11-39 and Tc-99m ChF11-39 possessed satisfactory immunoreactivity for CEA in the cell binding assay,[26] but a large proportion of Tc-99m antibody remained in the circulation and other normal tissues and obscured specific tumor imaging.

We examined the subtraction of the initial Tc-99m image from the Tc-99m localization image after a few hours. The initial image corresponds to the vascular activity, and the localization image after a few hours

corresponds to the distribution activity. After equalization of the count or the time (considered the attenuation of radioactivity) required for collection of the image, the initial image is subtracted from the localization image after a few hours to show the distribution of the antibody. The subtracted images are equal to the *in vivo* behavior of the antibody involving distribution, metabolism, excretion and ligand exchange reaction. There are several advantages in this subtraction method. We can detect the small fraction of monoclonal antibody localized by the tumor. It is not necessary to accelerate the disappearance of the background, and this method can also ignore the effect of the immune complex in the circulation. Furthermore, the physical properties of Tc-99m are ideal for gamma camera imaging. It does not have the problems of different distribution and energy, nor the disadvantage of giving additional radiation exposure to the patient compared with the subtraction method introduced by Goldenberg et al.,[1,6] but the technique of subtraction of the initial image requires painstaking positioning.

Subtracted scintigrams of athymic nude mice bearing MKN-45 xenografts demonstrated that the transplanted tumor and bladder were clearly visible after a few hours with both Tc-99m F11-39 and Tc-99m ChF11-39. The congested sites in the legs were faintly visualized by the ties. Tumor uptake increases with time after administration of the antibody, and the best time for imaging is 2–4 hr for consideration of tumor accumulation of the antibody, measurement time after injection, and attenuation of radioactivity. It would take much longer for the antibodies to cross tumor capillaries in the case of a 60 kg human subject. Subtracted scintigrams for both Tc-99m mouse IgG1 and human IgG1 showed that tumor sites were obscured at 1 hr and then tumor sites were slightly visible at 3 hr, but the counts in the tumor sites for both Tc-99m mouse IgG1 and human IgG1 were lower than those for Tc-99m F11-39 and ChF11-39. The vascularization and permeability of tumor capillaries are often observed with radiolabeled IgG1. We found that MKN-45 tumor tissues have a high blood flow and leakage from blood vessels by subtraction. Various radiolabeled nonantigen-specific IgGs have been shown to localize at sites of infection or inflammation in animals and human subjects.[27–29] There was good evidence, therefore, that tumor imaging with Tc-99m labeled antibodies was usually dependent on the specificity of the antibody, and the vascularization and permeability of the tumor tissue. Although the method of subtraction of the initial image served as a useful diagnostic method for an abnormal site, imaging results did not distinguish between affinity and vascularization in tumor tissue.

In these animal models, the location of the tumor tissue was known and mostly external to the mice. Early-late subtraction methods often have artifacts close to major blood vessels due to the distribution of antibody into extravascular spaces. These artifacts would not be apparent in a mouse model where these vessels were not visualized. The small quantity of radioactivity bound specifically by the tumor causes a problem in the manipulation of the data.

Technetium-99m should remain firmly bound to the antibody after administration. Technetium-99m F11-39 remained in the circulation long enough to be incorporated into the tumor site in the distribution study. On the other hand, a higher excretion of Tc-99m ChF11-39 was observed when compared to the corresponding Tc-99m F11-39. These findings could be explained by ligand exchange, immune response, and the metabolism of ChF11-39. Technetium-99m labeled antibody should diffuse into the blood where considerable concentrations of other chelating molecules and metal ions are present. An *in vivo* breakdown of Tc-99m ChF11-39 in the circulation may contribute to significant urinary excretion at some time after injection. The thin layer chromatography of urine activity showed another peak compared with the Tc-99m antibody peak, indicating that ligand exchange had occurred. The chromatographic peak in the urine analysis had the same Rf value as the peak due to Tc-99m cystinate.[26] In mice, the lower chelating ability of chimeric antibody results in the subsequently administered antibody being rapidly cleared from the circulation and thus not efficiently reaching the tumor sites. This intense bladder radioactivity appears to reduce the detection sensitivity in the lower abdomen. Although lower tumor uptake and rapid clearance of Tc-99m ChF11-39 were greater than with Tc-99m F11-39, the absence of extra tumor activity promised high immunoreactivity for this agent. The use of mouse-human chimeric antibody reduces the human immune response, so that Tc-99m ChF11-39 is suitable for human clinical use.

