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Effect of Circulating Antigen on Immunoscintigraphy of Ovarian Cancer Patients Using Anti-CA125 Monoclonal Antibody

Harumi Sakahara,[1] Makoto Hosono,[1] Hisataka Kobayashi,[1] Zhengsheng Yao,[1] Tsuneo Saga,[1] Shinsuke Yano,[1] Keigo Endo,[2] Takahide Mori[3] and Junji Konishi[1]

Departments of [1]Nuclear Medicine and [3]Gynecology and Obstetrics, Faculty of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-01 and [2]Department of Nuclear Medicine, Gunma University School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371

The murine monoclonal antibody (mAb) 145-9 recognizes an epitope present on CA125 but different from the epitope defined by the mAb OC125. To evaluate the clinical usefulness of the 145-9 antibody, immunoscintigraphy was performed in ovarian cancer patients and the effect of circulating CA125 on tumor imaging was investigated. Two milligrams (74 MBq) of ^{111}In-labeled 145-9 was injected intravenously into 11 patients with ovarian cancer. Pre-injection serum CA125 concentrations were between 166 U/ml and 7414 U/ml. Tumors were visualized in 10 of 11 patients. In two patients, lymph nodes that were not detected by other imaging modalities but were clinically suspected as metastases were visualized. There was no correlation between serum CA125 level and antibody uptake in the tumors. Immune complexes between the antibody and circulating antigen were observed in sera of all the patients, but the fraction of radioactivity in complex form did not correlate well with serum CA125 levels. The immune complexes survived in the circulation and the circulating radiolabel, including immune complexes, was still bound to solid-phase CA125. The plasma clearance rate and hepatic uptake of the antibody were not significantly affected by circulating CA125. In conclusion, the antibody 145-9 formed complexes with CA125 *in vivo* but this did not compromise the outcome of antibody imaging. The antibody 145-9 can be used in immunoscintigraphy of ovarian cancer irrespective of serum CA125 level.

Key words: CA125 — Immune complex — Immunoscintigraphy — Ovarian cancer

Several monoclonal antibodies (mAbs) have been tested for the detection of primary tumors, recurrences and metastatic disease of ovarian cancer.[1–9] One of these antibodies which is promising is the mAb OC125.[4, 10] The antibody OC125 recognizes a high-molecular-weight glycoprotein, CA125, detected in sera of more than 80% of ovarian cancer patients.[11, 12] When the OC125 antibody was administered to ovarian cancer patients, formation of immune complexes with circulating CA125 was observed.[13, 14] Although the immune complex formation does not affect the pharmacokinetics of the antibody in patients,[13, 14] it is not clear whether it interferes with tumor targeting of the antibody.

The murine mAb 145-9 recognizes an epitope present on CA125. The antibody 145-9, however, is not cross-reactive with the mAb OC125.[15] Previously we reported the clinical usefulness of ^{131}I-labeled 145-9 antibody in the immunoscintigraphy of ovarian cancer.[16] In the present study, the antibody was labeled with ^{111}In and the effects of circulating CA125 on tumor imaging and pharmacokinetics of the antibody were evaluated in patients with ovarian cancer.

MATERIALS AND METHODS

mAb The antibody 145-9 is a murine monoclonal IgG_{2b} that was generated by fusing myeloma cells and spleen cells of mice immunized with the human lung cancer cell line PC-9.[15] The antibody recognizes CA125, but the epitope is different from that recognized by the antibody OC125.[15] The antibody 145-9 was purified from culture supernatant of hybridoma by protein A affinity column chromatography and conjugated with diethylenetriaminepentaacetic acid (DTPA) by the cyclic DTPA anhydride method.[17, 18] After removal of unconjugated DTPA, the antibody solution was divided into aliquots. Each vial containing 2 mg of the DTPA-conjugated antibody (1.167 ml) was kept frozen until use. Labeling was performed by thawing and incubating the DTPA-conjugated antibody with 74 MBq of ^{111}In (1 ml in 0.1 *N* HCl, Nihon Mediphysics, Takarazuka). The labeling efficiency was determined by high-performance liquid chromatography (HPLC) with a TSK3000SW column (Tosoh, Tokyo) eluted at a flow rate of 1 ml/min. The labeling efficiency was also examined by silica-gel thin-layer chromatography (TLC). The immunoreactive fraction of the radiolabeled antibody was tested using CA125-coated beads. CA125-coated beads were prepared by incubating OC125-coated beads with ascites obtained from an ovarian cancer patient. OC125-coated beads were obtained from a CA125 immunoradiometric assay kit (ELSA CA 125, CIS Bio International, Gif-sur-Yvette, France). The radiolabeled antibody was incubated with CA125 beads. After exhaustive absorption (three times, each for 1 h at

