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Immunoscintigraphy of colorectal cancer using ^{111}In-labeled monoclonal antibody to mucin

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Abstract A murine monoclonal antibody MLS102 recognizes sialosyl-Tn antigen in mucin and immunohistochemically reacts with more than 80% of colorectal cancer tissues. The purpose of this study was to assess the usefulness of this monoclonal antibody for the immunoscintigraphy of colorectal cancer. Planar and SPECT images were obtained on day 2 or day 3 after injection of 2 mg and 74 MBq ^{111}In-labeled MLS102 antibody into 17 patients with colorectal cancer. Nine of 11 primary tumors and 4 of 6 locally recurrent tumors were detected. Positive images were obtained in all tumors larger than 4.5 × 2.7 cm. Three tumors of less than 2.5 cm and 1 recurrent tumor, which was missed by other imaging modalities, were negative. There were no adverse reactions. Human anti-(mouse Ig) antibody developed in 4 patients. Although improvement of detectability for smaller tumors needs to be pursued, the antibody MLS102 is potentially promising for use in immunoscintigraphy of colorectal cancer.

Key words Sialosyl-Tn antigen · Mucin · Antibody imaging · Colorectal cancer

Introduction

Specific procedures for the diagnosis of postoperative cancer recurrence are required in patients because of the need to differentiate between recurrence and scar tissue or fibrosis. Immunoscintigraphy is a very specific technique based on the antigen/antibody interaction. Monoclonal antibodies recognizing carcinoembryonic antigen (CEA) and TAG-72 have been successfully used for immunoscintigraphy of colorectal cancer. The murine monoclonal antibody MLS102 was obtained by immunizing mice with the human colon cancer cell line LS180, and reacts with sialosyl-Tn, a cluster of NeuAcα2→6GalNAc-Ser/Thr disaccharides (NeuAc, *N*-acetylneuraminic acid; GalNAc, *N*-acetylgalactosamine), in mucin [7, 10]. Sialosyl-Tn is strongly expressed on human carcinoma cells [9], and may be associated with cancer progression and metastasis of human carcinomas. Active immunotherapy trials have been performed using sialosyl-Tn vaccines in patients [14]. Immunohistochemical analysis using MLS102 revealed positive staining in more than 80% of colorectal cancer cases [16]. In normal tissues, the antibody MLS102 reacts weakly with the epithelium of the esophagus, stomach and colon. Goblet cells of the duodenum strongly express the antigen defined by the MLS102 antibody [16].

In the present study, we labeled the antibody with ^{111}In, and examined the pharmacokinetics and lesion detectability of the ^{111}In-labeled MLS102 in patients with colorectal cancer.

Materials and methods

Monoclonal antibody

The antibody MLS102 is a murine monoclonal IgG3. The antibody was purified from ascitic fluid of hybridoma-bearing mice by protein-A affinity column chromatography and was conjugated with diethylenetriaminepentaacetic acid (DTPA) by the cyclic DTPA anhydride method [8, 18]. After removal of unconjugated DTPA, the antibody solution was divided into aliquots. Each vial containing 2 mg 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 ^{111}In (1 ml 0.1 M HCl; Nihon Mediphysics, Takarazuka, Japan). To adjust pH, 0.5 ml 10% sodium citrate was added. The labeling efficiency was determined by both high-performance liquid chromatography (HPLC) and silica-gel thin-layer chromatography (TLC). The values obtained by the HPLC method corre-

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sponded well to those by the TLC method, and 91.1±5.0% (mean±1 SD) of the radioactivity was associated with the IgG. Radioactivity not associated with protein was ^{111}In-DTPA. To avoid steps that increase the possibility of microorganism or pyrogen contamination and to avoid loss of the antibody, post-labeling purification was omitted. Because ^{111}In-DTPA was rapidly cleared from the circulation and excreted in the urine, it would not interfere with the interpretation of the scintigrams. After incubation of ^{111}In-labeled MLS102 with beads that had been coated with ovine submaxillary mucin, 41.7±7.5% of the added radioactivity bound to the beads (mean±1 SD). Nonspecific binding of an irrelevant ^{111}In-labeled monoclonal antibody to the beads was 6.3±0.5% of the added radioactivity (mean±1 SD).

