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Imaging of Recurrent Intestinal Carcinoma with Indium-111-labeled Anti-carcinoembryonic Antigen Monoclonal Antibody CEA102

Kazuhiko Nakada,[1] Junichi Sakamoto,[2] Tadashi Watanabe,[3] Katsuki Itoh,[1] Seiji Akiyama[1] and Hiroshi Takagi[1]

[1]Second Department of Surgery, Nagoya University, 65 Tsurumai-cho, Showa-ku, Nagoya 466, [2]Department of Surgery, Aichi Prefectural Hospital, 18 Kuriyado, Kakemachi, Okazaki 444 and [3]Department of Surgery, Fujita Health University, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake 470-11

CEA102 is a mouse immunoglobulin G1 monoclonal antibody (mAb) that detects an epitope of carcinoembryonic antigen (CEA). The biodistribution and imaging characteristics of indium-111-labeled (111-In)-mAb CEA102 were studied in 1 primary and 9 extrahepatic recurrent intestinal carcinoma patients. Evaluation included antibody pharmacokinetics and assessment of antibody distribution in surgical specimens, in comparison with whole body imaging using a gamma camera, and imaging with single photon emission computed tomography. Selective mAb CEA102 localization to tumor tissue was demonstrated in 7 patients with tumors over 2 cm in size, and the external images correlated well with the results of surgical inspection, pathological examination, and tissue radioactivity measurements. Tumor : serum ratios ranged from 0.20 : 1 to 3.22 : 1, and serial biodistribution study of "regions of interest" also demonstrated a high radioactivity in the tumor. These results indicated the potential exploitability of the 111-In-labeled mAb CEA102 in radioimmunodetection of primary and extrahepatic recurrence of CEA-positive intestinal carcinomas.

Key words: Monoclonal antibody CEA102 — Intestinal carcinoma — Radioimmunodetection — SPECT

The early detection and therapy of carcinoma lesions are important goals of monoclonal antibody (mAb) research. For cancers in the gastrointestinal tract, carcinoembryonic antigen (CEA) is the best characterized tumor-associated antigen, and mAbs against CEA have been utilized for radioimmunolocalization to differentiate specifically between benign and malignant lesions.[1–3] To date, most studies in humans with mAbs directed against CEA have utilized iodine isotopes, and the use of antibody fragments,[4,5] combinations, second antibodies for clearance of the blood pool,[6,7] and transaxial tomography[8] has been examined in attempts to obtain more accurate imaging results.

The Nagoya University study group has developed a murine immunoglobulin G1 (IgG1) anti-CEA mAb CEA102 by immunizing mice with purified CEA.[9] This mAb CEA102 was shown to have high antigen-binding affinity with minimal cross-reactivity in immunohistochemical, immunocytochemical, and radioimmunoassay studies,[9,10] and was proven to be potentially useful for radioimmunodetection and autoradiographic studies in colorectal cancer patients.[11] More recently, the labeling of mAbs with 111-In has been documented by a number of investigators.[12–15] 111-In has several advantages over 131-I as an imaging agent: short half life (67 h), two emissions per disintegration, lower energy emission (173 and 247 kev), favorable dosimetry characteristics with no high-energy beta emissions, and stability of binding.

The purpose of the present investigation was to extend our radioimmunolocalization study in order to allow more accurate diagnosis, especially for the detection of local recurrence. We therefore labeled mAb CEA102 with 111-In by the diethylenetriaminepentaacetic acid (DTPA) method.[16] This 111-In-labeled mAb CEA102 was then used in patients with recurrent intestinal cancer to confirm the qualitative diagnosis of the tumor and to determine the need for extensive secondary surgery, including pelvic exenteration. A systematic comparison of imaging results with gross and histological findings was also performed to obtain a better understanding of how the tumor, host, and antibody characteristics affect the radioimmunolocalization of the tumor.

PATIENTS AND METHODS

Preparation of mAb CEA102 Establishment of the hybridoma, and the serological and immunohistochemical characteristics of the mAb CEA102 have been described.[9,11] CEA102 was purified from ascitic fluid by ammonium sulfate precipitation and protein A affinity chromatography. Once purified, IgG was filtered and all lots tested according to the guidelines of the U.S. Food and Drug Administration.[17] These included tests to confirm the absence of mycoplasma, adventitious viruses, and pyrogenicity, as well as tests of sterility and general safety.

