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A human/mouse chimeric monoclonal antibody against CA125 for radioimmunoimaging of ovarian cancer

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Abstract. Murine monoclonal antibody 196-14 recognizes the ovarian-cancer-associated antigen CA125, but the epitope it recognizes is different from that of monoclonal antibody OC125. We developed a human/mouse chimeric 196-14 using the variable regions of the murine 196-14 and human heavy-chain (γ1) and light-chain (κ) constant regions. Cell binding and competitive inhibition assays using chimeric 196-14 labeled with ^{125}I, ^{111}In or ^{99m}Tc demonstrated that the in vitro immunoreactivity of the chimeric antibody was identical to that of the parental murine monoclonal antibody. However, in mice bearing human ovarian cancer xenografts, the clearance from blood was faster and absolute levels of accumulation in the tumor were lower for the ^{125}I-labeled or ^{99m}Tc-labeled chimeric antibody than for the murine antibody labeled with the corresponding radionuclides. The tumor-to-blood radioactivity ratio was not significantly different between the chimeric antibody and the murine antibody, regardless of the radionuclide used for labeling. Chimeric antibody 196-14 labeled with ^{131}I, ^{111}In or ^{99m}Tc is promising for the radioimmunoimaging of ovarian cancer.

Key words: Monoclonal antibody – Immunodetection – CA125 – Ovarian cancer

Introduction

Most of the monoclonal antibodies (mAb) currently employed in immunoscintigraphy are of murine origin, and so are antigenic in humans, provoking an immune response to themselves when exposed to the human immune system. Anti-antibody production decreases the amount of radiolabeled antibody that is accumulated in the target [28]. Although the use of human mAb may solve these problems, the stable production of human mAb is quite difficult and only a few human are available at present [21, 32].

Recent advances in gene technology have made it possible to exchange mouse constant-region domains with human constant-region domains in the construction of a human/mouse chimeric antibody (Ab) [4, 18, 20]. The use of human/mouse chimeric Ab may reduce or eliminate the problems associated with murine mAb. By ligating the human heavy-chain enhancer element to chimeric light- and heavy-chain genes, we have been able to produce human/mouse chimeric Ab stably and efficiently [23].

Murine mAb 196-14 reacts with ovarian-cancer-associated antigen CA125 [2, 8]. The epitope recognized by 196-14 is, however, different from that recognized by mAb OC125. We have demonstrated that radiolabeled 196-14 specifically accumulated in human ovarian cancer xenografts in nude mice [24]. Human/mouse chimeric 196-14 was generated from the variable regions of murine 196-14 and human heavy-chain (γ1) and light-chain (κ) constant regions, and its in vitro and in vivo tumor targeting ability was tested.

Materials and methods

Cells. The human ovarian cancer cell line OVCAR3 [10] was obtained from American Type Culture Collection, Rockville, Md. The human uterine adenocarcinoma cell line TMCC1 [29] was kindly donated by Dr. Sakamoto (Tokyo Medical College, Tokyo). The cells expressed CA125 on the cell surface and were grown in RPMI-1640 medium (Nissui, Tokyo, Japan) containing 10% fetal calf serum (Gibco Laboratories, Grand Island, N.Y.) and 0.03% L-glutamine at 37°C in 5% CO_2. Subconfluent cells were removed using calcium and magnesium-free phosphate-buffered saline containing 0.02% EDTA.

Monoclonal antibodies. Parental murine mAb 196-14 (IgG_1 isotype) was raised against the human endometrial cancer cell line HOUA-I [13]. This mAb has been shown to be reactive with ovarian-cancer-associated antigen CA125 [24]. The epitope recognized by the 196-14 Ab was different from that recognized by the mAb OC125 [24].