Patients

A group of 17 patients who had histologically confirmed colorectal cancer were examined (Table 1). 11 patients had primary tumors and 6 had recurrent tumors. Four patients had two or three metastatic lesions in the liver. One patient had one metastatic lesion in an inguinal lymph node and another patient had one metastatic nodule in the lung. 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.

Study protocol

The patients were given 74 MBq and 2 mg ^{111}In-labeled MLS102 intravenously over a period of 5 min. Anterior and posterior whole-body images were obtained 10 min, 1, 2, and 3 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. All scintiscans were obtained on a gamma camera with a large field of view and tomographic capability (Gamma View-150E, Hitachi Medical Co., Tokyo, Japan). A medium-energy collimator was used with 20% windows centered over 173-keV and 247 keV photon peaks. Photons were collected for 15 cm/min on whole-body images and for 300 s per spot image. SPECT images were acquired over 360 degrees with a 64 × 64 matrix using 64 stops of 20 s each. SPECT projections were spatially smoothed and reconstructed using a filtered back-projection technique with a Butterworth filter.

Blood was drawn at 5 min, 1 h, 3 h, 16 h, 2 days, 3 days, 4 days, and 7 days after the antibody injection. Urine was collected daily up to 72 h after infusion. The radioactivity of tumor specimens was counted in 14 patients. Tumors were resected after 6–10 days in 13 patients and after 28 days in 1 patient (patient 17 in Table 1). Normal colon or muscle was selected as a nontumor tissue for comparison with primary or recurrent tumors and normal liver tissue was used as a control of liver metastasis. 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 the percentage of the injected dose per gram (%ID/g), and the tumor-to-nontumor radioactivity ratio was obtained by dividing the uptake (%ID/g)into the tumor by the uptake (%ID/g) into the nontumor tissue.

Human anti-(murine Ig) antibody (HAMA) was assayed using an enzyme-linked immunosorbent assay (ELISA) method (ImmuSTRIP HAMA IgG; Immunomedics Inc., Warren, NJ). Blood samples were taken prior to the antibody injection and at 1, 3–4, and 12–16 weeks after administration. Values above 400 ng/ml were considered positive [12].

Immunohistochemical staining

Immunoperoxidase staining of surgically resected tumor was performed according to an earlier report [16]. Briefly, deparaffinized sections of formalin-fixed, paraffin-embedded blocks were incubated with the antibody MLS102. After rinsing, the sections were incubated with biotinylated horse anti-(mouse IgG) (Vector Labs, Burlingame, Calif.) and then incubated with horseradish-peroxidase-linked avidin D (Vector). Non-immunized mouse IgG was used as the negative control. The extent of staining was assessed as the percentage of the carcinoma cells stained. To detect the murine immunoglobulin bound to tumors in vivo, immunohistochemical staining was performed by the same procedures, except that phosphate-buffered saline was used instead of MLS102 antibody solution in the first step, in tumor specimens obtained from five patients.

Results

Table 1 summarizes the patients' profiles and the results of imaging. Nine of 11 primary tumors (82%) and 4 of 6 locally recurrent tumors (67%) were visualized as areas of increased radioactivity (Figs. 1–3). The overall detectability rate was 76% (13/17). Ten tumors were detected both with planar images and with SPECT images but 3 tumors were only detected with SPECT. Tumors larger than 4.5 × 2.7 cm were all visualized. Three tumors of less than 2.5 cm (patients 5, 11, and 13) and 1 recurrent tumor, which was missed by CT and MRI (patient 4), were negative. In primary and recurrent tumors, the percentage of the injected radioactivity accumulated in the tumor was between 0.00057%/g and 0.00491%/g (mean 0.00292%) and the tumor-to-normal tissue radioactivity ratio ranged from 0.80 to 4.81 (mean 2.09).