Radiolabeling of mAb CEA102 Purified mAb CEA102, isotype IgG1, was diluted in phosphate buffer (pH 7.4) and concentrated to 10 mg/ml in 0.1 *M* citrate buffer (pH 5.7). The solution was mixed with DTPA dianhydride (MW 357, Dojindo Laboratories, Tokyo) at a DTPA: mAb molar ratio of 10 for 10 min at room temperature to prepare DTPA-conjugated mAb. Unconjugated DTPA was removed by filtration on MOLCUT (cut-off, 30,000; Millipore, Tokyo). Equal volumes of sterile 111-In-chloride solution (185 MBq/ml, Nihon Mediphysics, Chiba) and 0.1 *M* citrate buffer were added to the DTPA-conjugated mAb preparation. The extent of labeling of the mAb CEA102 was tested by thin-layer chromatography.

Patients Ten patients who had primary or recurrent intestinal carcinoma were considered eligible. Patients gave written informed consent prior to participation in the study. The protocol and consent procedures were approved by the Institutional Review Board and Ethical Committee of the Nagoya University Hospital.

Table I. Characteristics of Patients

Patient No.	Age	Sex	Diagnosis	Site of cancer	Surgical procedure	Plasma CEA (ng/ml)
1	56	M	Transverse colon cancer	Transverse colon	Right hemicolectomy	1.9
2	50	M	Recurrence of small intestinal cancer	Lymph node of mesentery	Tumor resection	24.8
3	50	F	Recurrence of rectal cancer	Anastomotic site	APR[a]	2.6
4	70	M	Recurrence of rectal cancer	Presacral space	(−)	11.4
5	65	M	Recurrence of rectal cancer	Pelvis, Inguinal lymph nodes	(−)	68.7
6	60	F	Recurrence of rectal cancer	Anastomotic site Left ovary	Posterior TPE[b]	9.0
7	38	M	Recurrence of rectal cancer	Left femoral bone	(−)	1.6
8	65	F	Recurrence of rectal cancer	Lumbar supine (III) Paraaortic lymph node	(−)	3.2
9	57	M	Recurrence of rectal cancer	Presacral space	TPE	4.6
10	36	M	Recurrence of rectal cancer	Pelvis	APR	6.1

a) APR, abdominoperineal resection.
b) TPE, total pelvic exenteration.

Table II. Distribution of Radiolabeled mAb CEA102 and Results of Radioimmunodetection

Patient No.	Tumor size (cm)	Dose of CEA102/^{111}In	%ID ($\times 10^3$)[a]	Tumor : serum ratio	Immunoperoxidase with CEA102	Imaging
1	6.0×5.0	10 mg/44 MBq	1.10	1.17	(+)	(+)
2	5.0×4.0	10 mg/31 MBq	0.87	2.41	(+)	(+)
3	1.5×1.0	10 mg/105 MBq	0.11	0.20	(+)	(−)
4	5.0×4.0	10 mg/131 MBq	NT[b]	NT	NT	(+)
5	7.0×6.0	10 mg/105 MBq	NT	NT	NT	(+)
6	4.0×3.0	10 mg/109 MBq	1.41	3.22	(+)	(+)
7	1.0×1.0	10 mg/105 MBq	NT	NT	NT	(−)
8	1.0×0.5	10 mg/149 MBq	NT	NT	NT	(−)
9	2.0×1.0	10 mg/128 MBq	2.68	1.47	(+)	(+)
10	2.5×1.5	10 mg/133 MBq	1.88	1.76	(+)	(+)

a) %ID, % injected dose ($\times 10^3$) per gram of tumor tissue.
b) NT, not tested.

