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B Delaloye, … , J Pettavel, J P Mach

J Clin Invest. 1986;77(1):301-311. https://doi.org/10.1172/JCI112291.

Research Article

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Detection of Colorectal Carcinoma by Emission-computerized Tomography after Injection of ^{123}I-labeled Fab or F(ab')$_2$ Fragments from Monoclonal Anti-Carcinoembryonic Antigen Antibodies

Bernard Delaloye, Angelika Bischof-Delaloye, Franz Buchegger, Vladimir von Fliedner, Jean-Philippe Grob, Jean-Claude Volant, Jacques Pettavel, and Jean-Pierre Mach

Division of Nuclear Medicine, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland; Ludwig Institute for Cancer Research, Lausanne Branch, CH-1066 Epalinges, Switzerland; Department of Surgery, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne; and Centre pluridisciplinaire d'Oncologie, Policlinique Médicale Universitaire, CH-1011 Lausanne, Switzerland

Abstract

This clinical study was based on experimental results obtained in nude mice grafted with human colon carcinoma, showing that injected ^{131}I-labeled F(ab')$_2$ and Fab fragments from high affinity anti-carcinoembryonic antigen (CEA) monoclonal antibodies (MAb) gave markedly higher ratios of tumor to normal tissue localization than intact MAb. 31 patients with known colorectal carcinoma, including 10 primary tumors, 13 local tumor recurrences, and 21 metastatic involvements, were injected with ^{123}I-labeled F(ab')$_2$ (n = 14) or Fab (n = 17) fragments from MAb anti-CEA. The patients were examined by emission-computerized tomography (ECT) at 6, 24, and sometimes 48 h after injection using a rotating dual head scintillation camera. All 23 primary tumors and local recurrences except one were clearly visualized on at least two sections of different tomographic planes. Interestingly, nine of these patients had almost normal circulating CEA levels, and three of the visualized tumors weighed only 3–5 g. Among 19 known metastatic tumor involvements, 14 were correctly localized by ECT. Two additional liver and several bone metastases were discovered by immunoscintigraphy. Altogether, 86% of the tumor sites were detected, 82% with F(ab')$_2$ and 89% with Fab fragments. The contrast of the tumor images obtained with Fab fragments suggests that this improved method of immunoscintigraphy has the potential to detect early tumor recurrences and thus to increase the survival of patients. The results of this retrospective study, however, should be confirmed in a prospective study before this method can be recommended for the routine diagnosis of cancer.

Introduction

After experimental work in nude mice and in hamsters heterografted with human colon carcinoma (1, 2), large series of patients have been injected with ^{131}I-labeled, purified polyclonal antibodies against carcinoembryonic antigen (CEA)[1] (3) in order to detect tumors by planar scintigraphy (4–10) (Delaloye et al., unpublished results). In these studies some striking cases of tumor detection were obtained with what should be called the first generation of immunoscintigraphy (polyclonal antibodies and planar scintigraphy). Major differences emerged, however, in the interpretation of the clinical results obtained. Goldenberg's group claimed that the results obtained with the first generation of immunoscintigraphy were already clinically useful (4, 9, 10), whereas our group, despite the use of goat anti-CEA antibodies giving specific tumor to normal tissue ratios in 58 colorectal carcinoma patients, concluded that the technology of immunoscintigraphy should be improved before it could be usefully applied in the diagnosis of carcinoma (5, 6).

In 1981, the first monoclonal anti-CEA antibody (MAb) 23, which had an affinity of 5.7×10^8 M^{-1} (11), was studied in a series of 31 colorectal carcinoma patients tested by planar scintigraphy (12). The results were slightly improved as compared with polyclonal antibodies, in the sense of less nonspecific accumulation of the tracer in the reticuloendothelial system, but they were not yet considered satisfactory for clinical application. The same MAb 23 was tested in an additional series of 16 patients with colorectal carcinoma and medullary thyroid carcinomas, using for the first time emission-computerized tomography (ECT) for detection of labeled antibodies (12, 13). With this second generation of immunoscintigraphy, the results were encouraging in terms of sensitivity (16–17 tumors detected) but the signal-to-noise ratio was not very high, in part due to the poor detection efficiency of ^{131}I by the camera and in part to the relatively low affinity of MAb 23.

As a further way of improving this technique we produced an additional series of 26 new anti-CEA MAb, which were selected first in vitro on the basis of high affinity for CEA and absence of crossreactivity with granulocyte glycoproteins (14, 15), and second, in vivo in the nude mouse model for good localization in human colon carcinoma heterograft (14). The MAb with the highest affinity for CEA (5.8×10^9 M^{-1}) and no crossreactivity with granulocytes was MAb 35. In addition, F(ab')$_2$ and Fab fragments from MAb 35 were shown to give 3 and 12 times, respectively, higher tumor to normal tissue ratios as compared with intact MAb 35, in the nude mouse model (16). Thus, in this experimental model, Fab fragments, which have a single

This work was presented in part at the 31st Annual Meeting of the Society of Nuclear Medicine in Los Angeles, CA, and has also appeared in abstract form (1984, *J. Nucl. Med.* 25:17).

Address reprint requests to Dr. Delaloye, Division of Nuclear Medicine, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland.

Received for publication 9 July 1985.

J. Clin. Invest.

0021-9738/86/01/0301/10 $1.00
Volume 77, January 1986, 301–311

1. *Abbreviations used in this paper:* CEA, carcinoembryonic antigen; CT scan, x-ray computerized tomography; ECT, emission-computerized tomography (also called SPECT, single photon emission-computerized tomography); MAb, monoclonal antibodies.

binding site and about one-third the size of an intact antibody, gave the most clear-cut tumor localization.

Here we present the results obtained in a series of 31 colorectal carcinoma patients injected with ^{123}I-labeled F(ab')$_2$ or Fab fragments from MAb 35 and tested by ECT, using a dual head rotating camera. This third generation of immunoscintigraphy has four advantages over the first generation: (*a*) the use of a highly selected MAb, with high affinity for CEA and no crossreaction with granulocytes (a crossreaction which has been reported to provoke major problems with many anti-CEA MAb) (17); (*b*) the use of MAb fragments, which have been shown to be more rapidly eliminated from the circulation and to penetrate better into tumors (16, 18); (*c*) the use of ECT, eliminating the necessity of subtraction techniques which are susceptible to the formation of artifacts (19); (*d*) the use of ^{123}I, which appears to be one of the most convenient isotopes for imaging with gamma cameras (20, 21).

The purpose of the retrospective clinical study reported here is to evaluate the improvement in the detection of known colon carcinoma sites by this new generation of immunoscintigraphy. A prospective clinical trial is ongoing to compare this method with other modern methods of tumor detection.