A rearranged murine heavy-chain variable-region gene was cloned from a murine hybridoma that secreted 196-14. A constant-region human

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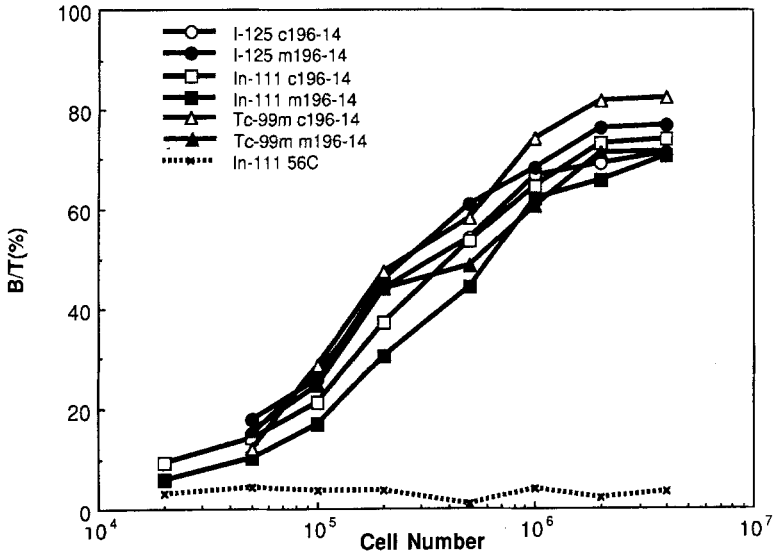

Fig. 1. Binding of ^{125}I-, ^{111}In-, or ^{99m}Tc-labeled murine and chimeric 196-14 Ab and ^{111}In-labeled control 56C antibody to TMCC1 cells. Percentage of radioactivity bound to cells was plotted against the number of cells. *c196-14*, chimeric 196-14; *m196-14*, murine 196-14

γ1 heavy-chain gene was obtained from human plasma cell leukemia ARH77 cells. These two genes were linked together to construct a human/mouse chimeric heavy-chain gene. A rearranged murine light-chain variable-region gene, cloned from the same hybridoma, was joined to a human κ light-chain constant-region gene. The chimeric heavy-chain gene and the chimeric light-chain gene were inserted into a plasmid pSV_2gpt to construct a single vector, and a non-producer mouse myeloma cell line, X63Ag8.653, was transfected with this vector. The stable transformants cells thus obtained produced 1–2 μg/ml chimeric 196-14 Ab in RPMI-1640 medium, containing 5% fetal calf serum, from which bovine immunoglobulin had been removed with protein-G–Sepharose 4B (Zymed Laboratories Inc., San Francisco, Calif.), and 0.03% L-glutamine at 37° C in 5% CO_2, and the chimeric Ab was purified by applying the culture medium to a goat anti-(human IgG) affinity column (American Qualex, La Mirada, Calif.).

mAb 56C (IgG_1), which recognized human chorionic gonadotropin, was used as an isotype-matched control Ab [27].

Radiolabeling of mAb. mAb were radioiodinated using the chloramine-T method, which has been previously reported in detail [24, 27]. The specific activity of the ^{125}I-labeled murine or chimeric 196-14 Ab was about 222 MBq/mg and that of the ^{131}I-labeled antibodies was 111 MBq/mg.

mAb were labeled with ^{111}In using diethylenetriaminepentaacetic acid (DTPA) as a bifunctional chelating agent. The method has been previously reported in detail [26]. The specific activity of the ^{111}In-labeled antibodies was about 111 MBq/mg.

mAb were labeled with ^{99m}Tc using the direct method [16, 31]. The mAb solution (5 mg/ml) in 0.05 M phosphate-buffered saline was incubated with 2-mercaptoethanol at a 2-mercaptoethanol : mAb molar ratio of 1 : 10000 for murine 196-14 or 1 : 1000 for chimeric 196-14 at room temperature for 30 min. The reduced antibody was purified on a PD-10 column. Purified Ab at a concentration of about 1 mg/ml was immediately divided into aliquots and stored at –40° C. The frozen Ab was thawed immediately prior to labeling. One vial from a HMDP bone-scanning kit (Nihon Medi-Physics Co., Takarazuka, Japan) was reconstituted with a 0.9% sodium chloride solution. A 5 μl sample of the solution was added to 50 μg reduced Ab, followed by 11.1 MBq pertechnetate eluted from a ^{99}Mo/^{99m}Tc generator (Daiichi Radioisotope, Tokyo, Japan). The labeling efficiency was more than 97% and ^{99m}Tc-labeled antibodies with specific activities of 222 MBq/mg were used without post-labeling purification.