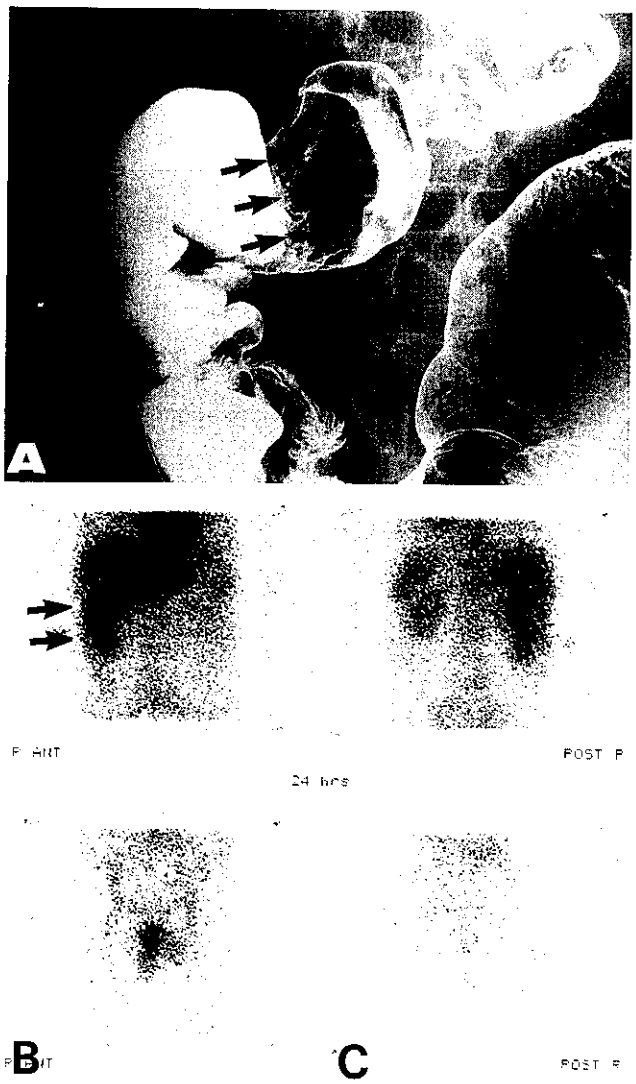

Fig. 1. Barium enema studies (A) and anterior (B) and posterior (C) planar scintigraphies at 24 h of patient #1 with primary transverse colon carcinoma showing a hot lesion at the hepatic flexure.

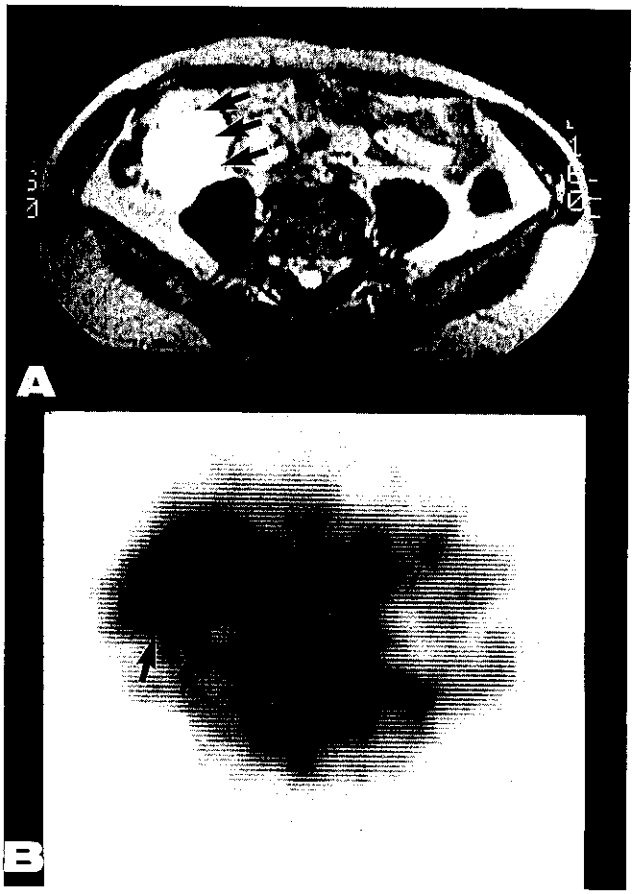

Fig. 2. MRI image (A) and SPECT image (B) of patient #2 with recurrent CEA-producing cancer of the small intestine. A large lymph node metastasis is visualized by the CEA102 scintigraphy.

Nine patients had histologically documented colorectal cancer and one patient had a cancer of the small intestine. Their ages ranged from 36 to 70 years old, with a mean age of 56.5 years. The group consisted of 7 men and 3 women. Baseline complete blood counts, liver function test, and renal function test were obtained prior to the radioimmunolocalization study and were repeated weekly after the injection of the radiolabeled mAb. The initial workup included chest X-ray, abdominal computed tomography (CT), and bone and liver/spleen scans. One patient had a primary colon tumor, one patient had a recurrent CEA producing cancer of the small intestine, and the other 8 patients had a local recurrence of rectal cancer (Table I).

Imaging and biodistribution All patients were initially negative to a 48 h skin test using 20 μg of mAb CEA102. Radiolabeled mAb CEA102 dissolved in 100 ml of physiological saline was administered intravenously over an hour at a dose of 10 mg of antibody and 31 to 131 MBq of 111-In. Vital signs were monitored for each patient during and after the infusion.

