INVESTIGATIVE NUCLEAR MEDICINE

Immunoscintigraphy of Colon Carcinoma

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Two I-131 labeled monoclonal antibodies that react specifically with human gastrointestinal cancers in cell cultures were administered to 90 cancer patients for the scintigraphic detection of cancer sites. Antibody 17-1A, or its F(ab')$_2$ fragments, accumulated significantly in 27 of 46 (59%) colorectal cancer sites, but not in 21 nonepitheliomatous colon cancers and cancers at other sites. Antibody 19-9, or its F(ab')$_2$ fragments, showed significant accumulation in 19 out of 29 (66%) colorectal cancer sites. In 17 patients, immunoscintigraphy with antibody 19-9 correlated with an immunoperoxidase study with the same antibody on resected tissue specimens. In 12 patients injected with two antibodies (17-1A + 19-9, or anti-CEA + 19-9), ten of 13 colorectal cancer sites were positive.

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The use of radiolabeled polyclonal antibodies directed against tumor-associated antigens, especially the carcinoembryonic antigen (CEA), has permitted scintigraphic detection of human tumors, though until now with controversial results (*1–3*). Development of monoclonal antibodies defining human tumor antigens (*4*) has led to the isolation, and sometimes the identification, of new tumor-associated antigens. Thus, Koprowski et al. (*5*) obtained hybridomas secreting monoclonal antibodies that bind specifically to human gastrointestinal cancers in cell culture. One of these antibodies, designated 17-1A, recognizing as yet an undefined antigen that is not shed into the circulation by tumor cells, is also capable of inhibiting tumor growth in nude mice (*6*). Another antibody, designated 19-9, recognized a monosialoganglioside (*7*) that is shed into the circulation, where it can be detected in radioimmunoassay (*8*).

In the present study, radioiodinated antibodies 17-1A and 19-9 were evaluated for their ability to localize specifically in tumor tissue of 90 patients with gastrointestinal and other cancers. These antibodies were initially administered separately to determine their sensitivity and specificity, and the results were correlated with those of an immunohistochemical study performed on surgically resected tumor and normal tissue specimens. In the second phase of the study, two antibodies recognizing different antigens were administered simultaneously in an effort to increase detection sensitivity.

METHODS

The antibodies used (antibodies 17-1A and 19-9* and anti-CEA 202†) have been characterized previously (*5–9*). The F(ab')$_2$ fragments were obtained from purified 17-1A and 19-9 antibodies by pepsin digestion (*10*).

The purified monoclonal antibodies, or their F(ab')$_2$ fragments, were radioiodinated with I-131 by the iodogen method (*11*). Briefly, 50 μl of iodogen (10 μg) was placed into a test tube and the methylene chloride evaporated. Three hundred μg of whole antibody, or 500

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μg of $F(ab')_2$ fragments, and 2.5 mCi of I-131 were added in a total volume of 150 μl, and the reaction was allowed to proceed for 10 min. Unbound iodine was removed by glass-column chromatography (column 10 cm by 1 cm diam, filled with Biogel P_2). The column was eluted with phosphate-buffered saline containing 0.3% human serum albumin. The labeled protein was collected between the third and sixth milliliters. The yield of purification was about 85%.

The immunoreactivity of the radioiodinated antibodies 17-1A and 19-9 was tested in a cell-binding assay (*5*) in which human melanoma and colorectal carcinoma cell lines (5×10^5 cells per well) were incubated for 4 hr with the labeled antibodies. Cells were spun down in the plate for 10 min and washed twice in 0.3% bovine serum albumin. Radioactivity of the cells was determined in a gamma counter and results were expressed as the percentage of binding of total radioactivity. Binding varied from 7 to 50%, depending on the colorectal carcinoma cell lines, whereas binding to melanoma cells was only about 2%. Binding of an irrelevant immunoglobulin to colorectal carcinoma and melanoma cell lines was consistently less than 3%.