Radioactivity in the liver and bone marrow was high. Tumors that were detected with SPECT but not detected with planar images were located at the midline and their radioactivity overlapped bone marrow radioactivity on planar images. Two liver metastases over 2.5 cm in diameter were recognized as filling defects (patients 2 and 7) but other metastatic tumors in the liver of less than 2 cm were not identified. Inguinal lymph node metastasis (patient 5) and pulmonary metastasis (patient 12) were not seen with the antibody imaging. The uptake in the liver metastases was no different from that in the primary lesions but the tumor-to-liver radioactivity ratio was very low.

The plasma clearance curve of radioactivity was biphasic with a biological half-life of 0.9–12.7 h (mean 4.4 h) for the first component and 29.1–70.0 h (mean 47.0 h) for the second component. Cumulative urinary excretion of ^{111}In at days 0–1, 1–2, and 2–3 were 9.4±2.0%, 11.9±2.5% and 13.5±3.2% of the injected dose (mean±1 SD) respectively.

Immunohistochemical analysis revealed that more than 20% of the carcinoma cells were stained in 14 of 16 tumors by the MLS102 antibody. Murine immunoglobulin, which was bound to tumors in vivo, was not detected in any of the specimens examined (patients 2, 6, 10, 11, and 12).

There were no adverse reactions. HAMA developed in 4 patients (24%; patients 9, 12, 13, and 16), being first detected 7–28 days after the antibody injection. The titer declined under the cut-off value in 2 patients 93–101 days after injection, but the titer remained high 89–150 days after injection in another 2 patients whose peak titers were very high.

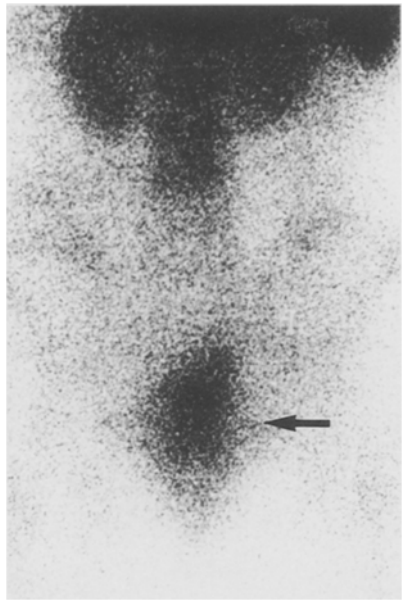

Fig. 1 Patient 6. Posterior planar image of the pelvic region 2 days after injection of ^{111}In-labeled MLS102 showing accumulation of the antibody in the sigmoid colon cancer (*arrow*)

Discussion

The results of the present study indicated that ^{111}In-labeled MLS102 had a high sensitivity for primary and recurrent colorectal cancers over 2.5 cm in diameter, especially with the use of the SPECT technique. SPECT provided better anatomical definition of lesions and was helpful for detection of tumors that were not detected with planar images. Although the number of case in this study was small, the detection rate of primary and recurrent tumor (76%) was comparable to those reported by others using different antibodies. Beatty et al. reported that the sensitivity of ^{111}In-labeled anti-CEA antibodies for imaging primary colon cancer and extrahepatic intra-abdominal metastases was 78% and 48% respectively [1]. Using another ^{111}In-labeled anti-CEA antibody, Divgi et al. demonstrated 60% sensitivity on a lesion-by-lesion basis to detect extrahepatic abdominal disease [6]. Collier et al. reported that the sensitivity of ^{111}In-labeled B72.3 in the detection of pelvic tumors and extrahepatic abdominal tumors was 74% and 66%, respectively [4]. Smaller tumors, although they expressed the antigen, were not detected in the present study and we could not obtain positive images for liver metastases. Improvement of the tumor-to-nontumor radioactivity

Table 1 Patients' profiles and results of immunoscintigraphy, direct tissue counting, and immunohistochemical studies. *T/NT* tumor-to-nontumor radioactivity ratio; *ND* not determined