The patients were imaged within 4 h of mAb administration and daily for 2 weeks or until the day prior to surgery. A GCA501S digital gamma camera (Toshiba Inc., Tokyo) was utilized to obtain anterior and posterior whole-body images as well as multiple spot views including chest, abdomen, and pelvis. Single photon emission CT (SPECT) was also used to visualize the transectional view of the tumor. Serial images were analyzed in patient No. 6 with manually drawn "regions of interest" (ROI)

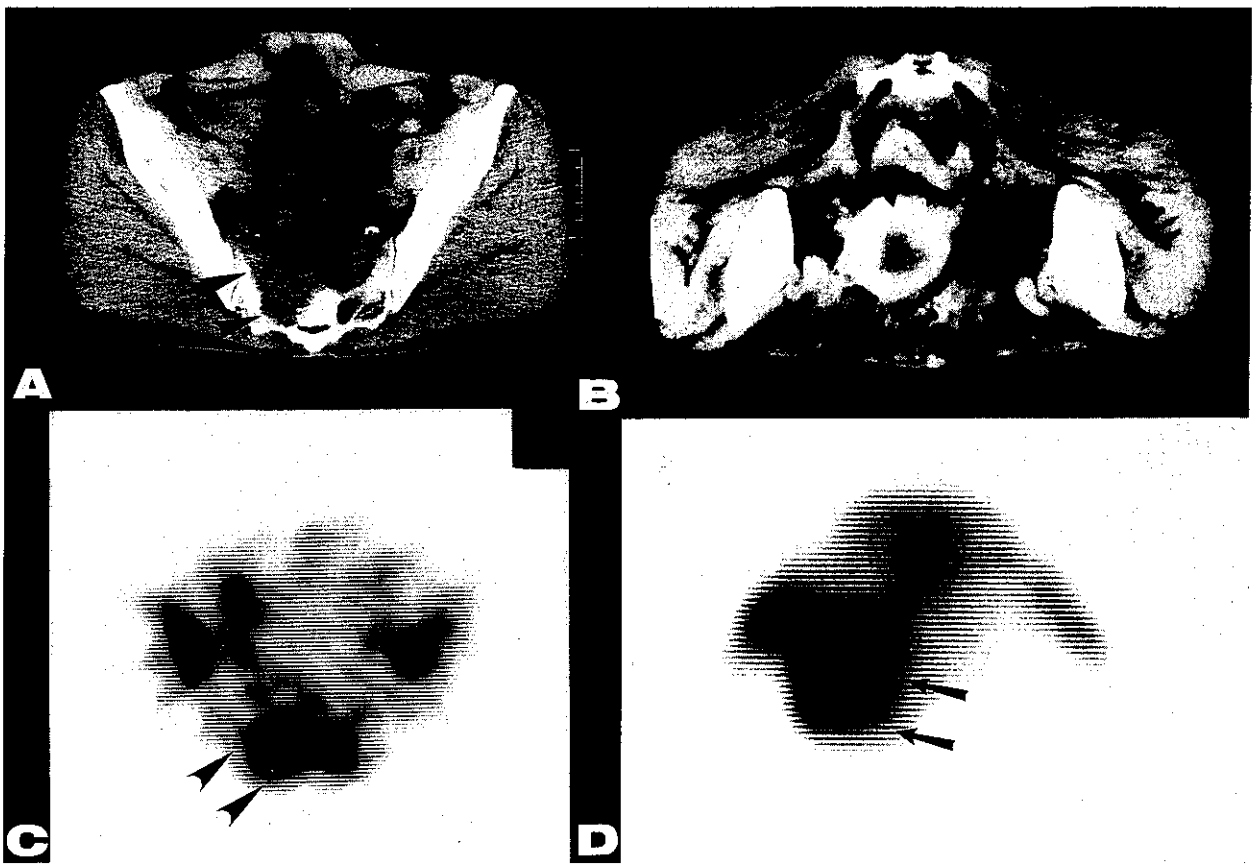

Fig. 3. CT scans (A, B) and SPECT images of patients #4 (C) and #6 (D) with recurrent rectal cancer in the pelvic lesion. Recurrent tumors (indicated by the arrows) were clearly visualized by the CEA102 scintigraphy.

over the major organs and tumor. Values were expressed as counts per minute (cpm), and corrected for isotope decay. The rate of isotope clearance from individual organs was compared to that from the tumor.