Informed consent was obtained from the 90 patients included in this study. Of 52 patients who were injected with I-131-labeled 17-1A, 40 received intact antibody and 12 the I-131-labeled $F(ab')_2$ fragments. The retrospective study of these 52 patients comprised 46 colorectal tumors (14 primary tumors, eight local recurrences, and 24 metastases), two nonepitheliomatous colon tumors (colonic localizations of lymphomas), and 19 tumors other than colonic. The I-131-labeled 19-9 antibody was administered to 26 patients, two receiving intact antibody and 24 the $F(ab')_2$ fragments. In this scintigraphic application the 19-9 antibody was tested after the 17-1A antibody. An experimental study in the nude mouse, carried out at the same time as the clinical study, demonstrated that the use of $F(ab')_2$ fragments was preferable to that of the intact antibody because of faster clearance of radioactivity from normal tissues (unpublished data). These findings, since confirmed (*12*), led us to prefer use of the $F(ab')_2$ fragments of the 19-9 antibody. These 24 patients comprised two cases of pancreatic tumors and 20 of colorectal cancers (14 primary tumors, two local recurrences, and ten metastases). All but two of the tumors studied retrospectively were 3 cm in diameter or larger. Two patients received labeled antibody in an effort to detect a suspected recurrence. Finally, 12 patients received a combination of two antibodies: either I-131-labeled 19-9 $F(ab')_2$ + intact I-131-labeled anti-CEA 202 (n = 5), or I-131-labeled 17-1A $F(ab')_2$ + I-131-labeled 19-9 $F(ab')_2$ (n = 7). In the first combination, the proportion of anti-CEA antibody was approximately 40%. In the second combination, the antibody concentrations were about equal.

Thyroid uptake of I-131 was blocked by oral administration of Lugol's solution, starting 3 days before injection of antibody and continued for 10 days. In patients with negative skin tests (0.05 mg of antibody in 0.1 ml injected intracutaneously), the radioiodinated antibody or its $F(ab')_2$ fragments were diluted in 100 ml of physiological saline, and injected slowly during 15 min. Patients received between 0.8-3 mCi (29.6-111 MBq), corresponding to between 0.3 and 1.5 mg of antibody. The studies were performed with an LFOV gamma camera connected to a data processing system, at 24 hr, 4 to 5 days, and sometimes 9 to 12 days after injection of the intact antibody, and at 24 hr and 2 to 7 days after injection of the $F(ab')_2$ fragments. Before each scintigraphic study, patients received Tc-99m-labeled red blood cells, Tc-99m-labeled sulfur colloid, or Tc-99m diethylene-triamine pentacetic acid (DTPA); this was done to detect activity in the vascular compartment, liver, kidneys, and bladder as an aid to the more precise definition of I-131 accumulation. Computerized subtraction was used only to improve the contrast of an image already considered positive. Images were classified as "well contrasted" (++), "moderately contrasted" (+) or "doubtful" (±). Because the contrast of the "doubtful" images was not sufficient to indicate tumor localization in the context of a prospective study, "±" images were scored as negative in that set of data.

To demonstrate the specificity of antibody accumulation in tumor tissue, four patients were injected simultaneously with either I-131-labeled 17-1A or I-131-labeled 19-9 (intact or $F(ab')_2$ fragments) together with an irrelevant I-125-labeled immunoglobulin (either an immunoglobulin IgG2a secreted by a murine myeloma, designated A5C3 and of the same class as the 17-1A antibody, or an antihepatitis immunoglobulin IgG1, designated A2C6 and of the same class as the 19-9 antibody).‡ A serum sample and fragments of tumor, mucosa, and normal colon serosa were obtained during surgery, and the radioactivity of both isotopes was measured. A localization index (*13*) was calculated as the ratio of I-131 to I-125 in tumor or normal tissue relative to this ratio in blood.