In vitro binding assay. ^{125}I-labeled parental or chimeric 196-14 Ab (3–5 ng/50 μl) and unlabeled parental or chimeric Ab (0.01–1000 μg/50 μl) were incubated with 2×10^5 TMCC1 cells in 100 μl phosphate buffer for 2 h at 4° C. After centrifugation, the supernatant was aspirated and the tubes were cut. The radioactivity that was bound to the cells was counted in an auto-well gamma counter. Affinity constants and epitope densities of both Ab were calculated by Scatchard plot analysis [30].

In another experiment, radiolabeled parental murine and chimeric 196-14 Ab (3–5 ng/100 μl) were incubated with 5×10^4–4×10^6 TMCC1 cells suspended in 100 μl phosphate buffer in 5.7 × 46-mm microcentrifuge tubes for 2 h at 4° C. After centrifugation, the radioactivity that was bound to the cells was counted. The immunoreactive fraction of the radiolabeled Ab was calculated by the Lindmo method [15].

In vivo biodistribution study and scintigraphy of tumor-bearing mice. After the subcutaneous inoculation of male BALB/c-*nu/nu* mice with 5×10^6 OVCAR3 cells, mice bearing tumors weighing 500–800 mg at 8–11 weeks were used in the study.

Nonradioactive iodine was administered to mice from 1 day before the injection of radioiodinated Ab until the end of the experiment. Mice were anesthetized by ether inhalation and 50–100 μl blood was taken from the orbital cavity before injection of Ab. Serum CA125 levels were measured using a commercially available kit. Serum samples sufficient for use in assays were obtained from 31 mice. A dose of 37 MBq ^{125}I-, ^{111}In- or ^{99m}Tc-labeled parental or chimeric Ab was injected into mice via the tail vein. The Ab dose was adjusted to 10 μg/mouse by the addition of the corresponding unlabeled Ab. At designated times after injection, groups of mice were killed, the organs and tumor were weighed, and the radioactivity was counted. Biodistribution data were expressed as the percentage of the injected dose per gram of tissue normalized to a 20 g mouse, and tumor-to-normal tissue radioactivity ratios were calculated. Statistical analysis was done with Student's *t*-test.

After the injection either of 3.7 MBq ^{131}I- or ^{111}In-labeled chimeric 196-14, or of 37 MBq ^{99m}Tc-labeled chimeric 196-14 Ab, mice with xenografted tumors were imaged using a gamma camera equipped with a pinhole collimator. Mice were anesthetized by intraperitoneal injections of sodium pentobarbital and exposures of 50 kilocounts per image were taken.

Results

The binding of ^{125}I-labeled chimeric or murine 196-14 Ab to TMCC1 cells was inhibited in a similar fashion by both unlabeled parental and chimeric 196-14 Ab (data not shown). The affinity constant and the number of binding sites of murine and chimeric 196-14 mAb for TMCC1 cells were 1.6×10^9 M^{-1}, 2.2×10^4/cell for murine 196-14 and

Table 1. Biodistribution of ^{125}I-labeled chimeric 196-14 Ab and ^{125}I-labeled murine 196-14 Ab in the slow-clearance group of nude mice bearing OVCAR3 (mean ± SD)

Tissue	Distribution (% dose/g)					
	^{125}I-labeled chimeric 196-14			^{125}I-labeled murine 196-14		
	24 h (n = 9)	48 h (n = 10)	96 h (n = 7)	24 h (n = 12)	48 h (n = 11)	96 h (n = 6)
Blood	11.63±3.66*	7.57±2.15	3.04±0.94*	15.57±2.58	11.52±3.98	9.11±3.31
Liver	2.65±0.31*	2.02±0.42**	0.79±0.30*	3.64±0.94	2.83±1.07	2.36±0.26
Kidney	3.44±0.36*	2.10±0.64*	0.97±0.36*	4.54±1.10	3.54±1.25	2.27±0.72
Intestine	1.15±0.25**	0.70±0.15	0.26±0.08*	1.44±0.32	0.98±0.32	0.61±0.14
Stomach	1.76±0.81***	1.38±0.49***	0.32±0.26***	1.88±1.07	1.37±0.71	0.26±0.05
Spleen	2.72±0.80***	1.47±0.46***	0.70±0.17*	2.89±0.42	1.97±0.85	1.64±0.35
Lung	5.53±1.22**	3.79±1.16**	1.98±0.62*	6.96±1.54	5.47±2.02	5.00±1.93
Muscle	0.70±0.13*	0.71±0.20***	0.42±0.14**	1.06±0.19	0.88±0.23	1.12±0.62
Bone	1.07±0.23*	0.80±0.30**	0.38±0.10*	1.53±0.35	1.20±0.41	0.90±0.12
Tumor	4.11±1.09***	6.51±1.56***	4.99±1.34*	5.09±2.21	8.09±2.39	10.26±2.69

