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Autoradiographic Analysis of Radiolabeled Anti-carcinoembryonic Antigen Monoclonal Antibody CEA102 in Colorectal Cancer Using Computed Radiography

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Anti-carcinoembryonic antigen monoclonal antibody (MAb) CEA102 was produced by immunization with purified CEA and the specific accumulation of radiolabeled CEA102 in colorectal cancers was investigated by autoradiography of surgical specimens using Fuji Computed Radiography (FCR). Five patients with colorectal cancer were injected intravenously with ^{131}I-labeled intact CEA102 or its F(ab′)$_2$. Primary tumor and liver metastases were successfully detected by external scanning with a gamma camera in 4 cases. Autoradiographic study of the surgical specimens using FCR showed predominant localization of ^{131}I-labeled CEA102 in primary tumors and liver metastases in all cases. Even a small liver metastasis (0.5 cm) was clearly visualized in the autoradiogram by FCR. The pixel distribution curves of the density of the respective tissues in the autoradiograms by FCR showed the heterogeneity of the distribution of administered radiolabeled MAb in individual tumors, but the density of the tumors was higher than that of the normal tissues. In the quantitative distribution analysis of CEA102, the uptake of the primary tumor (mean 1.10%ID/kg) was ten-fold greater than that of the normal colon mucosa (mean 0.10%ID/kg). These results revealed that the application of MAb has great potential in radioimmunodetection as well as in antibody-directed therapy.

Key words: Radioimmunodetection — Anti-CEA monoclonal antibody — Autoradiography — Colorectal cancer

Recently, remarkable progress in diagnosing tumors has been achieved by medical imaging, for example, computed tomography (CT), ultrasonography (US), and magnetic resonance imaging. These types of imaging techniques are useful for anatomic diagnosis of a tumor site but are not sufficient to diagnose whether the visualized mass is malignant or not. Imaging using radiolabeled monoclonal antibody (MAb) to tumor-associated antigen is a potentially specific approach to this problem. Development of these technologies may also allow therapy by using radionuclides, antitumor drugs or toxins conjugated with antibodies. In radioimmunodetection (RAID) of colorectal cancer, MAbs against carcinoembryonic antigen (CEA) or various other tumor-associated antigens have been used.[1–18] We have also performed imaging with ^{131}I labeled anti-CEA MAb CEA102 for detecting recurrent colorectal cancer. But the main problem of imaging with radiolabeled MAb is that the accumulation of administered MAb in the tumor (and hence the tumor-to-normal tissue ratio) is not sufficiently high. This problem prevents the obtaining of a high quality image, the detection of small tumors and the use of MAb for therapy. Several factors such as the form of antibodies, and circulating antigens may influence tumor uptake of MAbs. To evaluate trials of RAID, it is thought to be important to confirm the specific accumulation and affinity of radiolabeled MAb in tumors at the histological level. Autoradiographic analysis has been used for this purpose and experimental models using human tumor xenografts have been reported. In regard to clinical studies, only a few analyses have been reported, because autoradiography requires a long duration, and short half-life radionuclides have been used for RAID.[19, 20] We have applied Fuji Computed Radiography (FCR) (Fuji Photo Films, Tokyo) to autoradiographic analysis of surgical specimens. This method has the advantage of being a rapid technique that can provide high-quality images. The present study was aimed at investigating the specific accumulation of CEA102 in the tumors of patients with colorectal cancer by autoradiography using FCR.

MATERIALS AND METHODS

Monoclonal antibody CEA102 The MAb CEA102 used in this study was produced by the following methods. Splenic cells of the BALB/c mice immunized with

purified CEA (Accurate Chemical, Westbury, NY) were hybridized with murine myeloma cell MOPC 21NS/1 and one of the hybridomas producing anti-CEA MAbs was selected by the ELISA method and designated as CEA102 (IgG1). The tissue distribution of the antigen recognized by CEA102 was analyzed by the avidin-biotin immunoperoxidase technique as described by Hsu *et al.*[21] (Vector Laboratories, Burlingame, CA). Deparaffinized sections were treated with 0.3% H_2O_2 in methanol to inhibit endogenous peroxidase activity, and preincubated with 5% normal horse serum in phosphate buffer to minimize non-specific binding. The sections were then incubated with CEA102 culture supernatant (2–3 mg/ml) diluted at 1:10 and 1:100. Finally, the sections were developed with diaminobenzidine and counterstained with methyl green. The cases were considered as positive when the antigen recognized by CEA102 was present in the apical surface or cytoplasm of cells, or was shed into surrounding stroma, as shown previously in pancreatic cancer.[22] As a negative control, non-immune mouse sera at a 1:1000 dilution were used in place of CEA102.