We conclude that our technique appears to be convenient for routine investigations with Tc-99m antibodies in animals. Subtracted images of the same count reflected the *in vivo* behavior of the Tc-99m radioactivity. The subtracted scintigrams provided excellent tumor images with no significant extrarenal background. Visualization of the tumor site was dependent on antigen-specific binding and nonspecific exudation. These results demonstrate that a method of subtraction of the initial image may serve as a potentially useful diagnostic method for labeled antibodies with slow pharmacokinetics.

ACKNOWLEDGMENT

This work was supported in part by a fund from the Central Research Institute of Fukuoka University.

REFERENCES

1. Goldenberg DM, Kim EE, DeLand FH, Bennett S, Primus FJ. Radioimmunodetection of cancer with radioactive antibodies to carcinoembryonic antigen. *Cancer Res* 40: 2984–2992, 1980.

2. Larson SM. Clinical radioimmunodetection, 1978–1988: Overview and suggestions for standardization of clinical trials. *Cancer Res* 50: 892–898, 1990.
3. Mach J-P, Carrel S, Merenda C, Sordat B, Cerottini J-C. *In vivo* localisation of radiolabelled antibodies to carcinoembryonic antigen in human colon carcinoma grafted into nude mice. *Nature* 248: 704–706, 1974.
4. Zimmer AM, Kazikiewicz JM, Rosen ST, Spies SM. Pharmacokinetics of ^{99m}Tc(Sn)- and ^{131}I-labeled anti-carcinoembryonic antigen monoclonal antibody fragments in nude mice. *Cancer Res* 47: 1691–1694, 1987.
5. Haruno M, Kuroki M, Matsunaga K, Takata J, Karube Y, Senba T, et al. Tumor-Specific Accumulation of ^{125}I-Labeled Mouse-Human Chimeric Anti-CEA Antibody in a Xenografted Human Cancer Model Demonstrated by Whole-body Autoradiography and Immunostaining. *Nucl Med Biol* 23: 821–826, 1996.
6. Goldenberg DM, DeLand F, Kim E, Bennett S, Primus FJ, van Nagell Jr JR, et al. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. *N Engl J Med* 298: 1384–1388, 1978.
7. Begent RHJ, Searle F, Stanway G, Jewkes RF, Jones BE, Vernon P, et al. Radioimmunolocalization of tumours by external scintigraphy after administration of ^{131}I antibody to human chorionic gonadotrophin: preliminary communication. *J R Soc Med* 73: 624–630, 1980.
8. Dykes PW, Hine KR, Bradwell AR, Blackburn JC, Reeder TA, Drolc Z, et al. Localisation of tumour deposits by external scanning after injection of radiolabelled anti-carcinoembryonic antigen. *Br Med J* 280: 220–222, 1980.
9. Green AJ, Begent RHJ, Keep PA, Bagshawe KD. Analysis of radioimmunodetection of tumors by the subtraction technique. *J Nucl Med* 25: 96–100, 1984.
10. Halsall AK, Fairweather DS, Bradwell AR, Blackburn JC, Dykes PW, Howell A, et al. Localisation of malignant germ-cell tumours by external scanning after injection of radiolabelled anti-alpha-fetoprotein. *Br Med J* 283: 942–944, 1981.
11. Mach J-P, Carrel S, Forni M, Ritschard J, Donath A, Alberto P. Tumor localization of radiolabeled antibodies against carcinoembryonic antigen in patients with carcinoma, a critical evaluation. *N Engl J Med* 303: 5–10, 1980.
12. Searle F, Bagshawe KD, Begent RHJ, Jewkes RF, Jones BE, Keep PA, et al. Radioimmunolocalisation of tumours by external scintigraphy after administration of ^{131}I antibody to carcinoembryonic antigen. *Nucl Med Commun* 1: 131–139, 1980.
13. Hawkins EB, Pant KD, Rhodes BA. Resistance of direct Tc-99m-protein bond to transchelation. *Antibod Immunol Radiopharm* 3: 17–25, 1990.