* P <0.01 compared with murine Ab
** P <0.05 compared with murine Ab
*** No significance

Table 2. Biodistribution of ^{99m}Tc-labeled chimeric 196-14 Ab and ^{99m}Tc-labeled murine 196-14 Ab in the slow-clearance group of nude mice bearing OVCAR3 (mean ± SD)

Tissue	Distribution (% dose/g)			
	^{99m}Tc-labeled chimeric 196-14		^{99m}Tc-labeled murine 196-14	
	6 h (n = 4)	24 h (n = 4)	6 h (n = 5)	24 h (n = 5)
Blood	9.37±1.30*	1.92±0.21*,**	17.75±3.52	6.92±0.44**
Liver	6.80±4.36***	2.22±0.05*,***	9.90±1.80	4.52±0.62
Kidney	10.25±0.83***	4.78±0.54***,***	9.16±1.96	4.91±0.70
Intestine	2.44±0.81***	0.65±0.16***,**	2.45±0.47	1.06±0.19
Stomach	0.74±0.27***	0.31±0.14*,**	1.08±0.37	0.86±0.27
Spleen	1.99±0.39***	1.16±0.11*,**	3.29±0.96	2.6 ±0.48
Lung	3.66±0.82***	1.16±0.11*,**	6.88±2.61	3.06±0.25
Muscle	0.43±0.13***	0.18±0.03*,**	0.48±0.08	0.53±0.11
Bone	1.03±0.38***	0.33±0.08*,**	1.08±0.26	0.96±0.25
Tumor	1.90±0.81***	1.42±0.18*,**	2.68±1.08	3.58±0.47

* P <0.01 compared with murine Ab
** P <0.01 compared with ^{125}I-labeled corresponding Ab
*** No significance

Table 3. Biodistribution of ^{111}In-labeled chimeric 196-14 Ab and ^{111}In-labeled murine 196-14 Ab in the slow-clearance group of nude mice bearing OVCAR3 (mean ± SD)

Tissue	Distribution (% dose/g)					
	^{111}In-labeled chimeric 196-14			^{111}In-labeled murine 196-14		
	24 h (n = 2)	48 h (n = 4)	96 h (n = 3)	24 h (n = 4)	48 h (n = 5)	96 h (n = 4)
Blood	11.40	6.51±1.01	2.6 ±1.12	10.82±3.04	6.24±2.19	1.42± 0.77
Liver	18.59	14.26±1.84	14.47±1.53	9.69±5.29	12.32±4.79	10.42± 3.80
Kidney	15.11	15.97±0.83	18.82±1.49	13.39±3.96	15.33±3.14	12.98± 3.59
Intestine	2.63	2.29±0.11	1.62±0.28	2.23±0.45	2.63±0.34	1.55± 0.51
Stomach	1.44	1.02±0.35	0.98±2.28	0.79±0.25	1.61±1.28	1.26± 0.33
Spleen	9.37	8.04±2.01	12.77±0.12	6.62±1.68	7.24±0.51	5.73± 2.33
Lung	5.20	5.32±2.75	3.73±1.12	5.73±1.85	4.59±1.10	2.63± 0.85
Muscle	0.97	0.92±0.18	0.87±0.41	1.17±0.24	0.99±0.17	0.67± 0.21
Bone	2.70	2.27±0.30	1.89±0.35	2.93±0.65	2.88±0.75	1.61± 0.59
Tumor	8.75	13.81±3.76	16.47±3.91	5.49±1.16	12.00±5.07	16.15±10.38

Table 4. Tumor-to-blood radioactivity ratio of radiolabeled chimeric 196-14 Ab and murine 196-14 Ab in nude mice of the slow-clearance group (mean ± SD)[a]