Autoradiographic analysis using a bioimaging analyzer The specific accumulation of the radiolabeled mAb CEA 102 in the surgically resected specimen was investigated by the use of a bioimaging analyzer, BAS2000 (Fuji Photo Film, Tokyo). Resected specimens were put on Imaging Plates (Fuji Photo Film), and exposed overnight. The autoradiogram was developed and analyzed on the bioimaging analyzer.

RESULTS

Analysis by patients All 10 patients received 111-In-labeled CEA102 and were subsequently scanned with a gamma camera and by SPECT. No adverse reaction was observed in any patient following the radiolabeled antibody. Surgery was performed for 6 patients, and resection or debulking of the tumor was possible in 6 patients. For 4 patients with inoperable disease, tissues were not accessible for direct determination of cpm per gram or autoradiographic study by the bioimaging analyzer (Table II).

Radioimmunolocalization by scanning Seven of the 10 patients had positive scans. No difference in tumor detection was seen at the different dose levels of 111-In. The smallest lesion detected was 2.0 cm in diameter, with most of the detected lesions being 2.0 cm or larger.

Normal tissues visualized included the blood pool, liver, spleen, kidneys, bone, colon, bladder, and testes. Normal tissues were visualized most intensely at 24 h and, except for liver, thereafter faded relative to the tumor. Liver showed a high uptake of the radiolabeled antibody and remained dominant in all subsequent scans. The uptake in bones diminished from 2 to 3 days.

Primary tumor of the transverse colon in case No. 1 was well imaged by planar scintigraphy (Fig. 1). Lymph node metastasis of recurrent CEA-producing cancer of