Radiolabeling of CEA102 The MAb fraction was extracted using Protein A affinity chromatography. Purified CEA102, isotype IgG1, was diluted in phosphate buffer (pH 7.4). $F(ab')_2$ was produced by digesting IgG with pepsin. Both the intact MAb and $F(ab')_2$ were concentrated to 2.5 mg/ml. The intact MAb and $F(ab')_2$ (2–4 mg) were labeled with ^{131}I (148–185 MBq) using the Iodogen method and the radiochemical purity was about 90% of total radioactive products as assessed by thin-layer chromatography.

Radioimmunodetection This study was approved by the Ethical Committee of Nagoya University School of Medicine and each patient had given informed consent in writing. Five patients with histologically confirmed advanced colorectal cancers were selected. Patients underwent premedication with lugol iodine solution for 3 days. No history of allergy was found in any of the patients. An intradermal skin test was performed with the unlabeled MAb before infusion of the radiolabeled MAb, but no positive reaction was found. Each patient underwent intravenous infusion with ^{131}I-labeled MAb (2–4 mg, 148–167 MBq) diluted in 100 ml of saline solution for an hour. Vital signs were monitored for each patient before and after infusion and they were observed for signs of any reaction for the next hour. No adverse reaction developed following the administration in any of the patients. After infusion, extra scanning was done with a digital gamma camera GCA501S (Toshiba, Tokyo) and the radioactivity counts of blood and urine were registered daily. Five to seven days after infusion the operations were performed. The patients' data are summarized in Table I.

Autoradiographic analysis using FCR We studied the specific concentration of CEA102 in the tumor by autoradiography using FCR. Imaging plates (IP) (Fuji Photo Film) were exposed by keeping fresh specimens resected at surgery on them for 1–3 h and formalin-fixed speci-

Table I. The Summary of the Patients' Data

Case	Sex	Age	Serum CEA	^{131}I-labeled CEA102 dose	Preoperative diagnosis	Operation	Site of cancer	RAID	Immunoperoxidase with CEA102
1	F[a]	58	746.3	$F(ab')_2$ 4 mg, 167 MBq	Sigmoid colon cancer Liver metastases	Sigmoidectomy and partial resection of liver metastases and the disseminated tumor	Sigmoid colon Liver metastases Dissemination Lymph nodes	Positive Positive[c] Negative Negative	Positve
2	F	58	27.0	$F(ab')_2$ 4 mg, 148 MBq	Sigmoid colon cancer	Sigmoidectomy	Sigmoid colon Lymph nodes	Positive Negative	Positive
3	F	40	109.2	Intact MAb 3 mg, 148 MBq	Rectal cancer	AP resection	Rectum Liver metastases Dissemination Lymph nodes	Positive Negative Negative Negative	Positive
4	M[b]	51	4.7	Intact MAb 2 mg, 152 MBq	Rectal cancer	AP resection	Rectum Lymph nodes	Positive Negative	Positive
5	M	58	7.2	Intact MAb 2 mg, 14.8 MBq	Liver metastasis	Right lobectomy of the liver	Right lobe of the liver	Negative	Positive

a) Female. *b*) Male.
c) Positive: large-sized tumors were detected but small-sized metastatic tumors were not detected.

mens on them for 24 h. Macroautoradiograms of the specimens were made by using FCR. Each tissue density in the autoradiogram by FCR was counted and pixel distribution curves were made and compared. Microautoradiograms of the paraffin sections of the same specimens were made using autoradiographic emulsion NR-M2 (Konica Medical, Tokyo). To confirm the reactivity of CEA102 with the surgical specimen, sections of the same specimens were studied by means of the immunoperoxidase technique.