Labeled Ab	Radioactivity ratio: tumor/blood			
	6 h	24 h	48 h	96 h
^{125}I chimeric 196-14	0.15±0.08	0.37±0.11	0.91±0.11	1.78± 0.75
^{125}I murine 196-14	0.14±0.03	0.33±0.14	0.79±0.35	1.17± 0.24
^{111}In chimeric 196-14	ND	0.87	2.11±0.41*	6.97± 2.23*
^{111}In murine 196-14	ND	0.54±0.16	1.90±0.41*	15.02±12.11**
^{99m}Tc chimeric 196-14	0.20±0.08	0.75±0.16*	ND	ND
^{99m}Tc murine 196-14	0.15±0.03	0.52±0.07**	ND	ND

[a] Number of mice is the same as in Tables 1–3

* P <0.01 compared with ^{125}I-labeled Ab

** P <0.05 compared with ^{125}I-labeled Ab

Table 5. Biodistribution of ^{125}I-labeled chimeric 196-14 Ab and ^{125}I-labeled murine 196-14 Ab in the rapid-clearance group of nude mice bearing OVCAR3 (mean and range)

Tissue	Distribution (% dose/g)				
	^{125}I-labeled chimeric 196-14			^{125}I-labeled murine 196-14	
	24 h (n = 4)	48 h (n = 3)	96 h (n = 3)	48 h (n = 2)	96 h (n = 3)
Blood	2.13 (1.39–2.88)	1.50 (0.71–2.97)	0.29 (0.09–0.51)	1.82 (0.66–2.97)	1.56 (0.86–2.25)
Liver	2.13 (1.80–2.86)	0.65 (0.32–1.22)	0.11 (0.06–0.15)	0.65 (0.32–0.97)	0.48 (0.29–0.63)
Kidney	0.88 (0.84–0.98)	0.47 (0.22–0.85)	0.12 (0.05–0.18)	0.51 (0.26–0.77)	0.53 (0.30–0.72)
Intestine	0.38 (0.24–0.51)	0.19 (0.09–0.35)	0.04 (0.02–0.05)	0.38 (0.26–0.50)	0.18 (0.12–0.23)
Stomach	1.36 (0.43–2.21)	0.93 (0.20–2.33)	0.05 (0.02–0.08)	0.32 (0.20–0.43)	0.66 (0.43–0.90)
Spleen	1.01 (0.30–1.51)	1.14 (0.32–2.50)	0.31 (0.16–0.45)	1.56 (0.50–2.62)	0.28 (0.13–0.36)
Lung	1.28 (0.69–1.62)	1.02 (0.51–1.90)	0.35 (0.27–0.50)	1.22 (0.49–1.95)	0.90 (0.66–1.16)
Muscle	0.38 (0.30–0.45)	0.24 (0.12–0.35)	0.15 (0.04–0.29)	0.25 (0.15–0.34)	0.23 (0.16–0.26)
Bone	0.49 (0.33–0.58)	0.22 (0.12–0.35)	0.09 (0.03–0.13)	0.34 (0.15–0.52)	0.25 (0.19–0.30)
Tumor	2.59 (1.31–3.77)	2.59 (1.32–4.82)	1.44 (0.33–2.57)	3.49 (1.79–5.18)	3.11 (1.35–4.50)

2.2×10^9 M^{-1}, 2.1×10^4/cell for chimeric 196-14 Ab respectively. ^{125}I-, ^{111}In- and ^{99m}Tc-labeled parental and chimeric 196-14 Ab had almost identical curves for binding to TMCC1 (Fig. 1), and there was no significant difference in the immunoreactivity of all the labeled Ab. The immunoreactive fraction of chimeric 196-14 mAb was estimated to be more than 85%.

The results of the biodistribution studies showed that mice could be divided into two groups based on the clearance from the blood of radiolabeled antibodies. The percentage of the injected dose per gram of blood in the nude mice belonging to the rapid-clearance group was below the value of the mean of the slow-clearance group by more than two standard deviations. The biodistribution data of the slow-clearance group is shown in Tables 1–3. The ^{125}I- and ^{99m}Tc-labeled chimeric 196-14 Ab were significantly more rapidly cleared from the blood than the ^{125}I- and ^{99m}Tc-labeled murine 196-14 Ab (Tables 1, 2). ^{111}In-labeled chimeric 196-14 was cleared from the circulation with kinetics similar to that of the ^{111}In-labeled murine 196-14 (Table 3). Although the absolute level of the accumulation of ^{125}I- and ^{99m}Tc-labeled chimeric 196-14 in the tumor was lower than that of murine 196-14 labeled with the corresponding radionuclides, the tumor-to-blood ratio was not significantly different between the chimeric and murine Ab, regardless of the radionuclide used for labeling (Table 4). Of the ^{125}I-, ^{111}In- and ^{99m}Tc-labeled chimeric 196-14 Ab, the ^{99m}Tc-labeled Ab showed the most rapid clearance 24 h after injection.