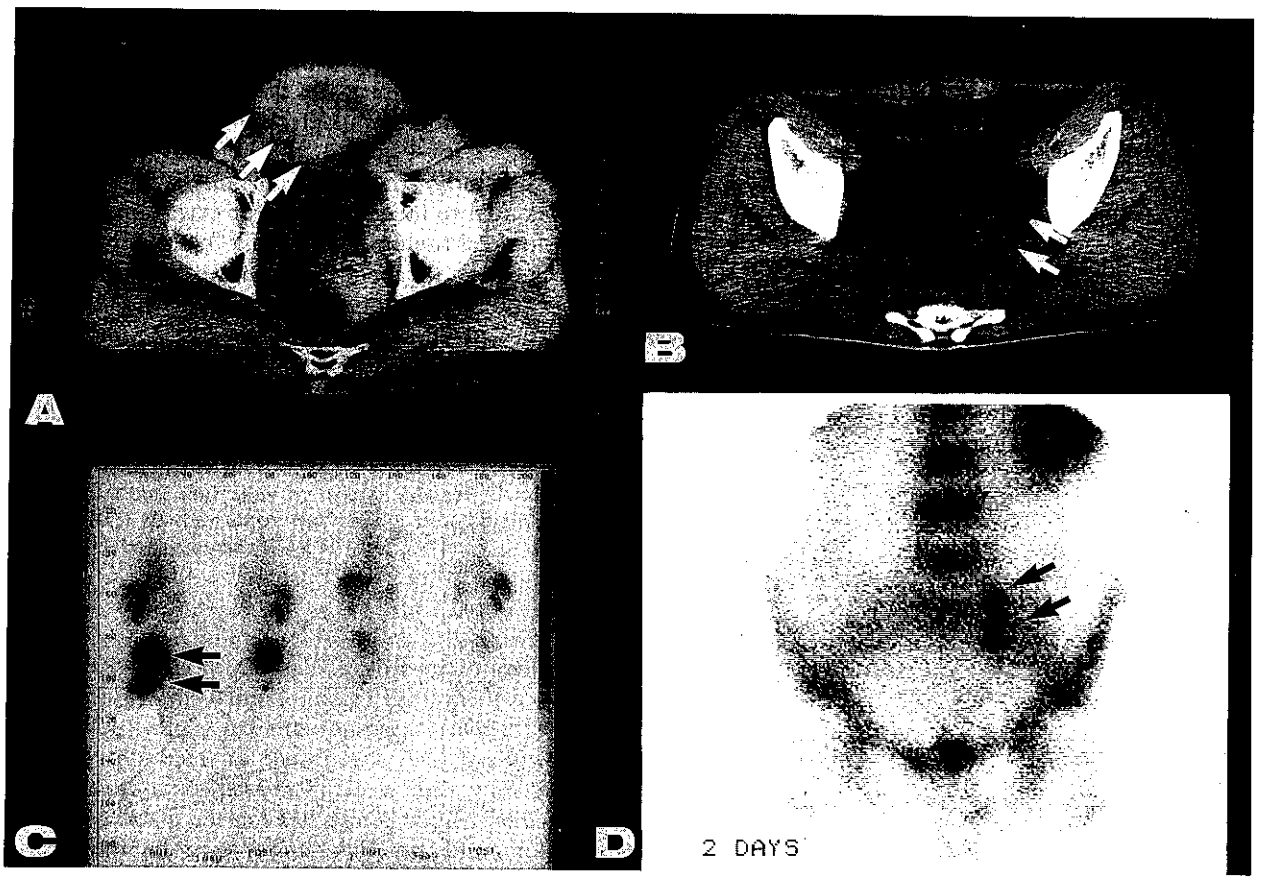

Fig. 4. CT images (A, B) and whole-body scintigraphy of patients #5 (C) and #10 (D) with recurrent rectal cancer. Both anterior and posterior views gave a clear tumor image at day 1 in patient #5. In patient #10, an anterior spot view of the antibody scan shows an image of the tumor consistent with the CT image.

the small intestine in case No. 2 was also clearly visualized (Fig. 2). Tumor recurrence of rectal cancer in the pelvic region was visualized in 4 out of 7 cases. In case No. 4 and No. 6, a tomographic scan was able to disclose an accumulation of radiolabeled antibody in the intrapelvic mass (Fig. 3). Likewise, whole-body scintigraphy as well as anterior and posterior spot views of the pelvis afforded relevant tumor images, especially in case No. 5 and No. 10 (Fig. 4). A small recurrence to the anastomotic site in case No. 3, metastases to paraaortic lymph nodes in case No. 8, and extraperitoneal metastasis to the femoral bone in case No. 7 were not visualized in this series of radioimmunodetection trials. The optimum time for imaging was found to be 2 to 4 days, when the background activity had decreased and tumor-to-nontumor ratios were greatest. There were no false-positive scans, and for all patients, the sensitivity was 70%, specificity 100%, accuracy 70%, and positive predictive value (PPV) 100%. For local recurrence of rectal carcinoma, the sensitivity of our 111-In-labeled mAb CEA102 was 67%, specificity 100%, accuracy 67% and PPV 100%.

Biodistribution study on radioimmunoscintigraphy Serial scans were performed in case No. 6, focusing on ROIs in the tumor, liver and bone (Fig. 5). Radioactivity of each ROI was evaluated daily for 4 days after the injection. Radioactivity in the tumor was higher than in the bone tissue or other intrahepatic organs but was always lower than that in the liver (Fig. 6).

Autoradiographic analysis using bioimaging analyzer Autoradiographic study of the surgical specimen was performed in 6 patients. The autoradiogram of the resected specimen from case No. 10 demonstrated that the radioactivity was specifically localized in the tumor, but not in the normal rectal tissues (Fig. 7). Evaluation of the radioactivity of the ROI in tumor, normal rectum, and skin by the bioimaging analyzer showed that the cpm of ROI of the tumor was over six times higher than that of the other tissues (Fig. 8).

DISCUSSION

Previous studies by the Nagoya University study group on radioimmunolocalization using 131-I-labeled mAb CEA102 in primary and metastatic colorectal carcinomas have afforded promising results.[9, 10] In a biodistribution study, they obtained 10-fold greater uptake of the antibody in the tumor compared to the normal tissue.[11] Although 131-I is inexpensive and easy to conjugate to mAbs, it has an excessively high-energy gamma emission (346 kev) and produces a certain amount of beta rays, both of which detract from its utility as an imaging radionuclide. The use of 111-In, which has ideal emission energy (173 and 257 kev) from the viewpoint of the imaging efficiency of gamma cameras, has been reported to afford better imaging quality and stability.[18] Despite its cost, difficulty of conjugation with mAb, and nonspecific accumulation in the liver,[19, 20] 111-In seemed to be an attractive agent for the development of our program for radioimmunodetection of recurrent colorectal cancers.

In this study, a highly specific mAb conjugated to 111-In was used to image primary or extrahepatic metastasis of colorectal tumors. We mainly focused on local recurrences of rectal cancer in the intrapelvic region, because conventional modalities such as ultrasonograms, CT, or MRI are of limited use for qualitative identification of

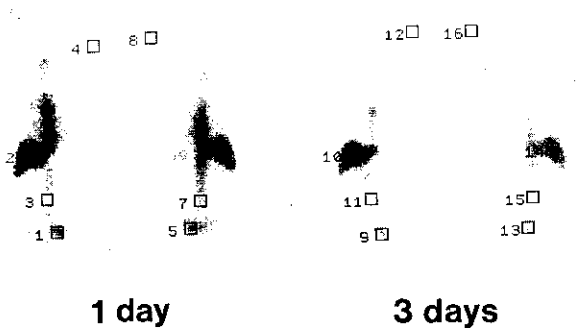

Fig. 5. "Regions of interest (ROI)" evaluated in the serial whole body scans in patient #6 on days 2 and 3. ROI were created in the tumor, liver and bone.