The quantitative distribution analysis The quantitative distribution analysis of radiolabeled MAb within the surgical specimen was investigated in case 1. The surgical specimen was divided into the respective tissues: primary tumor, normal colon mucosa, mesocolon, pericolic fat, normal lymph node, metastatic lumph node, and disseminated tumor. Tissue radioactivity was counted by using an auto well gamma system ARC 251 (Aloka Co. Ltd., Tokyo). We calculated % injected dose per kilogram (%ID/kg) and tissue-to-blood ratio.

RESULTS

Immunoperoxidase reactivity of CEA102 The results of immunoreactivity of CEA102 with human colon tissues and other tissues are summarized in Table II: 154/164 colorectal cances and 2/2 fetus colon mucosa showed a positive staining but normal colon mucosa did not.

Table II. Immunoperoxidase Reactivity of CEA102 with Various Tissues

Colorectum	
Normal colon mucosa	0/103
Mucosa of fetus (15,16 weeks old)	2/2
Adenoma	1/11
Polyp (early ca.)	26/37
Adenocarcinoma	154/164
Stomach	
Normal mucosa	0/54
Adenocarcinoma	38/54
Esophagus	
Normal and cancer	0/16
Pancreas	
Normal duct	0/7
Adenocarcinoma	4/7
Melanoma	0/2
Astrocytoma	0/2
Breast	
Normal mammary gland	0/10
Adenocarcinoma	7/61
Kidney	0/1
Sweat gland	2/2

CEA102 also reacted with 38/54 gastric cancers, 4/7 pancreatic cancers and 2/2 sweat glands. These results show that CEA102 is a suitable agent for RAID although a limited number of cancers other than colorectal cancer are reactive with this MAb.

Radioimmunodetection The procedure was well tolerated by all patients. No adverse reaction developed following administration of CEA102. Primary tumors were successfully detected by planar scintigraphy with ^{131}I-labeled CEA 102 and identified during the operations in cases 1, 2 (Fig. 1) and 4, but the scintigram of the rectal cancer of case 3 was unclear because of high uptake of the bladder. In case 1, large metastatic tumors in the liver were detected but small tumors and disseminated tumors were not detected (Fig. 1). Although there were some small tumors found in the left lobe of the liver in case 3 during the operation, they were not diagnosed by either RAID or the other preoperative examinations such as CT and US. The liver metastasis in case 5 was not detected because the labeling ratio of the administered MAb was low. The blood clearance of

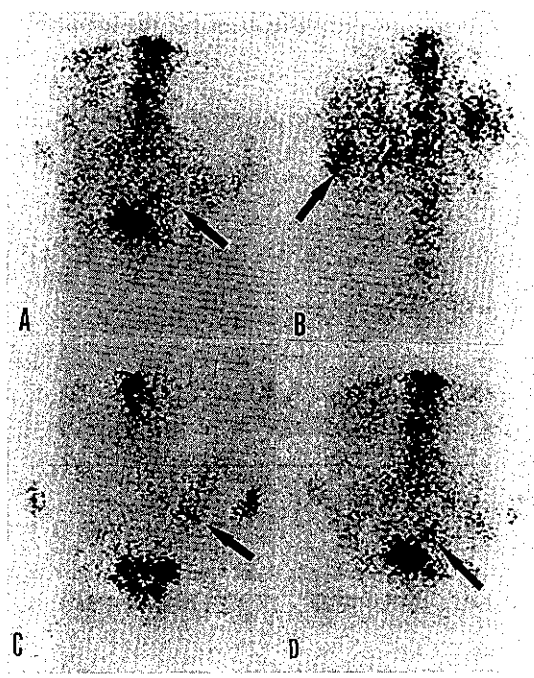

Fig. 1. (AB) Case 1. Scintiscans of pelvis (A) and upper abdomen (B) in anterior views 36 h after injection of ^{131}I-labeled CEA102 F(ab′)$_2$. High uptake is shown in the sigmoid colon lesion (arrow A) and in the right lobe of the liver (arrow B). (C) Case 2. Anterior view 36 h after injection. Positive accumulation of MAb is shown in the sigmoid colon lesion (arrow C). (D) Case 4. Anterior view 72 h after injection. The rectal cancer is visualized as a hot area above the bladder (arrow D).