In the rapid-clearance group, serum CA125 was measured in 7 mice and CA125 levels of over 25 U/ml in 6 of 7 mice, ranging from 25.7 U/ml to 85.1 U/ml, were observed. Serum CA125 levels were measured in 24 mice in the slow-clearance group. CA125 levels were below 25 U/ml in all 24 animals.

The biodistribution data of mice that received ^{125}I-labeled 196-14 in the rapid-clearance group are shown in Table 5. In the mice of the rapid-clearance group, tumor-to-normal tissue ratios were remarkably higher than those of the mice in the slow-clearance group, for both the chimeric and murine 196-14. The tumor-to-blood ratio 96 h after injection was 4.68±0.70 for chimeric 196-14 (P <0.01 compared to the slow-clearance group) and 1.93±0.30 for murine 196-14 (P <0.01 compared to the slow-clearance group). Although the number of mice that received ^{111}In- or ^{99m}Tc-labeled 196-14 and belonged to the rapid-clearance group was small, those mice tended to have high circulating CA125 levels and had high tumor-to-blood ratios.

The absolute uptake of radiolabeled Ab by the tumor and the tumor-to-blood ratios of all radiolabeled 196-14 Ab were sufficient for imaging, and the xenografted tumor was clearly elucidated by ^{131}I-, ^{111}In- and ^{99m}Tc-labeled chimeric 196-14 Ab (Figs. 2–4).

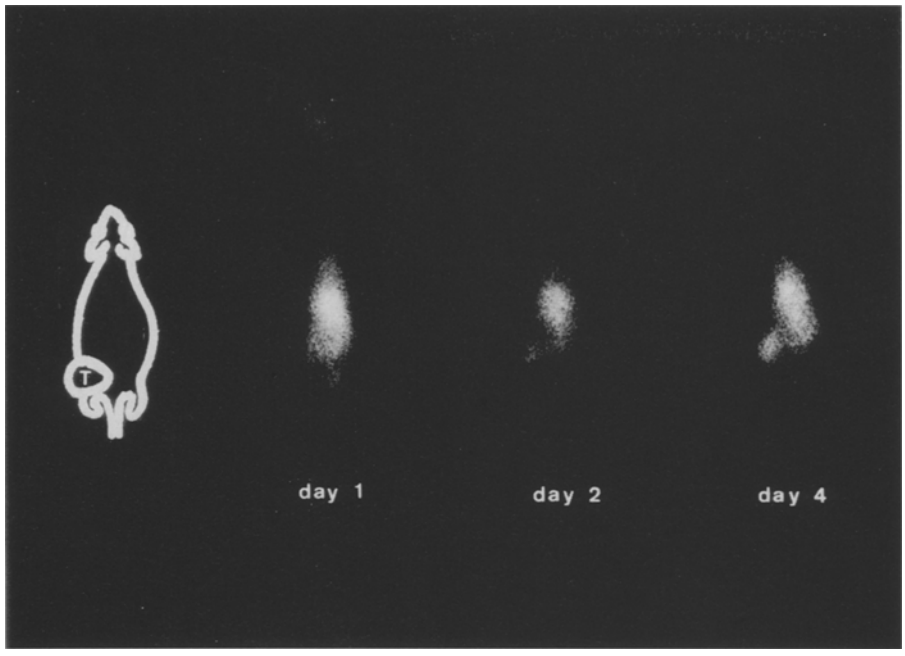

Fig. 2. Scintigrams of a mouse bearing an OVCAR3 human ovarian cancer cell xenograft. Images were obtained 24, 48 and 96 h after the injection of ^{131}I-labeled chimeric 196-14 Ab. *T*, the site of the xenografted tumor