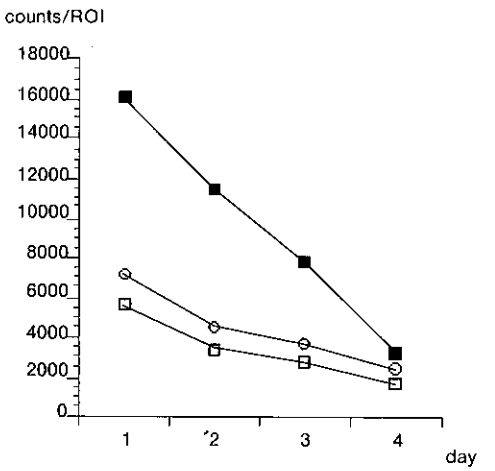

Fig. 6. Radioactivity of each ROI was evaluated daily for 4 days after CEA102 injection. Radioactivity in tumors was higher than in bone tissue, but was always lower than in liver throughout the follow-up period. ■ liver, □ bone, ○ tumor.

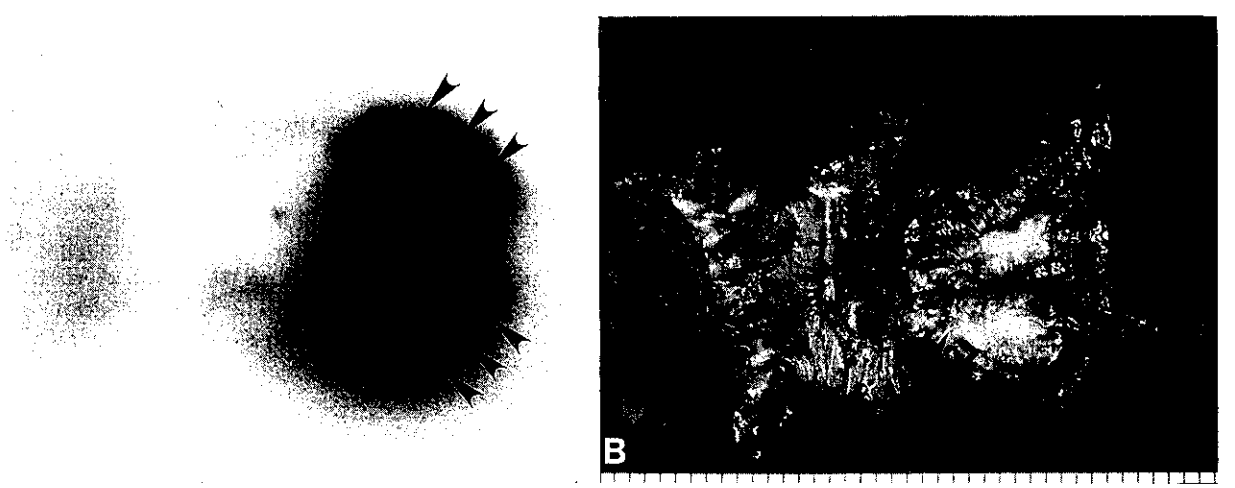

Fig. 7. Resected rectal carcinoma and surrounding normal rectal tissue from patient #10 following administration of mAb CEA102. *Ex-vivo* scan of the specimen (A) showing a distribution of 111-In-labeled mAb consistent with the locus of the tumor, indicated by arrows in the resected specimen (B).