Methods

An unselected series of 31 patients (21 males aged 35–82 [mean 63] yr, and 10 females aged 35–80 [mean 58] yr) with known colorectal carcinoma, diagnosed by contrast medium enema, rectosigmoidoscopy, colonoscopy, x-ray computerized tomography (CT scan), ultrasound, bone scan and/or radiography, was studied. Tables I–III show the clinical features for 10 patients with primary tumors (all of them were operated within 20 d after the present study), 13 patients having local recurrences with or without metastases (time between first operation and present study ranged from 1 to 28 mo; mean, 13 mo) and 8 with only distant metastases (time between first operation and study ranged from 5 to 50 mo; mean, 28 mo). The metastases were localized in the liver (n = 14), in the bone (n = 3), in the lung (n = 2), or in the peritoneum (n= 2). In four of these patients, metastatic involvement of the liver (n = 2), or bone (n = 2) was first detected by immunoscintigraphy and confirmed thereafter. Dukes' stage was determined at the first operation according to the original Dukes' classification with the addition of Dukes' D for distant metastases.

The thyroid was blocked by oral administration of Lugol's 5% Iodine solution (50 mg/d) for 2 d before and 3 d after injection. The thyroidal uptake was not more than 1% of the injected dose of ^{123}I as determined at 6 and 24 h.

To prevent allergic reactions, 16 and 1 h before injection the patients received an antihistaminic drug (Clemastine, 2 mg); in addition, 100 mg of prednisolone was injected intravenously 1 h before the administration of the antibody. No intracutaneous testing was performed. None of the 31 patients injected showed any adverse effect or any sign of discomfort during or after the injection of radiolabeled MAb.

One patient received the F(ab')$_2$ fragments of anti-CEA MAb 202, 13 patients F(ab')$_2$, and 17 patients Fab fragments of anti-CEA MAb 35. The fragments were prepared by pepsin or papain digestion, respectively, as previously described (16). 1.5 mg of F(ab')$_2$ or 1 mg of Fab fragments labeled with 2.3–8.0 (mean, 3.95 SD±1.04) mCi of ^{123}I were given in a 1-h i.v. perfusion. ^{123}I was produced from the ^{127}I (p, 5n) ^{123}Xe reaction and generously provided by the Swiss Federal Institute for Reactor Research, Würenlingen, Switzerland. The antibody fragments were simultaneously labeled with 0.07 mCi of ^{125}I to allow in vitro activity measurements of surgical specimens, as well as of late blood and urine samples. The labeling was performed at 4°C by the Iodogen method (Pierce Chemicals Co., Rockford, IL). Labeled antibody was separated from free iodine by chromatography on a Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden) column equilibrated in pyrogen-free 0.15 M saline and sterilized by filtration through a 0.22-μm Millipore filter (Millipore/Continental Water Systems, Bedford, MA) and tested for sterility and absence of pyrogenicity (in rabbits). All antibody preparations were tested for immunoreactivity by incubation with CEA coupled to cyanogen bromide (CNBr)-activated Sepharose (Pharmacia Fine Chemicals), as previously described (16). The percentage of binding of the labeled MAb fragments to insolubilized CEA ranged from 52 to 75% (mean 59%).

Anterior and posterior whole body scans were performed simultaneously at 1, 6, 24, and 48 h after injection by the means of a rotating dual head scintillation camera, ROTA camera (Siemens, Inc., Erlangen, West Germany) equipped with a whole body attachment and linked with an array processor (Computer Design and Applications Inc., Waltham, MA) and a computer (PDP 11/44, Digital Equipment Corporation, Maynard, MA). ECT studies of the lower and upper abdomen were performed at 6 and 24 h in all patients and also at 48 h in 11 patients. Additional regions were studied by ECT when other metastatic lesions were suspected.

For all tomographic reconstructions, 30 views per camera head were stored with an angular increment of 6°, using low energy all-purpose

Table I. Clinical Features and Immunoscintigraphic Results from Patients with Primary Tumors

Patient no./age/sex	Primary tumor	Dukes' stage	Tumor weight	Serum CEA	MAb fragments*	Injected activity	Scintigraphic results‡	
							Primary tumor	Liver metastases
			g	*μg/liter*		*mCi ^{123}I*		
1/80/M	Right colon	C	27	125	F(ab')$_2$	3.4	+	
2/60/M	Rectum	B	40	4	F(ab')$_2$	3.2	+	
3/60/M	Sigmoid	C	15	4	F(ab')$_2$	4.3	+	
4/75/M	Right colon	C	26	2	F(ab')$_2$	3.4	+	
15/76/M	Right colon	C	33	51	Fab	4.8	+	+
16/62/F	Sigmoid	B	18	3	Fab	2.3	+	
17/80/F	Left colon	B	3	2	Fab	4.2	+	
18/56/M	Rectum	A	4.5	25	Fab	5.1	+	
19/70/F	Sigmoid	D	50	1,370	Fab	4.2	+	+
20/55/F	Sigmoid	C	5	2	Fab	8.0	+	+

* All fragments were from monoclonal anti-CEA antibody no. 35 except for patient 1, who received F(ab')$_2$ from MAb 202. ‡ The + sign indicates primary or metastatic tumor sites that were visualized on at least two sections of different tomographic planes.

Table II. Clinical Features and Immunoscintigraphic Results from Patients with Local Recurrences

Patient no./age/sex	Primary tumor	Dukes' stage	Serum CEA	MAb fragments	Injected activity	Scintigraphic results*		
						Local recurrences	Distant metastases	
							Liver	Other
			μg/liter		*mCi ^{123}I*			
5/61/M	Sigmoid	C	240	$F(ab')_2$	3.6	+	+	
6/44/F	Rectum	C	86	$F(ab')_2$	2.9	+		
7/80/M	Rectum	B	1,200	$F(ab')_2$	3.3	+		
21/64/M	Sigmoid	C	13	Fab	3.7	+	+	
22/66/M	Rectum	A	11	Fab	4.0	+		
23/61/F	Caecum	C	1	Fab	8.0	+		
24/76/M	Sigmoid	D	7	Fab	4.3	+		− peritoneum
25/35/F	Right colon	B	140	Fab	3.8	−		+ bone − peritoneum
26/35/F	Sigmoid	B	48	Fab	4.1	+		
27/53/M	Rectum	B	415	Fab	4.5	+		
28/40/M	Rectosigmoid	B	800	Fab	2.5	+	+	
29/82/M	Right colon	B	3	Fab	4.9	+		
30/57/M	Sigmoid	C	2	Fab	4.2	+	+	

* The + sign indicates tumor sites that were visualized on at least two sections of different tomographic planes. The − sign indicates tumor sites that were not clearly visualized by immunoscintigraphy.

collimators. At 6 h post injection, an average of 70,000 counts per view were collected in about 30-s exposures; at 24 h, 50,000 counts in 60 s; and at 48 h, some 30,000 counts in 120 s. For all regions examined, transverse, sagittal, and coronal sections were reconstructed. A tumor site was considered as positive only when an uptake of radioactivity corresponding to the suspected tumor location was clearly detected on at least two sections from different tomographic planes and at two different time intervals. Following the last ^{123}I-ECT, 4 mCi of Tc-99m sulfur colloid was injected and another ECT of the liver was performed, with the patient remaining in the same position. No substraction techniques were used. The data were filtered during reconstruction. Smoothing and adjustment of lower and upper thresholds were performed when necessary for better visualization of tumors (voxels out of thresholds were set zero = black).