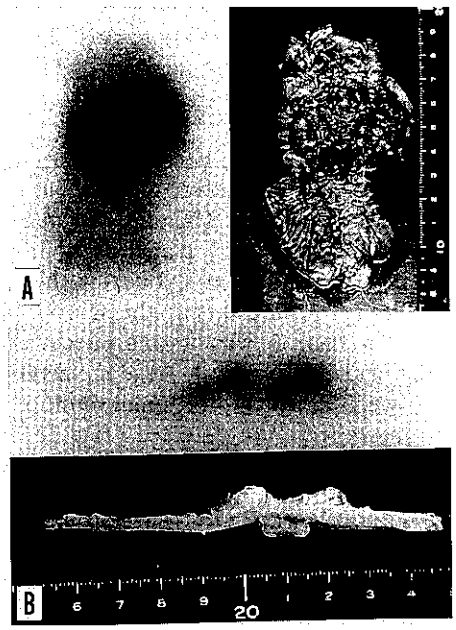

Fig. 2. (A) Case 1. Surgical specimen of the sigmoid colon (right) and its autoradiogram by FCR (left). Specific accumulation of MAb is apparent in the tumor. (B) Case 1. Slice of the primary tumor (lower) and its autoradiogram by FCR (upper). Accumulation of MAb is seen in the tumor.

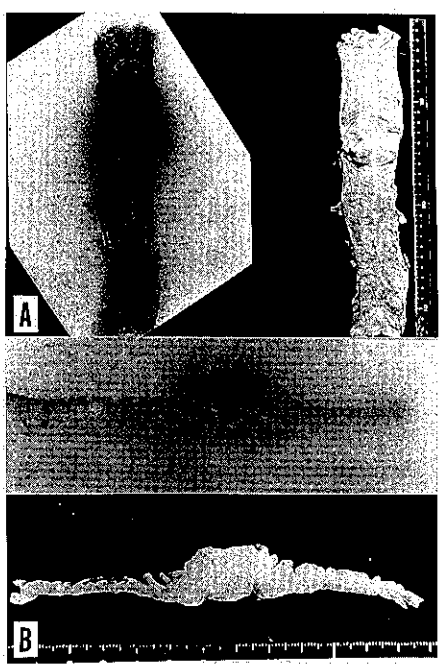

Fig. 3. (A) Case 2. Surgical specimen of the sigmoid colon (right) and its autoradiogram by FCR (left). Specific accumulation of the MAb is apparent in the tumor. (B) Case 2. The slice of the primary tumor (lower) and its autoradiogram by FCR (upper). The tumor has a high uptake of radiolabeled MAb.

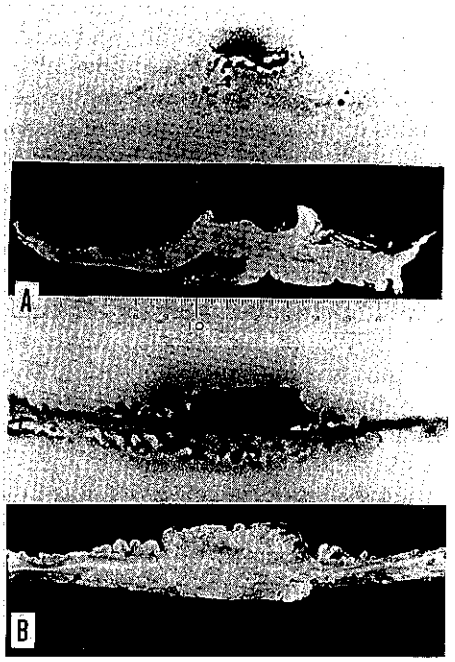

Fig. 4. (A) Case 3. Slice of the resected specimen of the rectum (lower) and its autoradiogram by FCR (upper). Specific accumulation of the MAb is apparent in the tumor. (B) Case 4. Slice of the surgical specimen of the rectum (lower) and its autoradiogram by FCR (upper). The result is similar to that of case 3.

radioactivity from the circulation showed that the blood radioactivity was reduced to less than 20% in 2 days (data not shown).