An index of approximately one indicates similar distribution of the specific antibody and of the irrelevant immunoglobulin. An index greater than one indicates preferential distribution of the specific antibody.

Finally, tissue specimens from 12 patients injected with I-131-labeled 17-1A, and from 17 patients injected with I-131-labeled 19-9, were fixed in formol and embedded in paraffin and tested with the avidin-biotin-peroxidase technique using the 19-9 and anti-CEA antibodies (*14*). This technique was not used to test the 17-1A antibody because preliminary results were all negative for reasons undetermined but probably related to the fragility of the antigenic determinant presumably destroyed by the fixation procedure. Slices (5 μm thick)

were deparaffinized and incubated with the antibody studied. The slides were then incubated with a biotin-conjugated goat anti-mouse antibody, treated with 0.06% diaminobenzidine in 0.01% H_2O_2, counterstained with hematoxylin, dehydrated, and mounted.

RESULTS

Table 1 summarizes the results of tumor imaging using the radioiodinated antibodies 17-1A and 19-9. Twenty-one of 35 (60%) documented colon carcinoma sites showed high accumulation (++ and +) of I-131-labeled intact 17-1A. Figure 1 shows the images obtained with this antibody in a patient with a lung metastasis from a previously resected colon carcinoma. Of the seven negative cases, one was attributed to the small size of the tumor (15 mm) and another to a highly undifferentiated epithelioma with extensive fibrosis. In two patients the results were different for the primary tumor and for a local recurrence or a metastasis: in one case the rectal tumor was not visualized even though it was large, whereas the liver metastases were clearly contrasted; in the other case, only the pelvic recurrence of a colon carcinoma was positive and not the liver metastases. Results were entirely negative for the 21 sites of nonepitheliomatous colon cancers or other types of cancer. Figure 2 shows that no I-131-labeled 17-1A accumulates in a confirmed lung metastasis from a prostate carcinoma. With the $F(ab')_2$ fragments of antibody 17-1A, results were positive for six of the 11 colon-cancer sites studied. The doubtful or negative results in two of the five cases may be attributed to a small (1–1.5 cm) metastasis and to a retroprostatic rectal tumor touching the bladder. One case of pancreatic cancer was negative at all time periods studied (1, 2, and 5 days).

Whereas only one of the two colon tumor sites explored with the intact I-131-labeled 19-9 antibody was positive, 18 out of 27 (67%) colorectal tumor sites explored using the labeled $F(ab')_2$ fragments of this antibody were positive, including one site examined prospectively (Table 1). Figure 3 shows the results of tumor imaging with I-131-labeled $F(ab')_2$ fragments of antibody 19-9. A local recurrence from a previously resected sigmoid carcinoma is clearly visualized. Three cases were uncertain and six cases were negative with the 19-9 $F(ab')_2$ fragments.

Regarding the nine uncertain or negative sites, scintigraphy was performed only 24 hr after injection in three cases, and in one case the sigmoid tumor was masked by a large shadow from urinary radioactivity that could not be eliminated by micturition before the examination.

Of the two cases studied prospectively, the first involved a clinically and radiologically suspected recurrence of rectal cancer in the absence of elevated serum levels of CEA or the 19-9-defined antigen. Results in immunoscintigraphy were negative, and the clinical and biological course of the case confirmed the absence of recurrence. The second case concerned a 35-yr-old woman with a suspected recurrence of a rectal cancer resected 2 yr before. She reported pelvic pains, and her serum CEA level was elevated, whereas 19-9-defined antigen levels remained normal. The endoscopic examination and biopsy were normal. Immunoscintigraphy revealed a radioactive spot behind the bladder (Fig. 4), indicating a local recurrence that was subsequently confirmed by second-look surgery. The results of immunoperoxidase staining were also positive for the 19-9-defined antigen and CEA. The fact that the recurrence was perivisceral accounts for the normal endoscopic appearance.