14. Hnatowich DJ, Mardirossian G, Rusckowski M, Fogarasi M, Virzi F, Winnard Jr P. Directly and indirectly technetium-99m-labeled antibodies—A comparison of *in vitro* and animal *in vivo* properties. *J Nucl Med* 34: 109–119, 1993.
15. Mather SJ, Ellison D. Reduction-mediated technetium-99m labeling of monoclonal antibodies. *J Nucl Med* 31: 692–697, 1990.
16. Pak KY, Nedelman MA, Tam SH, Wilson E, Daddona PE. Labeling and stability of radiolabeled antibody fragments by a direct ^{99m}Tc-labeling method. *Nucl Med Biol* 19: 669–677, 1992.
17. Rhodes BA. Direct labeling of proteins with ^{99m}Tc. *Nucl Med Biol* 18: 667–676, 1991.
18. Schwarz A, Steinstraßer A. A novel approach to Tc-99m labeled monoclonal antibodies. *J Nucl Med* 28: 721, 1987. (Abstract)
19. Mardirossian G, Wu C, Rusckowski M, Hnatowich DJ. The stability of ^{99}Tc^{m} directly labelled to an Fab′ antibody via stannous ion and mercaptoethanol reduction. *Nucl Med Commun* 13: 503–512, 1992.
20. Govindan SV, Goldenberg DM, Griffiths GL, Leung SO, Losman MJ, Hansen HJ. Site-specific modifications of light chain glycosylated antilymphoma (LL2) and anti-carcinoembryonic antigen (hImmu-14-N) antibody divalent fragments. *Cancer Res* 55: 5721s–5735s, 1995.
21. Behr TM, Becker WS, Bair HJ, Klein MW, Stuhler CM, Cidlinsky KP, et al. Comparison of complete versus fragmented technetium-99m-labeled anti-CEA monoclonal antibodies for immunoscintigraphy in colorectal cancer. *J Nucl Med* 36: 430–441, 1995.
22. Arakawa F, Haruno M, Kuroki M, Kanda H, Watanabe T, Misumi Y, et al. Construction and expression of two mouse-human chimeric antibodies with high specificity and affinity for carcinoembryonic antigen. *Hybridoma* 12: 365–379, 1993.
23. Haruno M, Kuroki M, Arakawa F, Kanda H, Watanabe T, Matsuoka Y. *In vitro* and *In vivo* characterization of two mouse-human chimeric antibodies with high specificity and affinity for carcinoembryonic antigen. *Antibod Immunol Radiopharm* 7: 133–148, 1994.
24. Kuroki M, Koga Y, Matsuoka Y. Purification and characterization of carcinoembryonic antigen-related antigens in normal adult feces. *Cancer Res* 41: 713–720, 1981.
25. Kuroki M, Arakawa F, Haruno M, Murakami M, Wakisaka M, Higuchi H, et al. Biochemical characterization of 25 distinct carcinoembryonic antigen (CEA) epitopes recognized by 57 monoclonal antibodies and categorized into seven groups in terms of domain structure of the CEA molecule. *Hybridoma* 11: 391–407, 1992.
26. Karube Y, Katsuno K, Takata J, Matsunaga K, Haruno M, Kuroki M, et al. Radioimmunoscintigraphy using technetium-99m-labeled parental mouse and mouse-human Chimeric antibodies to carcinoembryonic antigen in athymic nude mice-bearing tumor. *Nucl Med Biol* 23: 753–759, 1996.
27. Claessens RAMJ, Oyen WJG, van den Broek WJM, Corstens FHM, van der Meer JWM. Scintigraphic detection of inflammation sites with In-111 labeled polyclonal human gammaglobulin (IgG). *Eur J Nucl Med* 15: 454, 1989.
28. Rubin RH, Young LS, Hansen WP, Nedelman M, Wilkinson R, Nelles MJ, et al. Specific and nonspecific imaging of localized Fisher immunotype 1 Pseudomonas aeruginosa infection with radiolabeled monoclonal antibody. *J Nucl Med* 29: 651–656, 1988.
29. Serafini AN, Garty I, Vargas-Cuba R, Friedman A, Rauh DA, Neptune M, et al. Clinical evaluation of a scintigraphic method for diagnosing inflammation/infections using indium-111-labeled nonspecific human IgG. *J Nucl Med* 32: 2227–2232, 1991.