Autoradiographic analysis using FCR The autoradiograms of the surgical specimens using FCR are shown in Figs. 2, 3, 4 and 5. The autoradiogram of the resected specimen from case 1 demonstrates that administered radiolabeled MAb was localized in the tumor but not in the normal tissues (Fig. 2). The liver metastatic tumor 0.5 cm in diameter was clearly visualized by FCR, and the above results indicated that the MAb was concentrated in even the small liver metastasis (Fig. 5A). In case 2, the primary tumor had a higher radioactivity than normal lesions and was clearly visualized in the autoradiogram (Fig. 3). The autoradiograms of cases 3 and 4 gave similar results (Fig. 4). The liver metastasis of case 5 was visualized in the autoradiogram by FCR (Fig. 5B) although it was not detected by extra gamma scanning. Tissue density in the autoradiogram by FCR was found (Table III) and the pixel distribution curve is shown in Fig. 6. An uneven distribution of radioactivity within the tumors was observed. Although heterogeneity of distribution of the administered radiolabeled MAb in the tumor was found, the density of the tumors was higher

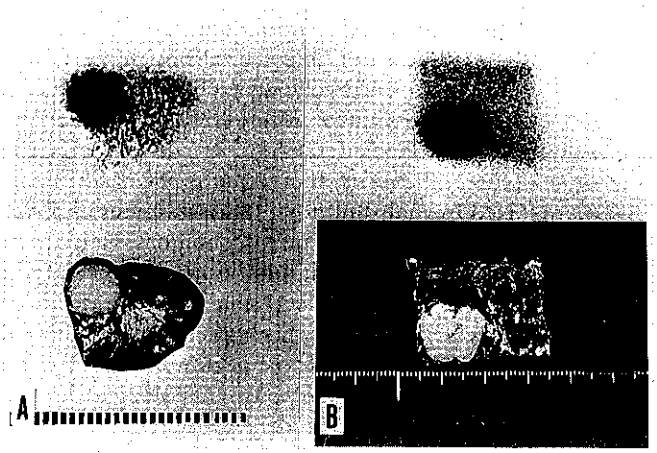

Fig. 5. (A) Case 1. Specimen of the partially resected liver (lower) and its autoradiogram by FCR (upper). A small liver metastasis (0.5 cm) is clearly visualized in the autoradiogram. (B) Case 5. Specimen of the liver metastasis (lower) and its autoradiogram by FCR (upper).

Table III. Tissue Density Counted in Autoradiogram by FCR

Case	Tissue	Density (mean±SD)
1	Primary tumor	2.10±0.10
	Normal colon	0.69±0.04
	Liver metastasis	1.30±0.15
	Normal liver	0.79±0.09
	Disseminated tumor	1.85±0.12
2	Primary tumor	2.30±0.13
	Normal colon	1.77±0.03
3	Primary tumor	1.33±0.05
	Normal colon	0.86±0.06

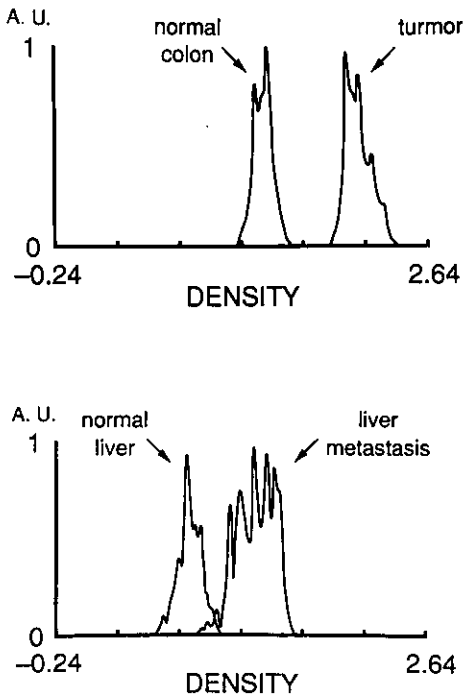

Fig. 6. The pixel distribution curves of the respective tissues in the autoradiograms by FCR of Case 1. The arbitrary unit on the ordinate in the frequency of the pixel with the highest frequency assigned a base of 1. Although heterogeneity within the tumor is apparent, the density of the tumor is higher than that of the mormal tissue.

than that of the normal tissues. The microautoradiograms of paraffin sections of the same specimens showed that clusters of high grain densities were distributed at the surfaces of the tumor cells (Fig. 7A). To confirm the reactivity of CEA102 with the surgical specimens, immunoperoxidase staining was performed in all specimens and the results were positive (Fig. 7B).