The camera was checked daily for center alignment, offset, uniformity, and sensitivity of both heads. The slice thickness of the reconstructed sections was 12 mm.

The serum CEA levels were measured by a solid-phase enzyme immunoassay using three different monoclonal anti-CEA antibodies (22). The normal level of serum CEA using this assay ranged from 0 to 5 μg/liter.

Results

Tumor status and immunoscintigraphy data for all patients are summarized in Tables I–III. Table I shows that all primary tumors have been visualized by this technique with both types of fragments and regardless of the level of circulating CEA.

Fig. 1 illustrates the detection of a carcinoma of the caecum on a coronal (i.e., frontal) ECT section of the lower abdomen and pelvis from patient 1. The $F(ab')_2$ fragments from MAb anti-CEA 202 injected in this patient were avidly taken up by the tumor, as confirmed by direct measurement of the radioactivity in resected tumor and adjacent tissues (Fig. 2 *A*). MAb

Table III. Clinical Features and Immunoscintigraphic Results from Patients with only Metastatic Disease

Patient no./age/sex	Primary tumor	Dukes' stage	Serum CEA	MAb fragments	Injected activity	Scintigraphic results*	
						Metastases to	
						Liver	Others
			μg/liter		*mCi ^{123}I*		
8/59/M	Rectum	C	1,150	$F(ab')_2$	4.4	+	
9/65/M	Sigmoid	C	2,500	$F(ab')_2$	3.5	−	− lung
10/68/M	Rectosigmoid	C	52	$F(ab')_2$	4.4	+	
11/47/F	Sigmoid	C	70	$F(ab')_2$	4.7		+ bone
12/76/F	Right colon	B	72	$F(ab')_2$	3.2	+	
13/70/F	Rectum	C	2,100	$F(ab')_2$	3	+	+ bone
14/59/M	Left colon	D	34	$F(ab')_2$	3.7	−	
31/46/M	Caecum	C	17	Fab	4.7	+	+ lung

* See Table II.

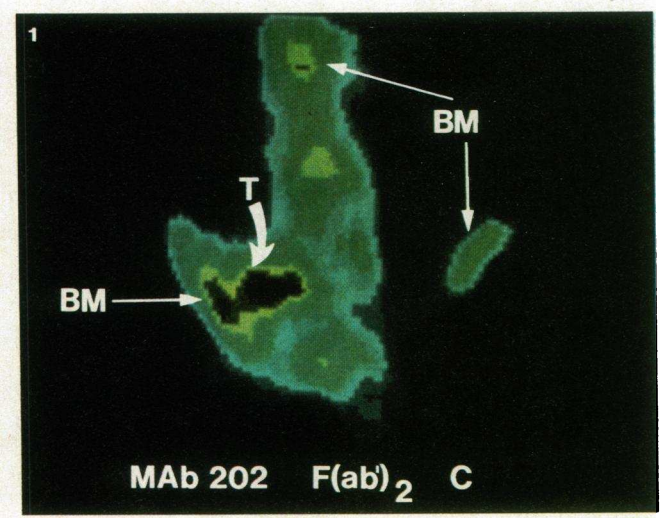

Figure 1. Uptake of MAb 202 F(ab')$_2$ fragments in tumor but also in bone marrow. The coronal section (*C*) of patient 1 with a carcinoma of the caecum obtained 24 h after injection shows avid uptake of the antibody by the tumor (*T*) as confirmed by in vitro counting of the surgical specimen (Fig. 2 *A*), but also visualization of bone marrow (*BM*) which seems to be related to the crossreaction of MAb 202 with granulocyte glycoproteins (15). The colors red, yellow, green, and blue correspond to the degree of concentration of radioactivity: red is high and blue is low.

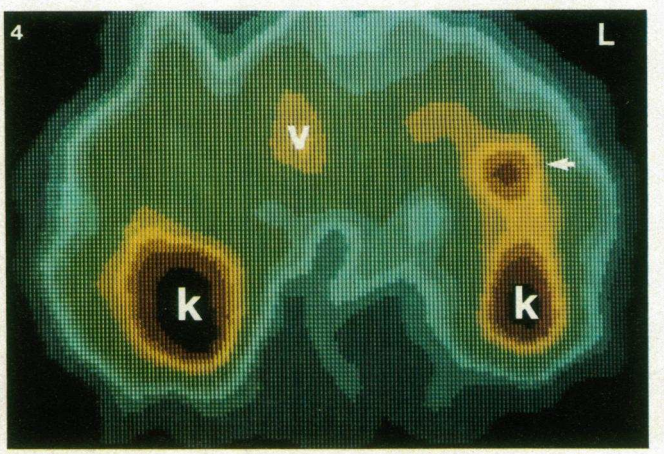

Figure 4. Detectability of small tumors. The tumor (arrow) presented on this figure weighed only 3 g and was located just distal to the splenic angle (patient 17). On the transverse section shown, the tumor is anterior to the left kidney (*k*). By 6 h after injection, tumor activity exceeded vascular activity (*V*). *L*, left.

202, however, was injected in only one patient because it was found to bind to granulocytes (15) and to be taken up by the normal bone marrow, as demonstrated in patient 1 (Fig. 1). Fragments of MAb 35 did not bind to granulocytes (15). They were injected in the next 30 patients and did not show uptake of radioactivity in the normal bone marrow.

Fig. 3 shows transverse sections taken at the same level of the middle abdomen at different times after injection of Fab fragments from MAb 35 in patient 15. The sections cut across a right colon tumor and the large abdominal vessels (aorta and inferior vena cava). At 6 h after injection vascular activity exceeded tumor activity, which was, however, already detectable. The tumor to blood circulation activity ratio increased with time, being near one at 24 h and definitely higher at 48 h. In our experience all but one primary tumor has been clearly distinguished on the 6-h scans, but the most reliable images have been obtained after 24 h. Images obtained more than 24 h after injection often had artifacts created by patient movement in the case of long data collection times or by statistical uncertainty in the case of shorter ones. The tumor of the patient presented in Fig. 3 was rather large, weighing 33 g and having a maximum diameter of 6 cm. Detectability of primary tumors by ECT, however, does not appear to be related to tumor size.

The smallest tumor in this series was detected in patient 17 after injection of Fab fragments from MAb 35. It weighed 3 g and was a primary tumor of the descending colon situated just distal to the splenic angle. In spite of its small size, it was easily distinguished on the 6-h transverse section as a unique hot spot anterior to the left kidney (Fig. 4). Kidneys take up radioactivity during 24 h after injection and can be used as landmarks.