Table I. Patients' Profiles and Results of Immunoscintigraphy

No.	Age	Primary or recurrent	CA125 (U/ml)	Diagnosis before immunoscintigraphy	Scan	%Dose/gram of tumor (day)[a]	Tumor /Serum[b]
1	60	Recurrent	166	Lymph node metastases	(+)	ND[c]	ND
2	40	Recurrent	415	Pelvic tumor	(+)	ND	ND
3	52	Recurrent	435	Pelvic tumor	(+)	ND	ND
4	62	Primary	5778	Pelvic tumor	(+)	0.00689 (6)	2.8
5	43	Primary	2230	Pelvic tumor	(+)	0.00474 (5)	2.7
				Peritoneal dissemination	(+)	0.00445 (5)	2.5
6	62	Primary	7414	Pelvic tumor	(+)	ND	ND
				Peritoneal dissemination	(−)	ND	ND
				Liver and lung metastases	(−)	ND	ND
7	64	Primary	710	Pelvic tumor	(+)	0.00288 (3)	0.8
8	26	Primary	4280	Pelvic tumor	(+)	0.00121 (7)	4.9
9	44	Primary	7065	Pelvic tumor	(+)	ND	ND
				Peritoneal dissemination	(+)	0.00953 (4)	4.3
				Liver metastases	(−)	ND	ND
10	36	Recurrent	248	Undetermined	(−)	ND	ND
11	72	Recurrent	2763	Lymph node metastases	(+)	ND	ND

a) Percentage of the injected dose per gram of tumor (day of operation).
b) Tumor-to-serum radioactivity ratio.
c) Not determined.

room temperature), the cumulative percentage of radioactivity bound to the beads was determined.

Patients Eleven patients who had histologically confirmed ovarian cancer were examined (Table I). Six patients had primary tumors and five had recurrent tumors. Serum CA125 concentrations were assayed using an immunoradiometric assay kit (ELSA CA125 or CA125II, CIS Bio International). The CA125 kit was employed for patients 1–3 and the CA125II kit for patients 4–11. Serum CA125 in the patients was between 166 U/ml and 7414 U/ml. Diagnoses before immunoscintigraphy are shown in Table I. Tumors were not visualized by conventional imaging studies in 2 patients before immunoscintigraphy. In patient 1, serum CA125 was still high after shrinkage of swollen para-aortic lymph nodes by external radiation therapy. No tumor was detected despite the elevation of serum CA125 in patient 10. All patients gave their informed consent to participation in the study, which was approved by the Ethical Review Committee of the Faculty of Medicine, Kyoto University.

Imaging protocol The patients were given 74 MBq and 2 mg of ^{111}In-labeled 145-9 intravenously over a period of 5 min. Anterior and posterior whole-body images were obtained 10 min, 1, 2, and 3 or 4 days after the injection. Planar digital spot images of anterior and posterior views and SPECT images of the abdomen were obtained on day 2 or day 3. One patient underwent repeat imaging on day 7. All scintiscans were obtained on a large-field-of-view gamma camera with tomographic capability (Gamma View-150E, Hitachi Medical Co., Tokyo). A medium-energy collimator was used with 20% windows centered over the 173 and 247 keV photon peaks. Photons were collected for 15 cm/min on whole-body images and for 300 seconds per spot image. SPECT images were acquired over 360 degrees with a 64×64 matrix using 64 stops of 20 seconds each. SPECT projections were spatially smoothed and reconstructed using a filtered back-projection technique with a Butterworth filter. To calculate the hepatic uptake of the labeled antibody, the geometrical mean of counts over the whole body and entire liver was obtained after correction for physical decay. The hepatic uptake was expressed as the percentage of the injected dose assuming the whole body counts at 10 min to be the injected counts.

Blood was drawn at 5 min, 1 h, 3 h, 16 h, 2 days, 3 days, 4 days, and up to 7 days after antibody injection, when feasible. Urine was collected daily up to 72 h after infusion. Tumors were resected after 3–7 days in 5 patients. Radioactivities of serum, urine and tissue specimens were counted in an auto-well gamma counter with the reference standard of the injectate. Uptake of the radiolabeled antibody in the tumor was expressed as percentage of the injected dose per gram (%ID/gram).