Quantitative distribution analysis We studied %ID/kg and tissue-to-blood ratio of the respective tissues of the resected specimen in case 1. As shown in Table IV, the primary tumor uptake was mean 1.10±0.88SD%ID/kg, whereas the normal colon mucosa uptake was mean 0.11±0.07SD %ID/kg. The tumor had a 10-fold greater uptake than normal colon mucosa. The tissue-to-blood ratio showed that both the primary tumor (mean 3.74± 3.27SD) and disseminated tumor (mean 2.99±2.27SD)

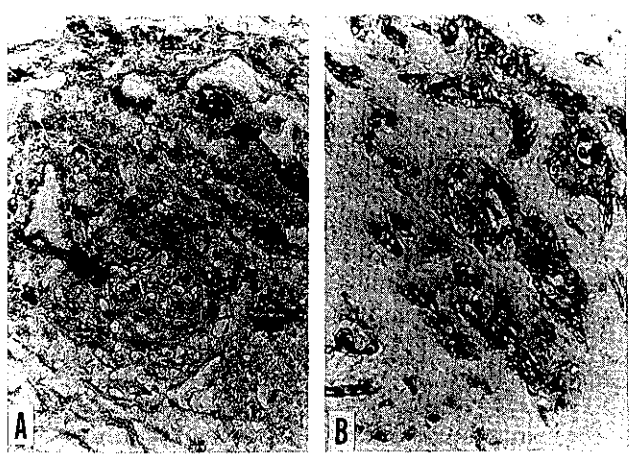

Fig. 7. (A) Microautoradiogram of the primary tumor of case 3 (HE stain, ×400). Clusters of high grain density are distributed at the surfaces of the tumor cells. (B) Immunoperoxidase staining with CEA102 of the primary tumor of case 3 (×400). The tumor has positive staining.

Table IV. Tissue Uptake of ^{131}I-labeled CEA102 in Case 1

Tissue	%ID/kg (mean±SD)
Primary tumor	1.10±0.88
Disseminated tumor	0.89±0.55
Normal colon mucosa	0.11±0.07
Pericolic fat	0.07±0.04
Mesocolon	0.22±0.06
Omentum	0.06±0.03
Normal lymph node	0.41±0.33
Metastatic lymph node	0.78±0.37

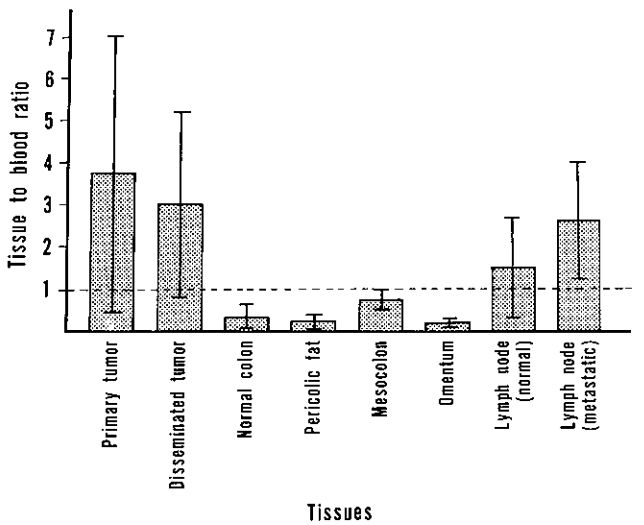

Fig. 8. Tissue-to-blood ratio. The radioactivity of the primary and metastatic tumor is higher than that of the normal tissues. The primary tumor has a 10-fold-greater uptake than normal colon mucosa.

were higher than the normal tissues (normal colon mucosa mean 0.35±0.31SD, pericolic fat mean 0.22±0.15, mesocolon mean 0.75±0.27SD, omentum mean 0.20±0.10SD). Metastatic lymph node (mean 2.63±1.43SD) was also higher than normal lymph node (mean 1.49±1.22SD) (Fig. 8). These results indicate that CEA102 was specifically accumulated in the tumors of both the primary and metastatic lesions.