It is sometimes difficult to distinguish tumor from bladder activity when the tumors are located in the lower pelvis, as in patient 18 who had a carcinomatous polyp of the recto-sigmoid weighing only 4.5 g, which was detected with Fab fragments of MAb 35. On the transverse section, tumor activity could not be separated from bladder activity, whereas on the 24-h sagittal section (Fig. 5) the tumor appeared as a well-delineated area of ectopic activity behind the bladder. The uptake of radioactivity by this tumor was confirmed by direct counting of the tissues after surgical resection (Fig. 2 *C*).

The detection of a 5-g sigmoid tumor in patient 20 is demonstrated in Fig. 6. In a coronal section of the lower abdomen obtained 24 h after injection of Fab fragments from MAb 35, the tumor is well defined on the upper left of the pelvis and

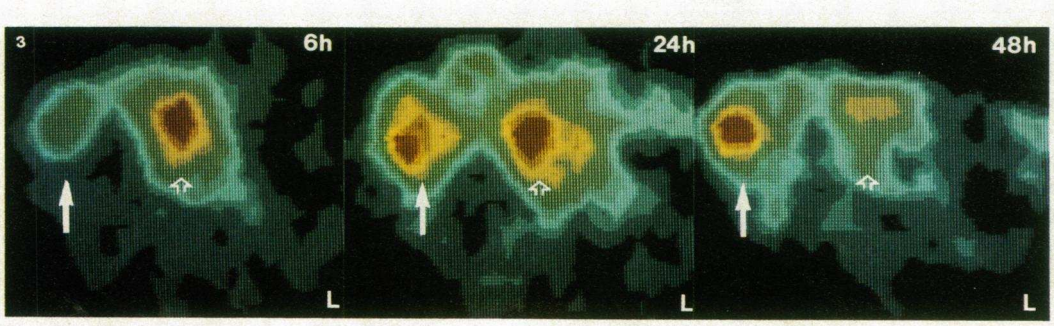

Figure 3. Change of tumor-to-blood activity ratio with time. The transverse sections of the lower abdomen of patient 15 across a right colon carcinoma (closed arrow) and large vessels (open arrow) at different time intervals after injection of labeled Fab fragments from MAb 35 show a progressive increase of tumor-to-blood ratio. Tumor activity is lower than blood at 6 h; it equals it at 24 h; and it exceeds it at 48 h. *L*, left.

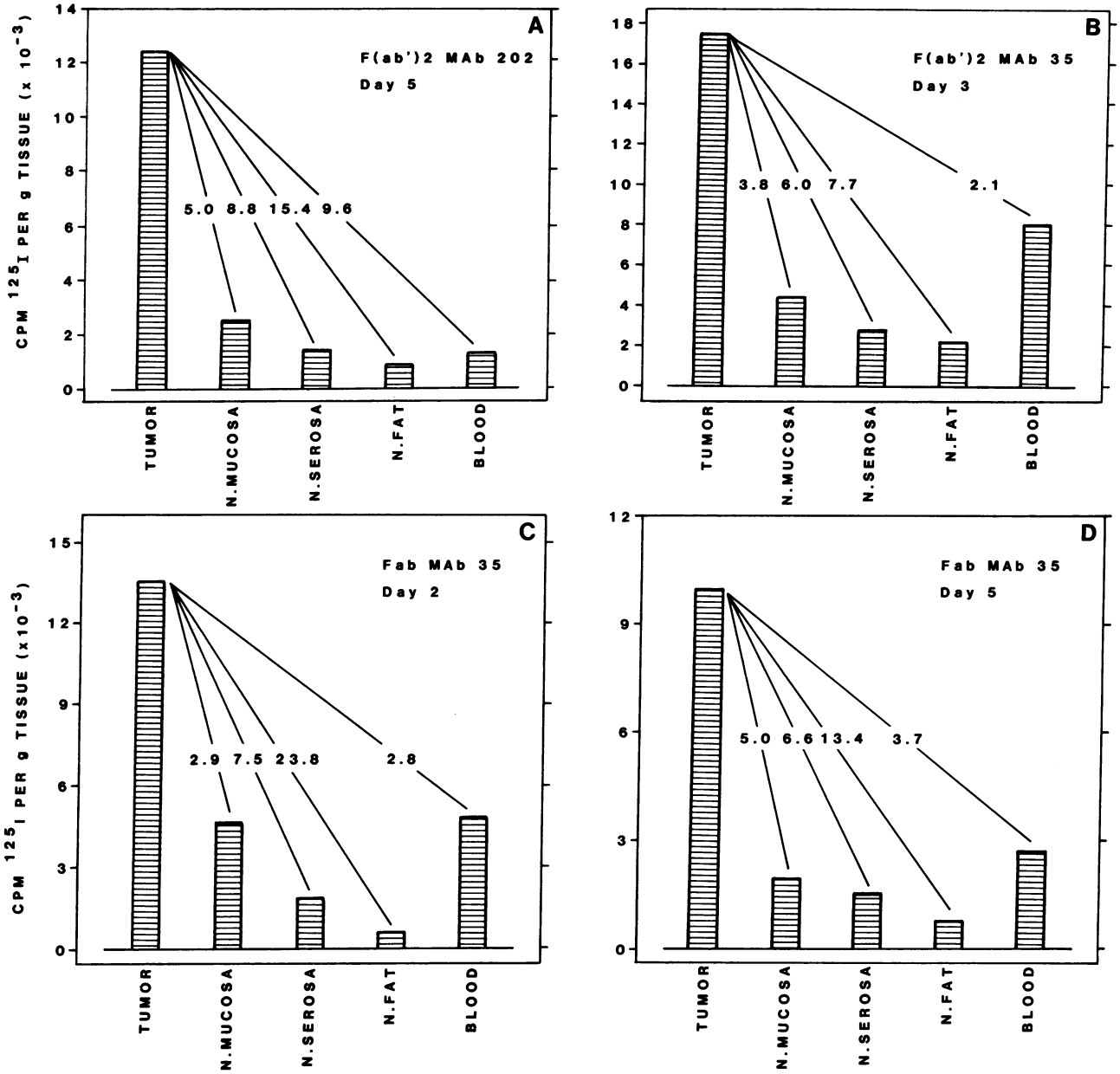

Figure 2. Demonstration of the tumor localization of MAb anti-CEA fragments by direct measurement of radioactivity in tumor and adjacent normal tissues. The results were obtained on surgically resected tissues from four different patients with colorectal carcinoma injected with (*A*) $F(ab')_2$ fragments from MAb 202 (patient 1), (*B*) $F(ab')_2$ fragments from MAb 35 (patient 4), and (*C* and *D*) Fab fragments from MAb 35 (patients 18 and 20). The concentration of radioactivity per gram of tissue is indicated by the vertical bars for tumor, dissected normal mucosa (N. Mucosa), remaining bowel wall (N. Serosa), normal fat (N. Fat), and whole blood taken preoperatively. The numbers in the middle of the oblique lines indicate the tumor-to-normal tissue ratios.

distinct from the radioactivity in the urinary bladder. This tumor was resected 5 d after injection and the radioactivity in tumor and adjacent normal tissues was compared (Fig. 2 *D*).