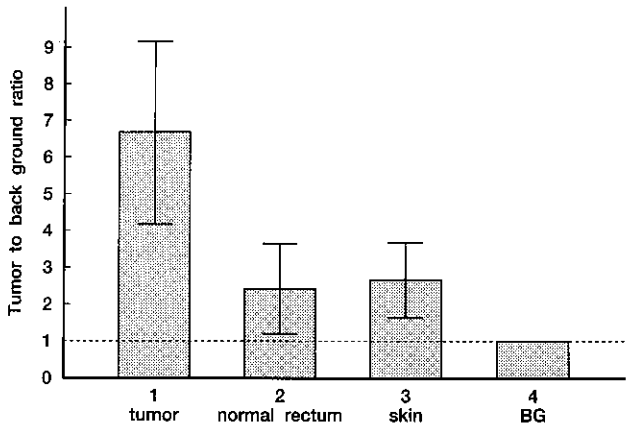

Fig. 8. Evaluation of radioactivity of the ROI in tumor, normal rectum, and skin by the bioimaging analyzer. The cpm of ROI of the tumor was over 6 times higher than the cpm of the background (BG).

local recurrence in the pelvis. For radioimmunodetection, both gamma camera and SPECT images were analyzed. Most of the patients scintiscanned had a laparotomy for treatment of the disease, which allowed for a more accurate correlation of the images with the extent of disease. Also, normal and tumor tissues were available for determinations of radioactivity and immunohistology with the mAb CEA102.

The radioactivity in normal tissues can cause difficulty in interpretation of scan images. However, most normal tissues show a decrease in intensity after 24 h. As expected, the liver uptake remained substantial, but this did not affect the imaging results in our study.

The ability to image successfully 67% of local recurrences in the pelvis is far superior to the sensitivity reported by other investigators.[21–24] Although detection of the primary tumor was about 70% in some studies,[25,26] previous imaging results demonstrated a very low detection rate with regard to intrapelvic recurrent lesions.[21,25] Furthermore, recurrence in the anastomotic site in case No. 3, who gave a negative scan, was less than 1 cm in diameter, and all the intrapelvic lesions over 2.0 cm were detected in our study. This high sensitivity to recurrent lesions in the pelvis is comparable to the previous results with 131-I labeled anti-sialyl Lea mAb (H-15) imaging, in which sensitivity was 71%, specificity 100%, accuracy 78% and PPV 100%,[27] and the tumor images in our 111-In CEA102 study were much clearer and more detailed than those in the 131-I H-15 study.

A possible explanation for this high sensitivity, compared to the reports of other investigators, is the difference of anti-CEA mAb utilized. CEA102 was selected from over 400 established anti-CEA antibody-producing hybridomas. Serological screening with normal and non-colon cancer cell lines and immunohistochemical studies with normal and various cancer tissues were employed to exclude broadly reactive anti-CEA producing clones. Although accurate epitope analysis has not yet been performed with CEA102, the mAb might be highly specific for a certain region of the CEA molecule.

Biodistribution studies of the radiolabeled mAb, performed on patients and resected specimens, demonstrated that the tumor had a more than 6-fold greater uptake than normal tissues, except liver. These results indicated that intravenously injected mAb was specifically accumulated in the primary tumor, metastatic lymph nodes and intrapelvic local recurrence, thus reconfirming the results of the radioimmunolocalization study.

Despite these favorable findings with 111-In-labeled mAb CEA102, one may argue that its usefulness in clinical diagnosis is limited by the inability to detect tumors less than 2 cm in size. A tumor of that size or even smaller can be visualized on routine imaging modalities such as CT or nuclear magnetic resonance (NMR). However, it should be stressed that one of the advantages of using mAb CEA102 for radioimmunodetection is the specific accumulation of the mAb in the locally recurrent tumor mass in the pelvis, which is hardly accessible by conventional biopsy methodology. At present, qualitative diagnosis of pelvic mass after a primary operation for colorectal cancer is quite difficult, even by sophisticated CT or NMR techniques, and patients are sometimes subjected to an unnecessarily extensive operation for benign granuloma mass detected by CT or NMR. Specific accumulation of the labeled antibody at the tumor in such cases would provide a strong and objective indication for radical operation.

Another point of interest arising from this radioimmunodetection study is its applicability for therapy. The ultimate aim of using mAbs in clinical practice is their application for targeting therapy. The physical behavior of yttrium-90 is known to be similar to that of indium-111. The only difference is that 90-Y is a strong beta emitter which can kill cancer cells, while 111-In is a gamma emitter, which is useful for radioimmunodetection, but not for therapy. Successful imaging of recurrent tumors using 111-In-labeled mAb CEA102 implies the feasibility of 90-Y radioimmunotherapy. Therefore, the proven ability of 111-In-labeled mAb CEA102 to detect recurrent tumor should be considered as providing a basis for the development of more sophisticated radioimmunodetection using 123-I or 99m-technetium for better sensitivity, and also for the possible specific treatment of recurrences by conjugation of the mAb with beta-radiating isotopes or chemotherapeutic agents.

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