DISCUSSION

Radioimmunodetection using MAbs against tumor-associated antigens has recently received a great deal of attention as a tool for qualitative diagnosis. In this study, primary tumors in cases 1, 2, and 4 and the large liver metastatic tumors in case 1 were successfully detected by extra scanning. Rectal cancer in case 3 was not clearly detected because of the high radiouptake of the bladder. The small liver metastatic tumors and the disseminated tumors in case 1 were not detected, although the tumors in all cases reacted positively with CEA102 by the immunoperoxidase technique. These results showed that imaging with ^{131}I-labeled CEA102 could detect CEA-positive colorectal cancer, but not in every case.

As reported by many authors, the assessment of RAID as one of the current imaging techniques is that this method is useful for detecting tumors qualitatively, but is limited in its ability to detect small-sized tumors and in its usefulness in immunotherapy. Therefore, it is of interest to investigate the specific accumulation of radiolabeled MAb in the tumors at the histological level in relation to the external imaging. Autoradiographic analysis is useful for this purpose, but only a few analyses have been performed in clinical studies.[19, 20] It has been reported that surgical specimens were imaged under the gamma camera, but the images were not of higher quality than those obtained by autoradiography.[5, 6] We tried imaging our specimens under the gamma camera and obtained poor images (data not shown). We then applied FCR to autoradiographic analysis. For comparative purposes, a biodistribution study was also performed. FCR was developed as a result of the progress of digital imaging, and it aims to replace the present screen/film system for analog X-rays.[23] IP, developed as a sensor, are exposed by placing specimens on them and autoradiograms are made by converting the signals read on the IP into digital signals. This method has the advantages of being a rapid technique and of providing high-quality images. Using this method, we could confirm the specific accumulation of radiolabeled MAb in the tumors in all cases, even the small distant metastasis (0.5 cm). Although the liver metastasis in case 5 was not detected by extra scanning due to the low labeling ratio of the administered MAb, the tumor was clearly visualized in the autoradiogram by FCR. The pixel distribution curves of the density of the primary tumor and the liver metastasis in case 1 showed a heterogenous accumulation of MAb in the tumors although these specimens were homogeneously imaged in autoradiograms. Autoradiography by FCR is, therefore, an important tool for observing the affinity of MAb to tumor and the distribution of MAb as a radioactivity map.

From the results of the biodistribution study, the primary tumor had a 10-fold-greater uptake than normal colon mucosa, metastatic lymph node uptake was higher than normal lymph node uptake, and disseminated tumor uptake was higher than normal tissue uptake. These results confirmed that CEA102 intravenously injected was specifically accumulated in primary tumors, liver metastases, disseminated tumors, and metastatic lymph nodes. However, normal lymph node uptake was report-

edly higher than metastatic lymph node uptake in studies using anti-CEA MAb.[3-5] The difference between those results and ours may be caused by several factors: subtle differences of characteristics of the anti-CEA MAb, the use of ^{111}In, or characteristics of the bifunctional chelating agents. In both autoradiographic analysis and the quantitative distribution study, the distribution of radioactivity within individual tumors was not uniform. This is considered to be mainly a result of heterogeneity of CEA distribution in the tumor. Additional important factors to be considered include tumor cellularity, capillary permeability, and local blood flow.

In the cases administered the intact MAb (3, 4 and 5), the tumors were detected by extra scanning at 72 h after infusion. But in the cases administered $F(ab')_2$ (1 and 2), the tumors were detected at 36 h after infusion. The rise of the radioactivity ratio of tumor to background in the case of $F(ab')_2$ was faster than that in the case of intact MAb. A difference between the intact MAb and $F(ab')_2$ was found in the clearance from the backgrounds. However, in autoradiographic analysis, no significant difference between intact MAb and $F(ab')_2$ was found regarding the distribution and the tumor uptake of the specimens.

To encourage research and clinical trials on RAID, observations concerning *in vivo* delivery of MAb to tumor and *in vivo* distribution of MAb are important and fundamental, and autoradiography using FCR is a suitable tool for this purpose. The results of this study are encouraging, suggesting that small metastatic tumors and lymph node metastases could be detected by using an appropriately chosen MAb, radionuclide, labeling method, scanning technology, etc. in the fairly near future. Moreover, such studies will be helpful in the development of immunotherapy with MAb alone or conjugated with radionuclides, chemotherapeutic agents or toxins.

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