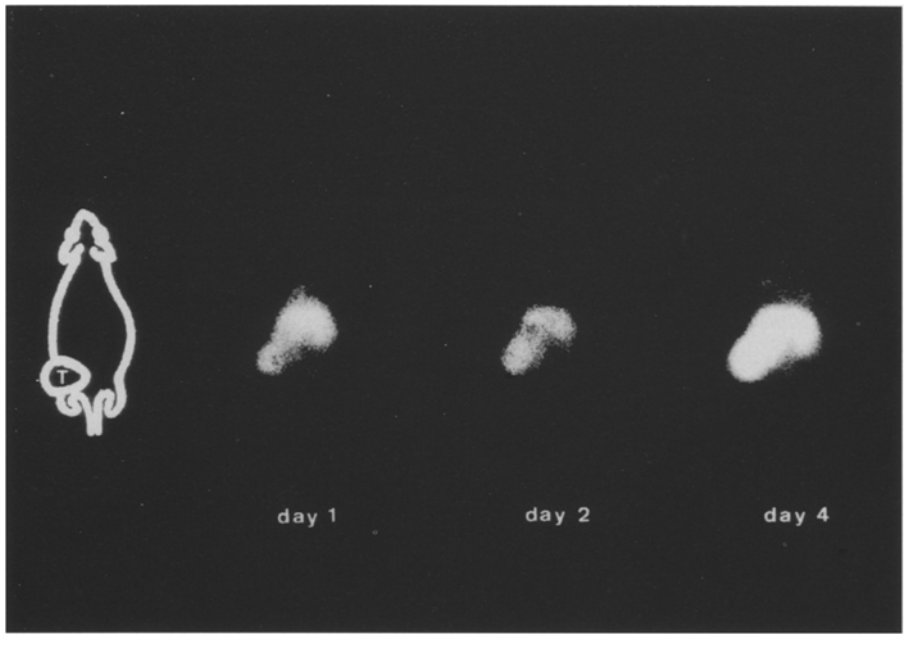

Fig. 3. Scintigrams of a mouse bearing an OVCAR3 human ovarian cancer cell xenograft. Images were obtained 24, 48 and 96 h after the injection of ^{111}In-labeled chimeric 196-14 Ab. *T*, the site of the xenografted tumor

Discussion

Advances in gene technology have made it possible to manipulate immunoglobulin genes and to produce human/mouse chimeric mAb [4, 18]. A chimeric Ab has advantages over a murine mAb when it is used in vivo. Several chimeric Ab have been produced and their characteristics have been reported. In humans, immune responses against most chimeric Ab are low compared to those against murine Ab [5, 14, 17, 25].

CA125 is a useful tumor marker in ovarian cancer patients. Parental murine mAb 196-14 recognized CA125 and accumulated well in a xenografted human ovarian cancer tumor that expressed CA125 [24]. A human/mouse chimeric mAb specific for CA125 has been constructed by ligating human heavy-chain enhancer elements to chimeric heavy- and light-chain genes. Cells obtained after transfection with this construct secreted high levels of chimeric Ab.

In vitro binding and competitive inhibition assays indicated that the parental murine and chimeric Ab competed for binding sites, and ^{125}I-, ^{111}In- or ^{99m}Tc-labeled chimeric 196-14 Ab had immunoreactivity similar to that of the parental murine 196-14 Ab. The replacement of the murine constant-region domain with its human counterpart did not reduce or alter the original immunoreactivity.

^{111}In and ^{99m}Tc have many advantages over radioiodine for clinical use and have been employed mainly in the labeling of antitumor or other mAb [1, 6, 12, 22]. After