Patient	Age (years)	Sex	Site	Size (cm)	Planar	SPECT	Ab uptake (%ID/g)	T/NT	Staining (%)
1	58	M	Rectum, recurrence	6 × 6	(+)	(+)	ND	ND	ND
2	68	M	Sigmoid colon	8 × 4	(+)	(+)	0.00277	1.53	80
			Liver[a]	2.5 × 2.5	(–)	(–)	0.00327	0.15	ND
3	64	M	Rectum	8 × 5	(+)	(+)	0.00332	1.70	20
4	60	F	Rectum, recurrence	ND	(–)	(–)	ND	ND	40
5	58	M	Rectum, recurrence	2 × 1	(–)	(–)	0.00154	1.64	90
			Inguinal lymph node	1.5 × 1.5	(–)	(–)	ND	ND	ND
6	67	F	Sigmoid colon	7 × 6	(+)	(+)	0.00219	2.24	90
7	59	M	Rectum	8.5 × 5	(–)	(+)	0.00306	4.81	10
			Liver[a]	3.5 × 3.5	(–)	(–)	0.00120	0.05	ND
8	71	F	Rectum	5 × 3	(+)	(+)	0.00057	0.80	20
9	67	M	Rectum	7 × 7	(+)	(+)	0.00379	1.93	40
			Liver[a]	1 × 1	(–)	(–)	ND	ND	ND
10	78	F	Sigmoid colon	4.5 × 2.7	(+)	(+)	0.00448	1.75	90
			Liver[a]	2 × 2	(–)	(–)	ND	ND	ND
11	61	F	Sigmoid colon	1.5 × 1.5	(–)	(–)	0.00414	2.25	90
12	41	F	Rectum	4.5 × 4	(+)	(+)	0.00320	2.17	80
			Lung	1 × 1	(–)	ND	ND	ND	ND
13	71	F	Rectum	2.5 × 2.5	(–)	(–)	0.00337	1.28	90
14	43	M	Rectum	7 × 7	(–)	(+)	0.00247	1.62	10
15	60	M	Rectum, recurrence	5 × 4.5	(+)	(+)	0.00109	2.87	30
16	37	M	Rectum, recurrence	7 × 6	(+)	(+)	ND	ND	90
17	84	F	Sigmoid colon, recurrence	6 × 4.5	(–)	(+)	0.00491	2.60	90

[a] The largest tumor is listed in the table

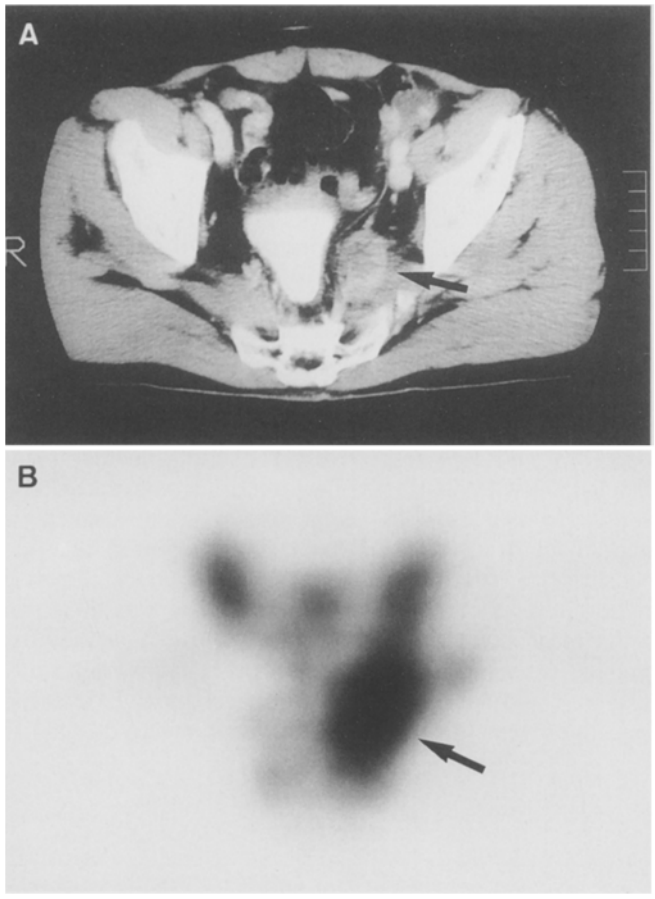

Fig. 2A, B Patient 15. **A** CT scan showing a recurrent tumor beside the bladder (*arrow*). **B** Transaxial SPECT image 2 days after injection of ^{111}In-labeled MLS102 showing accumulation of the antibody in the tumor (*arrow*)