Serum analysis Serum samples obtained after radiolabeled antibody injection were subjected to HPLC to assess immune complex formation. The fraction of high-molecular-weight complexes was determined by comparing the integrated radiation profiles of the complexed and free antibody. The antigen-binding ability of blood-borne

radioactivity was evaluated using CA125-coated beads and compared with that of the injectate.

Human anti-murine antibody (HAMA) was assayed using an ELISA method (ImmuSTRIP HAMA IgG; Immunomedics Inc., Warren, NJ). Blood samples were taken prior to the antibody injection and at 1 and 4 weeks or more after administration. Values above 400 ng/ml were considered positive.[19]

RESULTS

The labeling efficiency calculated by the HPLC method corresponded well with that by the TLC method, and 98.8±0.7% (mean±SD) of the radioactivity was associated with IgG. As the labeling efficiency was very high, post-labeling purification was omitted. The immunoreactive fraction estimated by serial adsorption of the labeled antibody with CA125-coated beads was estimated to be 76.8±4.3% (mean±SD).

Tumors were visualized in 10 of 11 patients (Figs. 1 and 2). SPECT provided better anatomical definition of lesions but was not helpful for detection of tumors that were not detected in planar images. In two patients, lymph nodes that were not detected by other imaging modalities were visualized (patients 1 and 2). Small but definite accumulation was noted in the abdomen in patient 1, who had enlarged nodes there before external radiotherapy. In patient 2, bilateral inguinal lymph nodes and left supraclavicular lymph nodes were positive on immunoscintigraphy and became clinically apparent metastases several months later (Fig. 2). Metastatic tumors in lungs and in the liver were less than 1 cm and less than 2 cm in diameter, respectively, and were not detected in patients 6 and 9. Patient 10 had high serum CA125 but recurrent tumors were not detected by either conventional imaging studies or immunoscintigraphy. Her serum CA125 declined after chemotherapy. In 6 tumors from 5 patients, the percentage of the injected radioactivity accumulated in the tumor was between 0.00121 and 0.00953% per gram (mean; 0.00495%) and the tumor-to-serum radioactivity ratio ranged from 0.8 to 4.9

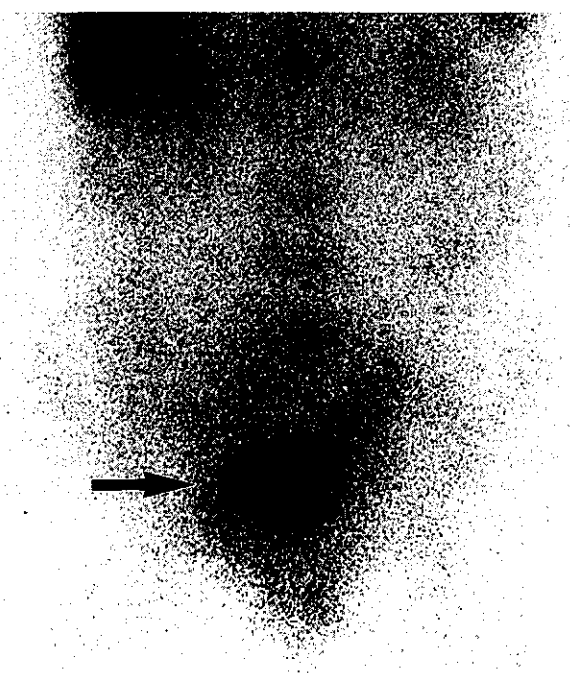

Fig. 1. Patient 6. Anterior planar image at the pelvic region 3 days after injection of ^{111}In-labeled 145-9 showing strong uptake of the antibody in primary ovarian cancer (arrow). Serum CA125 was 7414 U/ml.

Fig. 2. Patient 2. Recurrent tumor of 3 cm in diameter was found in the lower abdomen by ultrasonography. Immunoscintigraphy at 4 days revealed increased uptake in the left supraclavicular region, left iliac region and bilateral inguinal regions (arrows), as well as in the pelvic tumor (arrowhead). Lymph nodes in the regions of increased uptake were confirmed to be metastases several months later. Serum CA125 was 415 U/ml.