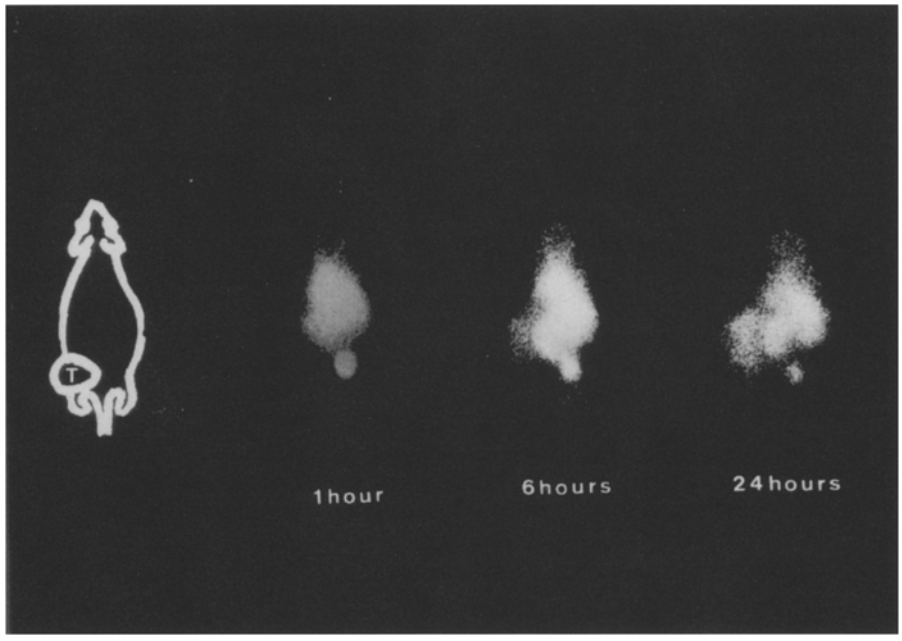

Fig. 4. Scintigrams of a mouse bearing an OVCAR3 human ovarian cancer cell xenograft. Images were obtained 1, 6 and 24 h after the injection of ^{99m}Tc-labeled chimeric 196-14 Ab. *T*, the site of the xenografted tumor

conjugation with cyclic DTPA anhydride, ^{111}In was efficiently coupled to the Ab through chelation with DTPA. After reduction of the Ab by 2-mercaptoethanol, ^{99m}Tc was efficiently coupled directly to the Ab. This makes it possible to construct a kit for radiolabeling Ab. In this study, we showed that both chimeric Ab and murine mAb could be directly labeled with ^{99m}Tc.

In vivo biodistribution studies demonstrated that ^{125}I-, ^{111}In- and ^{99m}Tc-labeled chimeric 196-14 Ab could be localized to the xenografted tumors in the same way as radiolabeled parental murine 196-14 Ab. The tumor-to-normal tissue ratios of all of the radiolabeled chimeric Ab increased with time, which indicated specific localization to the tumor site.

Of 31 mice bearing OVCAR3 tumors, 6 were thought to have considerable amounts of CA125 in the serum. Mice with higher serum CA125 levels had faster blood clearance and higher tumor-to-normal tissue ratios. Circulating CA125 may enhance tumor imaging when anti-CA125 Ab are used in ovarian cancer patients, or at least may not be a disadvantage.

Clinical trials using monoclonal Ab OC125 for imaging or therapy of ovarian cancer have been successful [3, 7, 9]. However, a high incidence of human anti-(mouse antibody) (HAMA) production was reported [19]. Anti-idiotype HAMA was also reported in patients who received the OC125 Ab [11]. Murine 196-14 Ab recognizes a different epitope from OC125, and chimeric 196-14 has three major advantages over OC125 for use in vivo. First, chimeric 196-14 is expected to be less immunogenic, the anti-antibody response may be reduced. Secondly, serum CA125 levels can be monitored, even immediately after injection of chimeric 196-14, circulating chimeric 196-14 would not interfere with the CA125 assay. Thirdly, anti-idiotype HAMA against OC125 resulted in false readings indicating high CA125 levels [11]. Even if anti-idiotype antibody against chimeric 196-14 develops, readings of serum CA125 levels will not be affected.

In mice from the slow-clearance group, the clearance from the blood of radiolabeled chimeric 196-14 Ab was more rapid than that of parental murine 196-14 Ab. The presence of human Fc may have resulted in faster blood clearance in mice. If so, it is expected that chimeric 196-14 would show slower blood clearance than murine Ab in humans. In fact, slower clearance of chimeric Ab has been reported in human studies [14, 17].

The human/mouse chimeric 196-14 Ab retained the full immunoreactivity of the parental murine 196-14 Ab after labeling with radioiodine, ^{111}In and ^{99m}Tc. Radiolabeled chimeric 196-14 localized well to xenografted tumors and is expected to be less immunogenic than the murine form. The clinical application of radiolabeled human/mouse chimeric 196-14 Ab appears promising.

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