Table II summarizes the results obtained in patients with local tumor recurrences. All but one local recurrence could be visualized by this technique, even in patients with very low or normal CEA levels. In a single patient (patient 25), the recurrent tumor at the ileo-colic anastomosis after right colectomy could not be visualized, whereas Fab fragments were taken up by multiple bone metastases that had not been suspected before and were only thereafter confirmed by a conventional bone scan (Tc-99m dicarboxy-propane-diphosphonate). Fig. 7 shows on the left panel a sagittal section through the thorax and upper lumbar spine and on the right panel a coronal section of the pelvis. Both sections showed multiple hot spots corresponding to uptake of the labeled antibody.

Altogether, 21 distant tumor involvements (several tumor sites in one organ or tissue were counted as one) were present and 16 were correctly localized by ECT (Tables I–IV). There were only two patients with lung metastases in this series. One (patient 31) showed two distinct sites of uptake of radioactivity which indicated the presence of two metastatic lesions corre-

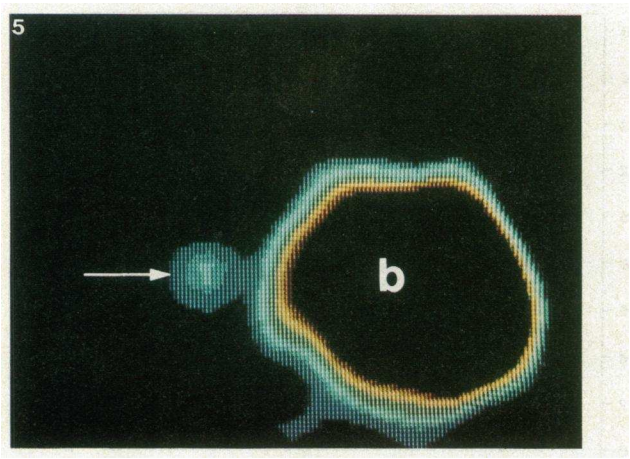

Figure 5. Interference of tumor and bladder activity. Small tumors near the bladder may not be easily differentiated from urinary activity. The sagittal section is often the most useful one, as shown here for patient 18, who presented a 4.5-g carcinomatous polyp of the rectum. 6 h after injection, tumor activity (arrow) is clearly distinct from urinary activity in the bladder (*b*), which is located anteriorly. 2 d after injection, the patient was operated on and specific tumor-to-normal tissues ratios were observed by direct counting of the radioactivity in tissues (Fig. 2 *C*).

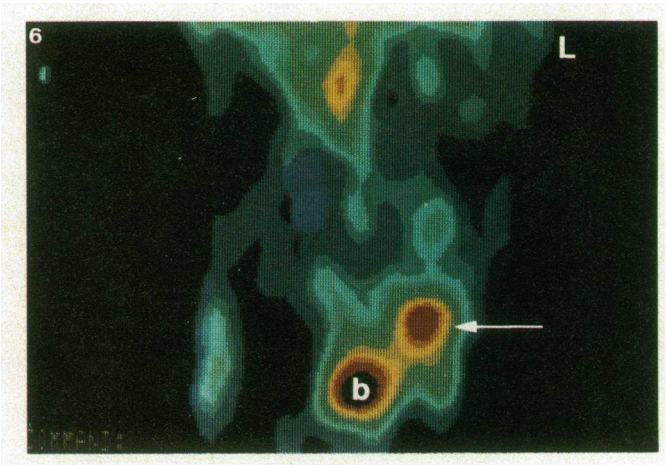

Figure 6. Visualization of a 5-g sigmoid tumor on a coronal section of the pelvis obtained 24 h after injection in patient 20. Tumor (arrow) and bladder (*b*) are well-defined. The relatively high tumor uptake of Fab fragments was confirmed by direct counting of the resected tumor and adjacent normal tissues (Fig. 2 *D*). *L*, left.

sponding exactly with the radiological findings. No uptake of radioactivity could be demonstrated in two small lung lesions of the other patient (patient 9), who had in addition a large liver metastasis which also undetected by ECT and a very high serum CEA level (2,500 μg/liter).

Two regional tumor metastases in the peritoneum were not detected. One was situated in the lower abdomen of patient 25, who had in addition multiple bone metastases which took up Fab fragments and the already mentioned single undetected local recurrence from this series. One may assume that the accumulation of labeled fragments in the bone metastases prevented their localization in the local and regional tumor recurrence. In the other patient with peritoneal involvement (patient 24), the sigmoid local recurrence was correctly identified, whereas the peritoneal carcinomatosis remained undetected by immunoscintigraphy.

Two patients (patients 15 and 20) with primary tumors had normal liver CT scan or ultrasound examinations, whereas immunoscintigraphy showed antibody uptake in an area of the liver which proved to be photon deficient on the Tc-99m sulfur colloid ECT. In both cases, the liver metastases were confirmed at surgery. Among 12 patients with known liver metastases (as detected by ultrasound and/or CT scan), 10 had lesions that took up antibody fragments. Liver involvement was undetected in two patients, one with liver cirrhosis (patient 9) and the other with a metastasis located at the site of resection after repeated partial hepatectomy (patient 14).

ECT studies from two patients with liver metastases will be presented in further detail. The first patient (patient 12) had a large metastasis in the posterior part of the right lobe of the liver easily detected by ultrasound. This case is presented (Fig. 8) because it demonstrates the slow and progressive penetration of the $F(ab')_2$ fragments within the tumor mass as well as the morphological accuracy of the ECT images.

Patient 15 was not known to have liver metastasis before immunoscintigraphy. Fig. 9 shows the corresponding transverse

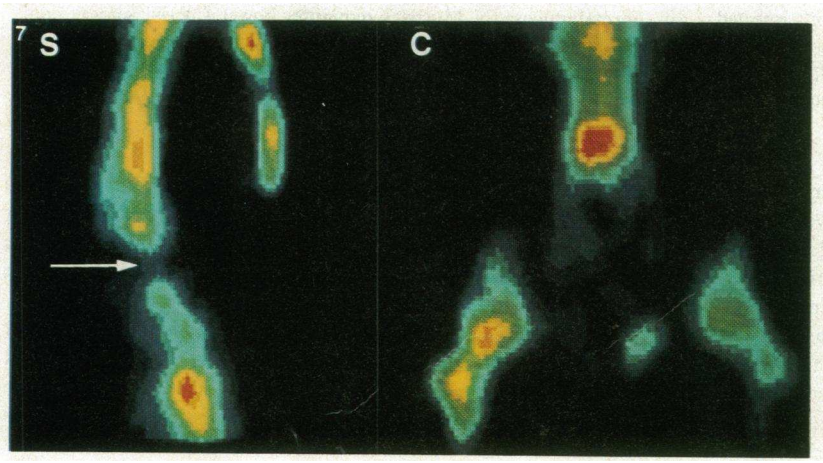

Figure 7. Uptake of MAb fragments in bone metastases. On the sagittal (*S*) section (*left*) through the dorsal and upper lumbar spine and the sternum as well as on the coronal (*C*) section (*right*) of the pelvis, numerous hot spots are suggestive of multiple bone metastases which were unsuspected at the moment of immunoscintigraphy and only subsequently confirmed by a Tc-99m diphosphonate bone scan. A single dorsal vertebra (arrow) was considered as free of tumor by both examinations.