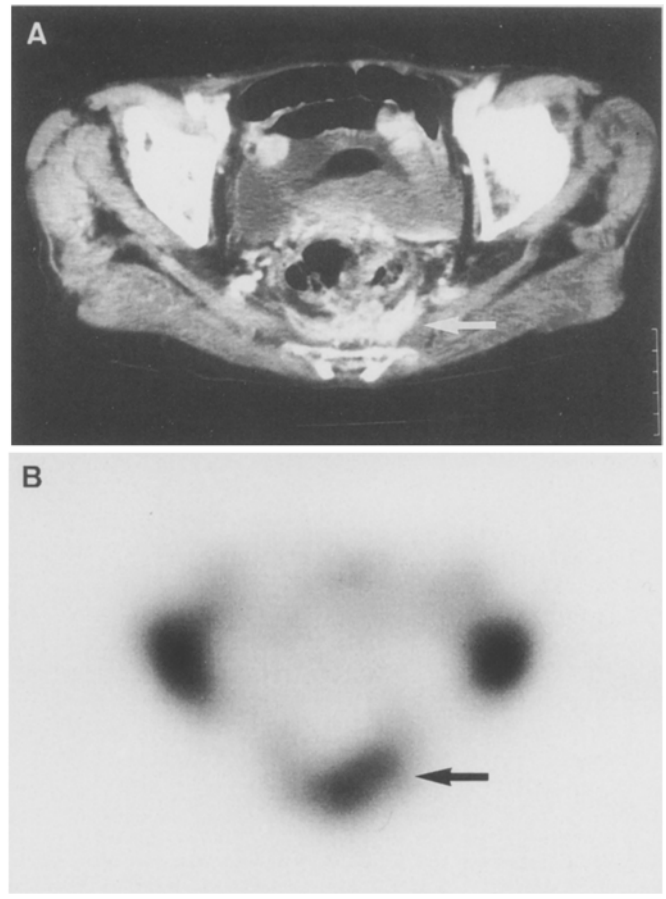

Fig. 3A, B Patient 17. **A** CT scan showing a recurrent tumor in the presacral area (*arrow*). **B** Transaxial SPECT image 2 days after injection of ^{111}In-labeled MLS102 showing accumulation of the antibody in the tumor (*arrow*)

ratio is necessary. It would be helpful to use Fab or F(ab′)$_2$ fragments to reduce the radioactivity in nontarget tissue [2, 11, 19]. Since bone marrow uptake of ^{111}In could be the result of transchelation of ^{111}In from antibody to transferrin [20], antibody-chelate conjugates prepared by benzyl-DTPA or benzyl-ethylenediaminetetraacetic acid (EDTA), which would be more stable against transchelation than unsubstituted DTPA conjugates, could decrease the bone marrow radioactivity [3, 5]. Benzyl-DTPA or benzyl-EDTA conjugates with the antibody may reduce also the liver and spleen uptake. Hepatic uptake of the radiolabel would be less if the antibody were labeled with ^{99m}Tc or ^{123}I [2, 13].

We could not demonstrate the injected antibody that accumulated in the tumors in vivo. The injected dose of the antibody (2 mg) may be too small. The antibody could be catabolized during the interval between its injection and tumor resection (6–9 days in the 5 patients). Schroff et al. reported that, in the biopsy specimens removed from the patients 24–96 h after the intravenous infusion of 9.2.27 antibody, little or no in vivo binding of the 9.2.27 antibody to tumor cells was found following 1- and 10-mg doses, whereas all specimens demonstrated in vivo binding of the antibody following 200- and 500-mg doses [22].

The HAMA response against MLS102 was low; experience with other monoclonal antibodies has indicated a generally higher incidence of HAMA [15, 17, 21]. The low response observed here could be due to the low dose of the antibody injected (2 mg).

The antigen recognized by MLS102 has been reported to be expressed in more than 80% of colorectal carcinoma cases, and this finding was also confirmed in the present study. Although more studies are needed to determine its sensitivity for detecting recurrent tumor or metastases, this antibody is potentially promising for use in immunoscintigraphy of colorectal cancer.

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