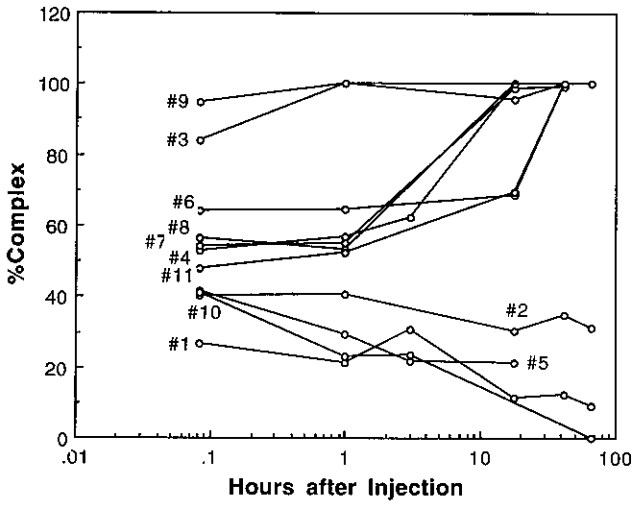

Fig. 3. Immune complex formation in patients' sera. Serum samples obtained after radiolabeled antibody injection were subjected to HPLC. The fraction of radioactivity present in high-molecular-weight complexes was determined by comparing the integrated radiation profiles of the complexed and free antibody. The numbers in the graph correspond to the patients' numbers.

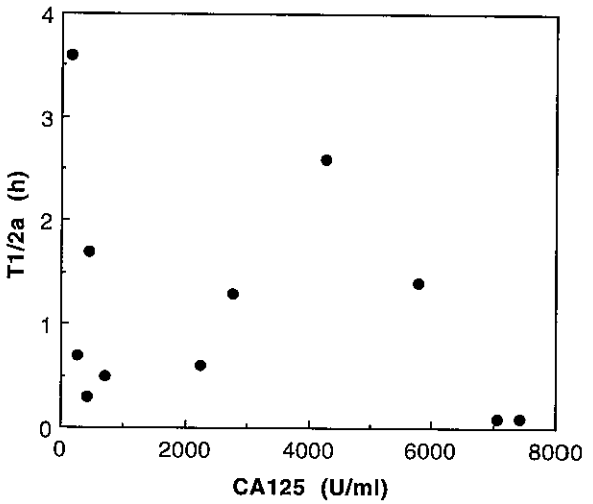

Fig. 4. There was no correlation between serum CA125 and T1/2α by the tests of simple correlation coefficient ($r=0.298$, $P>0.3$) and Spearman's rank correlation coefficient ($rs=0.421$, $P>0.1$).

(mean; 3.0). There was no correlation between serum CA125 levels and antibody uptake in the tumors either on gamma camera imaging or on direct tissue counting.

Immune complexes were observed in sera from all patients (Fig. 3). There was a tendency that the fraction of radioactivity in complex form was higher in patients with higher serum CA125. However, two patients with low CA125 (patients 3 and 7) had high complex formation and one patient with high CA125 (patient 5) had low complex formation. The plasma clearance curve was biphasic with a half-life of 0.1–3.6 h (mean; 1.2 h) for the first component (T1/2α) and 21.9–53.1 h (mean; 30.8h) for the second component (T1/2β). There was no correlation between serum CA125 and T1/2α by the tests of simple correlation coefficient and Spearman's rank correlation coefficient (Fig. 4). Although there was no statistically significant correlation between serum CA125 and T1/2β by the test of simple correlation coefficient, the Spearman's rank correlation procedure showed an inverse correlation (Fig. 5). The imaged biodistribution of the antibody in normal organs was not influenced by CA125 levels. The calculated hepatic uptake was also independent of serum CA125 (data not shown). Urinary excretions of ^{111}In at days 0–1, 1–2, 2–3 and 3–4 were 3.2 $\pm$1.0, 3.3$\pm$1.5, 3.6$\pm$1.7 and 3.6$\pm$1.9% of the injected dose (mean$\pm$SD), respectively. The circulating radiolabel, including the complex form, was bound to solid-phase CA125. The relative immunoreactivity of the

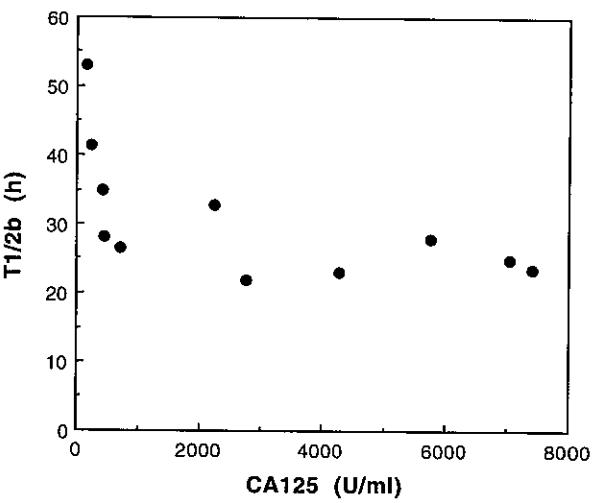

Fig. 5. Although there was no statistically significant correlation between serum CA125 and T1/2β by the test of simple correlation coefficient ($r=0.600$, $P>0.05$), the Spearman's rank correlation procedure showed an inverse correlation ($r=0.791$, $P<0.01$).

radiolabel in patients' sera was 59.5$\pm$35.7% at 1 h and 72.3$\pm$32.7% at 18 h (mean$\pm$SD).