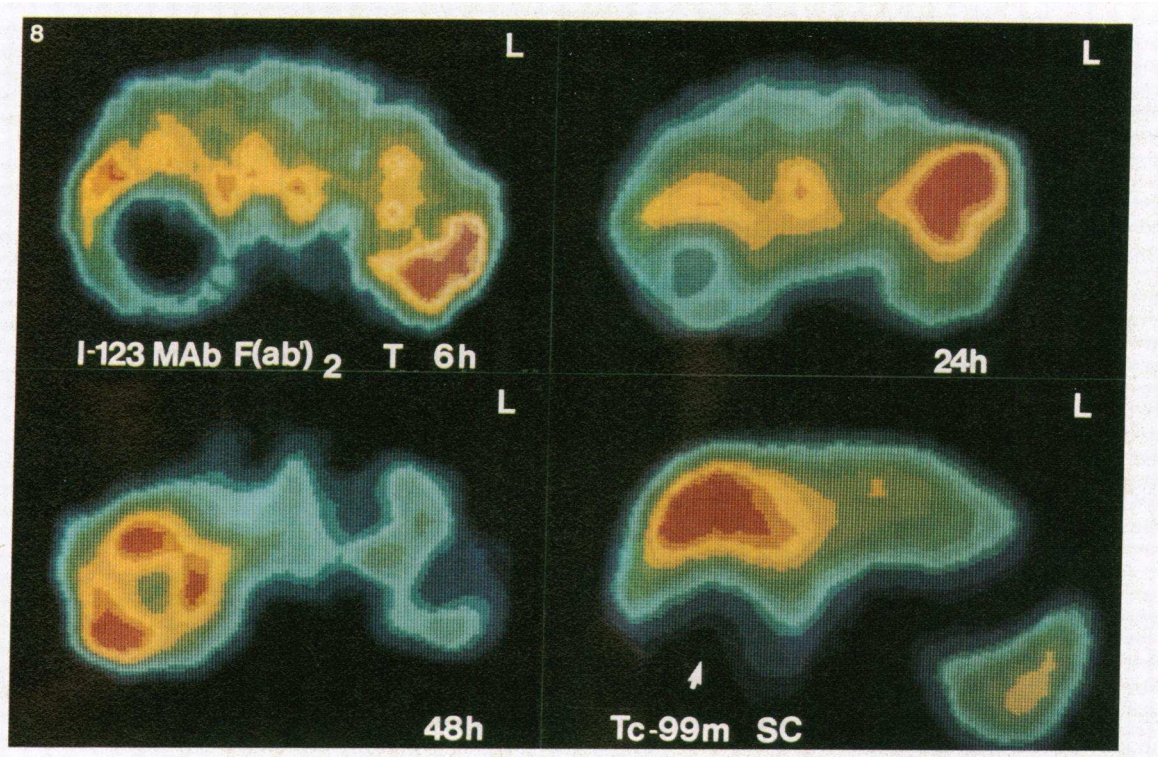

Figure 8. Progressive penetration of $F(ab')_2$ fragments into a large liver metastases. Transverse sections across the liver of patient 12 taken at intervals of 6, 24, and 48 h after injection of the antibody fragment (upper left and right, and lower left panels). A corresponding ECT section obtained after injection of Tc-99m sulfur colloid is shown on the lower right panel: the tumor appears as a large photon-deficient area in the posterior part of the right liver lobe on the sulfur colloid ECT (arrow). On the 6-h immunotomoscintigraphy a circular area of antibody uptake with a large central defect is shown, which gradually fills up, as demonstrated on the 24-h and especially on the 48-h ECT. At this moment the tumor mass is almost entirely filled with radioactivity except for a small, probably necrotic central region; whereas most of the antibody has cleared from normal liver parenchyma. *L*, left.