Regardless of whether intact or $F(ab')_2$ fragments of the antibody were used, images with the sharpest contrast in positive cases were obtained late after injection—7 to 8 days for the intact antibodies and 4 to 5 days for the $F(ab')_2$ fragments.

Four patients were injected simultaneously with either 17-1A, 19-9, or their $F(ab')_2$ fragments, together with an irrelevant immunoglobulin. Localization indices calculated from uptake counts ranged from 2.6 to 3.1 for the tumors, thus confirming the selective distribution of 17-1A or 19-9 in the tumors (Fig. 5). For one of the four patients who was injected with the intact I-131-labeled 19-9 antibody, it was possible to carry out a kinetic study

TABLE 1. IMAGE RESULTS OBTAINED WITH MONOCLONAL ANTIBODIES 17-1A AND 19-9

Image results	Antibody 17-1A: Documented colon Carcinoma sites Intact	Antibody 17-1A: Documented colon Carcinoma sites $F(ab')_2$	Antibody 17-1A: Nonepitheliomatous colon and Noncolon cancer sites Intact 17-1A	Antibody 19-9: Documented colon carcinoma sites Intact	Antibody 19-9: Documented colon carcinoma sites $F(ab')_2$
Positive ++	6	0	0	0	7
Positive +	15	6	0	1	11
Doubtful ±	7	2	0	1	3
Negative −	7	3	21	0	6
Total	35	11	21	2	27