There were no adverse reactions. Human anti-mouse antibody developed in 7 of 11 patients (64%, patients 2, 3, 4, 7, 8, 10 and 11).

DISCUSSION

The formation of immune complexes can occur between injected antibody and circulating antigen or between murine antibody and HAMA.[20] Since none of the patients had HAMA before antibody injection, immune complexes observed in these patients were between murine antibody and circulating antigen. The proportion of circulating radiolabel in complex form, however, did not correlate well with pre-administration levels of CA125. Hnatowich *et al.* reported similar results in a study using the $F(ab')_2$ fragments of the OC125 antibody.[13] Haisma *et al.*, however, reported that the rate of complex formation correlated with the pre-injection serum CA125 levels.[14] Circulating CA125 in patients formed complexes with anti-CA125 antibodies regardless of their epitopes. Although the slow component of blood clearance of the 145-9 antibody seemed shorter in patients with higher serum CA125 levels, the circulating radiolabel survived and complex formation did not significantly alter the pharmacokinetics of the 145-9 antibody. Furthermore, the present study demonstrated that immune complex formation did not interfere with tumor targeting of the 145-9 antibody. Similar results were reported with anti-CEA antibodies and the mAb B72.3.[21] In humans, the formation of immune complexes between murine antibody and circulating antigen may not restrict effective localization of the antibody.[21] The blood-borne radioactivity, including immune complexes, bound to CA125-coated beads *in vitro,* suggesting that the antibody even if complexed with antigen can still recognize the antigen in tumor tissue *in vivo*. Complexes themselves may bind to the antigen or the antibody may exchange the partner from liquid-phase to solid-phase antigen.[21]

In mice with human tumor xenografts, on the other hand, tumor uptake of $F(ab')_2$ fragment of the antibody OC125 decreased with increasing pre-injection level of serum CA125.[22] In another human tumor xenograft models, circulating antigen altered the pharmacokinetics of murine antibodies by forming immune complexes.[23] Pimm supposed that homology between the species of antibody complexing with antigen and the host is important and that the *in vivo* fate of complexes between murine antibody and antigen in mice could be different from that in humans.[21] In our previous study, however, circulating CA125 altered the blood clearance of an anti-CA125 human/mouse chimeric antibody in a mouse model.[24] If homology is important, the pharmacokinetics of chimeric or humanized antibody may be affected by circulating antigens in human.

The HAMA response against 145-9 was higher than that against the MLS102 antibody, which recognizes sialosyl-Tn and was administered to colorectal patients with the same form of conjugate and at the same dose (7/11, 64% vs.4/17, 24%).[25] The difference of the incidence of HAMA may be due to the difference in isotype of the two mAbs. Since the incidence of HAMA against the antibody OC125 was also high,[26] the antibody-antigen complex formation may be related to the production of HAMA. Chimeric or humanized antibodies would decrease the incidence of anti-antibody response.[24]

Several occult foci were detected with either ^{131}I-labeled 145-9 in the previous study[16] or ^{111}In-labeled 145-9 in the present study. Although the accumulation of ^{111}In in the liver and bone marrow is high, ^{111}In has many favorable nuclear properties compared with ^{131}I, such as low radiation dose to patients and suitable gamma-ray energy for scintigraphic imaging. Dehalogenation of radioiodinated antibody decreases the accumulation of radioactivity in tumors, while ^{111}In-labeled antibody is stable *in vivo*. Furthermore, rapid and efficient labeling of antibodies with ^{111}In makes it possible to construct an "instant" kit for clinical use in hospitals where no special facilities are available. Although more studies are needed to determine its sensitivity in detecting recurrent tumor or metastases, the mAb 145-9 is potentially promising for use in immunoscintigraphy of ovarian cancer.

In conclusion the mAb 145-9 formed complexes with CA125 *in vivo,* but this did not compromise the outcome of antibody imaging. The antibody 145-9 can be used in immunoscintigraphy of ovarian cancer irrespective of serum CA125 levels.

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