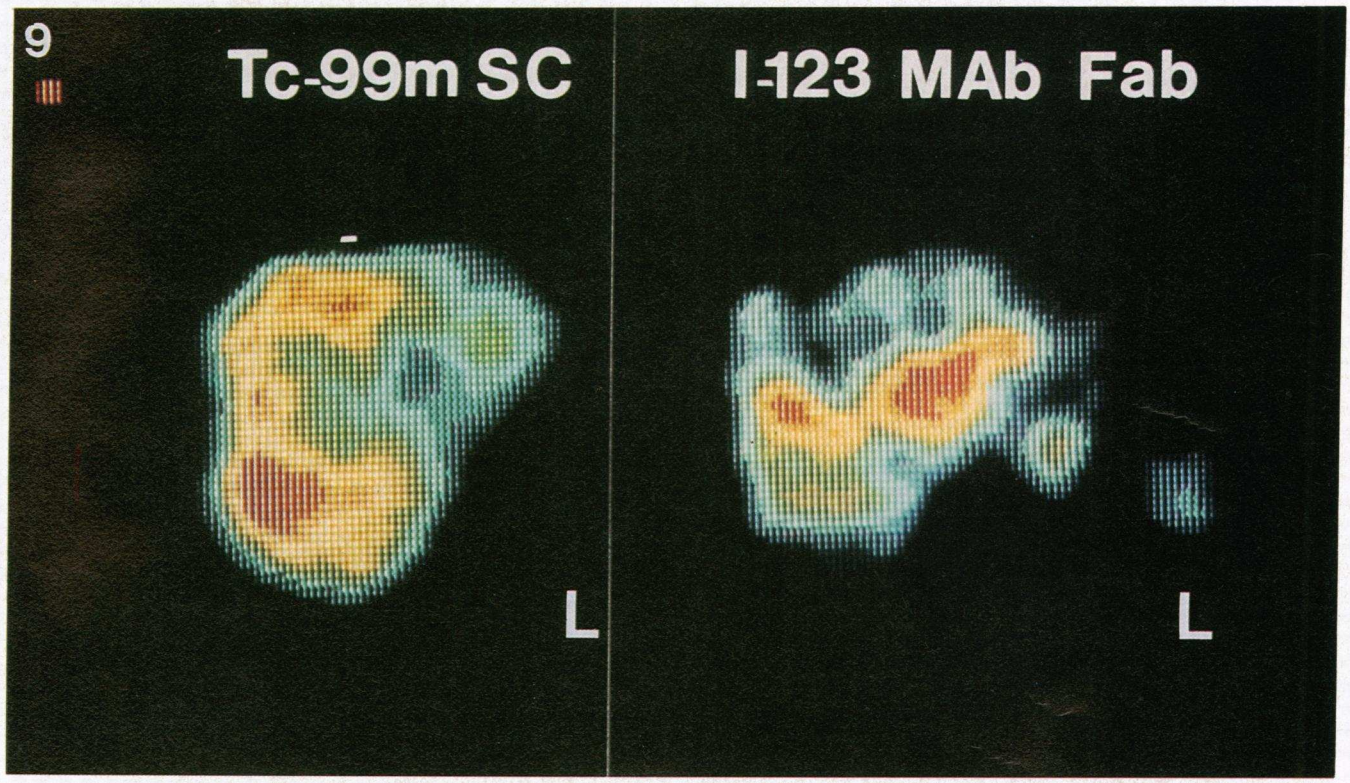

Figure 9. Detection of previously unknown liver metastases. The corresponding transverse sections of Tc-99m sulfur colloid ECT (*left*) and immunotomoscintigraphy (*right*) of the liver obtained from patient 15 show that the area of antibody uptake has the same shape as the photon-deficient area of the sulfur colloid ECT and corresponds to liver metastases, which were confirmed by surgery. The antibody uptake by normal liver parenchyma is already very low at 24 h after injection of Fab fragments from MAb 35. *L*, left.

sections of the liver after injection of Tc-99m sulfur colloid (left panel) and after injection of ^{123}I-labeled Fab fragment from MAb 35. The shape of the area of antibody uptake fits well into the photon-deficient area of the sulfur colloid ECT. The suspected tumor was confirmed at surgery but could not be resected. In the other case in which a liver metastasis was discovered by immunoscintigraphy (patient 20), the liver metastasis was surgically resected. Both of these cases were investigated with Fab fragments, which showed relatively less accumulation in normal liver parenchyma on early images than did $F(ab')_2$ fragments.

Our overall results are summarized in Table IV. 38 of 44 organs with tumor involvement were correctly identified. The detection rate proved to be somewhat higher with Fab (89%) than with $F(ab')_2$ fragments (82%).

Discussion

The concept of using radiolabeled antibodies to detect hidden tumor cells (23) has been revitalized by the development of the MAb technology. For the last 7 yr, however, several thousands of patients have received injections of radiolabeled polyclonal or monoclonal antibodies directed against various tumor markers, with relatively modest clinical results in terms of precision of tumor imaging (4–10, 24–26). We were among the first to make a critical evaluation of the results obtained with polyclonal and monoclonal anti-CEA antibodies labeled with ^{131}I (5, 12). We considered that the tumor detection obtained by this method, particularly after computerized subtraction of blood pool radioactivity, was not precise and reliable enough to influence clinical decisions.

In contrast, the tumor images reported here, obtained by using ECT and the smallest fragment (Fab) of highly selected anti-CEA MAb, labeled with ^{123}I represent a marked improvement over previously published results. The high resolution of the tomographic tumor images strongly suggests that this improved method of immunoscintigraphy has the potential to compete with the most modern methods of tumor detection. A controlled prospective clinical trial, however, including systematic comparison with results from CT scan, ultrasonography, and possibly nuclear magnetic resonance results, is still necessary to confirm this point. If immunoscintigraphy, improved by ECT, can reach the same sensitivity as the CT scan and nuclear magnetic resonance imaging, the immunological method will still have the advantage that the antibodies detect a tumor marker within a lesion, whereas the other methods only detect a morphological alteration.

Table IV. Visualization of Colorectal Carcinoma with ^{123}I-labeled Anti-CEA MAb Fragments and ECT

	Both fragments	$F(ab')_2$	
		Positive/Total	Fab
Primary tumors	10/10*	4/4	6/6
Local recurrences	12/13	3/3	9/10
Metastases			
Liver	12/14	5/7	7/7
Lung	1/2	0/1	1/1
Bone	3/3	2/2	1/1
Peritoneum	0/2		0/2
Total	38/44 (86%)	14/17 (82%)	24/27 (89%)

* Number of tumor sites visualized in at least two sections from different tomographic planes per number of tumors confirmed by conventional methods or by surgery. When several tumor sites were found in the same organ, or tissue or anatomical region (liver, lung, bone, or peritoneum) they were counted as one.

In order to obtain the necessary improvement of colon carcinoma detection by immunoscintigraphy, three factors appeared to be of particular importance: (*a*) the selection of the most favorable target tumor marker and the best MAb directed against this antigen, (*b*) the choice between intact antibodies and their $F(ab')_2$ or Fab fragments, and (*c*) selection of the most convenient isotope. These three factors will be discussed in sequence.

Despite the theoretical disadvantage of CEA, as an antigen released into the circulation, we chose this marker because our previous clinical experience showed that we could obtain relatively good ratios of tumor to normal tissue localization despite the presence of elevated circulating CEA levels (5, 6, 12, 13). Two other markers associated with colon carcinoma have been identified by MAb 17-1A (27) and MAb NS 19-9 (28). The antigen recognized by MAb 17-1A is not shed into the circulation, whereas the antigen recognized by MAb NS 19-9 is released into the blood. Our experience, based on 53 colon carcinoma patients injected with ^{131}I-labeled MAb 17-1A (29), was that the tumor to normal tissues ratios and the immunoscintigraphy results were not superior to those obtained with our ^{131}I-labeled anti-CEA MAb (12, 13). More recently, however, Moldofsky et al. (30) reported encouraging clinical results with ^{131}I-labeled $F(ab')_2$ fragments from MAb 17-1A. Chatal et al. (31) injected ^{131}I-labeled MAb 19-9 or its $F(ab')_2$ fragments and detected 66% of 29 colorectal carcinoma sites by planar scintigraphy. This might be because not all colon carcinomas express the 19-9 antigen. A large series of colorectal carcinoma patients have been tested by immunoscintigraphy using a MAb directed against human osteosarcoma cells (24). It appears unlikely, however, that a single MAb which has been found to react with very different types of tumors could be more specific than highly selected MAb against a well-defined marker such as CEA. Another MAb of potential interest for immunoscintigraphy of colon carcinoma is the MAb B72.3 anti-breast carcinoma. It localized well in colon carcinoma grafted into nude mice (32), but only preliminary clinical results have been reported (33).

The anti-CEA MAb 35 used in this study was selected out of more than 30 anti-CEA MAb by the criteria of high affinity (5.8×10^9 M^{-1}) for CEA and absence of crossreactivity with granulocyte glycoproteins (14–16).