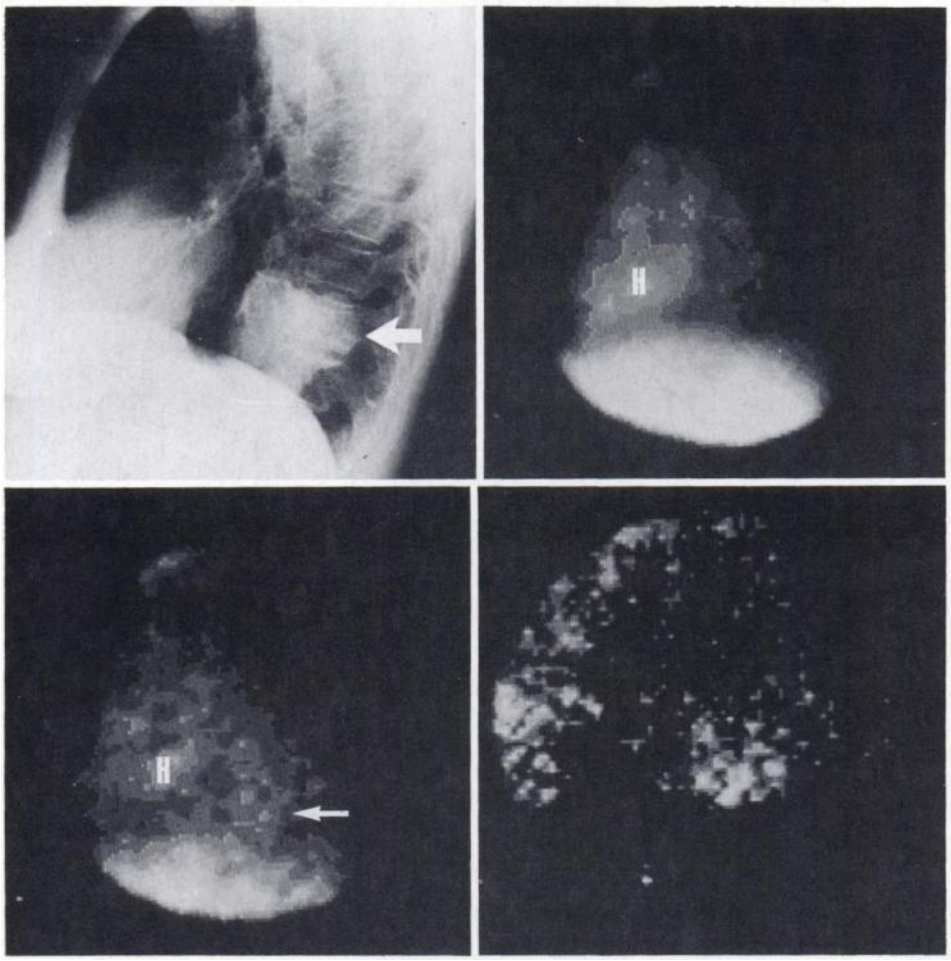

FIG. 1. Female patient, age 60, with left-lung metastasis from previously resected colon carcinoma. Left lateral views. Chest radiograph (upper left) shows large mass behind heart (arrow). Tc-99m red-blood-cell image (upper right) shows no focal uptake behind heart. I-131-labeled 17-1A image at 5 days after radioantibody injection (lower left) demonstrates increased focal uptake behind heart corresponding to mass (arrow). Subtracted image (lower right) enhances contrast of uptake in metastasis. H = heart; L = liver.

of radioactivity distribution, since a colonoscopy with biopsy was performed 2 days after injection and a hemicolectomy on the fifth day. During the 3-day interval, the localization index for the tumor rose from 1.9 to 3.2.

Table 2 presents a comparison of the results of immunoscintigraphy with those of the immunohistochemical study of tumors from the same patient. Of 13 cancers that were removed surgically from patients and bound 19-9 antibody in immunohistochemical reaction, ten were detected previously by immunoscintigraphy with 19-9 antibody while three were not. Of these three nonvisualized tumors, two were rectal and one was a sigmoid tumor masked by a large area of bladder radioactivity. No tumor that was negative by immunoperoxidase staining was positive in immunoscintigraphy. Of nine cancers that bound 19-9 antibody, only three were detected previously by immunoscintigraphy with 17-1A antibody, and six were not.

Analysis of the tumors tested with anti-CEA-202, removed from 17 patients injected with the I-131-labeled 19-9 antibody, showed that in two cases immunoscintigraphically 19-9-positive tumors did not express CEA. Of the six scintigraphically negative cases that bound anti-CEA-202 antibody, three did not bind 19-9 antibody. Only one tumor was negative in both assays. Of nine tumors that bound anti-CEA-202 antibody, only three were visualized by scintigraphy with 17-1A antibody, and six were not. These results demonstrate the diversity of tumor antigenic expression, and thus suggest the potential advantage of using combinations of antibodies to increase the chances of tumor detection. In a preliminary study, ten out of 13 colorectal tumor sites (77%) were immunoscintigraphically positive in 12 patients injected with a combination of two antibodies. Five of the positive sites were detected by the combined use of $F(ab')_2$ fragments of I-131-labeled 17-1A + I-131-labeled 19-9, and the five other positive sites were detected after injection of labeled, intact anti-CEA-202 + $F(ab')_2$ fragments of 19-9.