The selection of the antibody fragments was based on our experimental results obtained in nude mice grafted with human colon carcinoma. ^{131}I-labeled $F(ab')_2$ or Fab from MAb 35 gave 3.5 and 11.7 times higher tumor to normal tissue ratios, respectively, than intact MAb 35 (16). Furthermore, when we started to inject the Fab fragments into patients [after having used $F(ab')_2$ in the first 13 patients of this study], we noticed an improvement in the quality of the tumor images (earlier tumor uptake, less background radioactivity in spleen and liver); thus we injected all the following patients with Fab from MAb 35.

In fact, 6 out of 8 of the figures of ECT studies presented here were obtained after injection of Fab from MAb 35. Our selection of the Fab fragments is in agreement with Larson et al. (34), who also used Fab of high affinity MAb for imaging of melanomas. It should be noted, however, that in some experimental models, $F(ab')_2$ fragments and even intact antibodies have been reported to give better tumor localization than Fab (35, 36). The poor results with these Fab fragments may be explained by a too low affinity of the antibody. A relatively low affinity becomes a limiting factor when a fragment with a single binding site is used. In our experience, the Fab fragments from MAb with an affinity lower than 5×10^8 M^{-1} perform less well than $F(ab')_2$ (Buchegger et al., manuscript in preparation). In addition, large amounts of antibody fragments labeled with ^{111}In through the bifunctional chelating agent diethylenetriamine-pentaacetic acid may accumulate in the kidney (36), whereas kidney accumulation of ^{131}I-labeled MAb fragments was much less important in our experimental studies (16).

The choice of the isotope is controversial. Tc-99m, the most convenient and widely used isotope in nuclear medicine, would be ideal if it had a physical half-life slightly longer than 6 h. The methodology to label antibodies with a sufficiently high specific activity of this isotope has been developed and the in vivo localization of Tc-99m–labeled MAb anti-cardiac myosin in myocardial infarction has been reported (37). According to most experimental results, however, it takes at least 24 h to obtain a good localization in solid tumors; thus, the 6-h half-life of Tc-99m appears to be too short.

^{111}In has a favorable half-life of 2.7 d, and excellent experimental results have been obtained with ^{111}In-labeled intact anti–tumor antibodies (36). When ^{111}In-labeled antibodies have been injected into patients, however, a very high nonspecific uptake has always been observed in the reticuloendothelium, especially in the liver (26, 38), even when the DTPA coupling was performed under mild conditions (25, 39). Since high nonspecific liver uptake may interfere with the detection of liver metastases, we considered this label not to be optimal for the evaluation of colon carcinoma patients by immunoscintigraphy.

^{131}I has the advantage of a long half-life (8 d) and has been used by Chatal et al. (31) to detect a liver metastasis of colon carcinoma as late as 11 d after injection of 1.4 mCi of ^{131}I-labeled MAb. The major disadvantages of ^{131}I are the emission of beta rays, which limits its use for purely diagnostic tests, as well as the rather high energy (364 keV) of the principal gamma ray, which is not convenient for ECT because gamma cameras have a low efficiency for high energy photon emitters.

In our opinion, the best presently available isotope for antibody labeling is ^{123}I. Its energy of 159 keV is perfectly adapted for use in ECT (20). Although its half-life of 13 h is a little too short, it can represent an optimal tracer when it is coupled to the small Fab fragments which penetrate more easily into the tumor and are rapidly cleared from the circulation (16, 18).

The use of fragments of the anti–CEA MAb 35 along with ^{123}I has given very encouraging clinical results: almost all (23 out of 24) primary tumors or local recurrences of colorectal carcinoma were detected with both types of fragments within 24 h, and metastatic disease was detected in various organs, such as liver, bone, and lung. The overall percentage of detection was 82% for $F(ab')_2$ and 89% for Fab fragments.

It is of clinical interest that in nine patients with normal or borderline serum CEA values (<5 μg/liter), tumor deposits could be detected by radiolabeled MAb fragments and ECT. Furthermore, in four patients the improved immunoscintigraphy procedure described here was able to detect tumor deposits which were either not suspected clinically or not found by standard diagnostic methods, such as ultrasound or CT scan. In two of these cases, the liver metastases were confirmed by surgery, and in one of them the detected metastasis weighing ~8 g (patient 20) could be entirely resected.

One of the limitations of the ^{123}I-labeled MAb fragment method is the uptake of iodine by the stomach and its rapid elimination by the urinary tract. These regular nonspecific accumulations, however, may also be used as anatomical landmarks, as previously suggested by Berche et al. (13), and thus be helpful for the topographic characterization of hot spots. Interestingly, two of the small tumors reported here, weighing 3 and 4.5 g, were detected near a very radioactive organ, the first one near the left kidney (Fig. 4) and the second one behind the urinary bladder (Fig. 5). One of the greatest advantages of ECT is that it allows a distinction between tumor uptake of radiolabeled antibodies from physiological organ concentration and circulating radioactivity, without the need to use computerized subtraction techniques which are prone to artifacts (19).

Another, more important, limitation of antibody fragments which has to be mentioned is that the percentage of the injected dose which localizes in tumor remains relatively low (<1%). Even in the experimental animal, where we reached tumor to normal tissue ratios of 82 with Fab fragments from MAb 35, the percentage of injected dose was four times lower than for intact MAb 35 (16). Therefore, if one considers the injection of radioactive antibodies for therapeutic purposes, we are not certain that Fab fragments are the best carrier, as proposed by Larson et al. (33, 34). Intact antibodies with their longer biological half-life may have a better chance to deliver the necessary dose of radioactivity into the tumor, as suggested by Order et al. (40). Intact antibodies, however, will give higher background radioactivity in the reticuloendothelial system. Since the therapeutic index is determined by the ratio of radiation delivered to the tumor to that delivered to the sensitive normal organs (33), it is possible that a fragment of intermediary size such as $F(ab')_2$ will be the most appropriate carrier for antibody-guided radiotherapy. We have recently injected 100 mCi of ^{131}I coupled to 10 mg of $F(ab')_2$ from MAb 35 into the hepatic artery of four patients with advanced liver metastases of colon carcinoma. The treatment was well tolerated, that is, no bone marrow toxicity was observed, but no evidence of significant regression of the large hepatic metastases was obtained (41).

For diagnostic purposes, however, the use of Fab fragments labeled with ^{123}I and detected by ECT, as described here, appears to offer the best potential for the detection of early tumor recurrences, a necessary condition to improve curability and survival in patients with colon carcinoma.

Acknowledgments

We thank Drs. S. Halpern, C. M. Haskell, and J.-C. Cerottini for advice and suggestions. We also thank Drs. P. Burckhardt, F. Saegesser, R. Mosimann, L. Barrelet, F. Mosimann, A. Besson, and J.-C. Gertsch for referring patients in the study; Drs. J. Costa, J. Hürlimann, and P. Anani for histopathological diagnosis; and Drs. G. Candardjis and A. Anderegg for providing radiological and ultrasonographic interpretations. We are

indebted to Mrs. D. Minaidis, registered nurse, who followed all patients of the study with particular care.

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