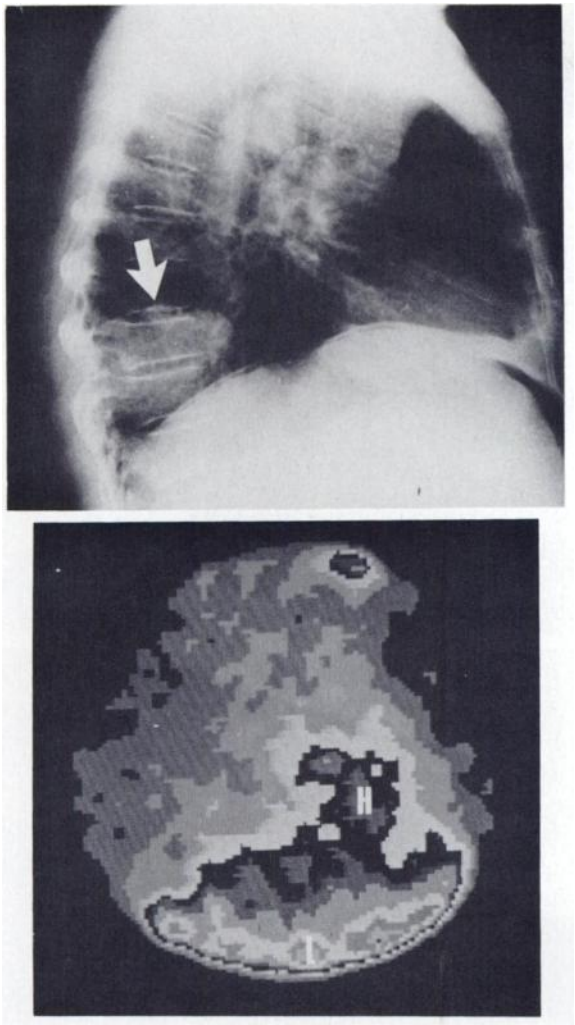

FIG. 2. Male patient, age 66, with right-lung metastasis from prostate carcinoma. Right lateral views. Chest radiograph shows large mass in approximately same location behind heart, and of same size, as that of patient in Fig. 1. I-131-labeled 17-1A image at 5 days after injection of radioantibody shows no I-131 activity in mass behind heart. H = heart; L = liver.

DISCUSSION

In the retrospective clinical study, in which each colorectal tumor site had been documented before injection of antibodies 17-1A and 19-9, the sensitivity of immunoscintigraphy was, respectively, 59% (27/46) and 66% (19/29). The sensitivity obtained with the 17-1A antibody was very close to the overall sensitivity of 54% obtained with the same antibody in a multicenter study that included some of the cases of our study (*15*). Moreover, these detection sensitivities, when compared with those obtained with polyclonal or monoclonal anti-CEA antibodies, are lower than the 85% positive colorectal cancers reported by Goldenberg (*16*) and higher than Mach's findings of 50% positive cases (*3*). In the latter study, the percentage was considerably higher with single photon emission tomography, though the advantage of that technique is limited by nonspecific uptake areas that are visible early. Our results were obtained after optimal labeling and detection parameters had been established in prior studies on cell cultures and in nude mice bearing grafted tumors (unpublished data). Iodogen labeling was used instead of the chloramine-T procedure, since the milder labeling conditions with the former technique lead to higher uptake percentages for the antibody or its fragments in colon-cancer cell lines. Moreover, percentages of antibody accumulation in tumors in nude mice were higher with iodogen than with chloramine-T. The specific activity used was low—less than 10 μCi/μg for the intact antibody and less than 5 μCi/μg for the $F(ab')_2$ fragments—since we have demonstrated that immunological activity declines progressively as specific activity rises. Finally, the tumor images with the sharpest contrast, and thus the easiest to interpret, were obtained late after injection: 6 to 7 days for the intact antibody and 4 to 5 days for the $F(ab')_2$ fragments. According to our studies and those of others (*17*) in the nude mouse, the improved contrast at late time intervals is the result of a faster radioactivity clearance from normal tissues than from the tumor. The absolute value of tumor uptake tends to remain stable or to decrease, which at late time intervals entails rather long recording periods, lasting more than 10 min per projection depending on the activity injected. The good scintigraphic contrast at late time intervals makes it generally unnecessary to resort to computerized subtraction, which may result in arti-

TABLE 2. CORRELATION BETWEEN IMMUNOPEROXIDASE STAINING AND RADIOIMMUNODETECTION RESULTS

Immunoperoxidase staining		Radioimmunodetection			
		19-9		17-1A	
		Positive	Negative	Positive	Negative
19-9-defined antigen	Positive	10	3	3	6
	Negative	0	4	0	3*
CEA-202	Positive	8	6†	3	6
	Negative	2	1	0	3*

* One of these three patients had a colonic infiltration by lymphoma.

† Three of the six cases were negative for the 19-9-defined antigen in immunoperoxidase staining.

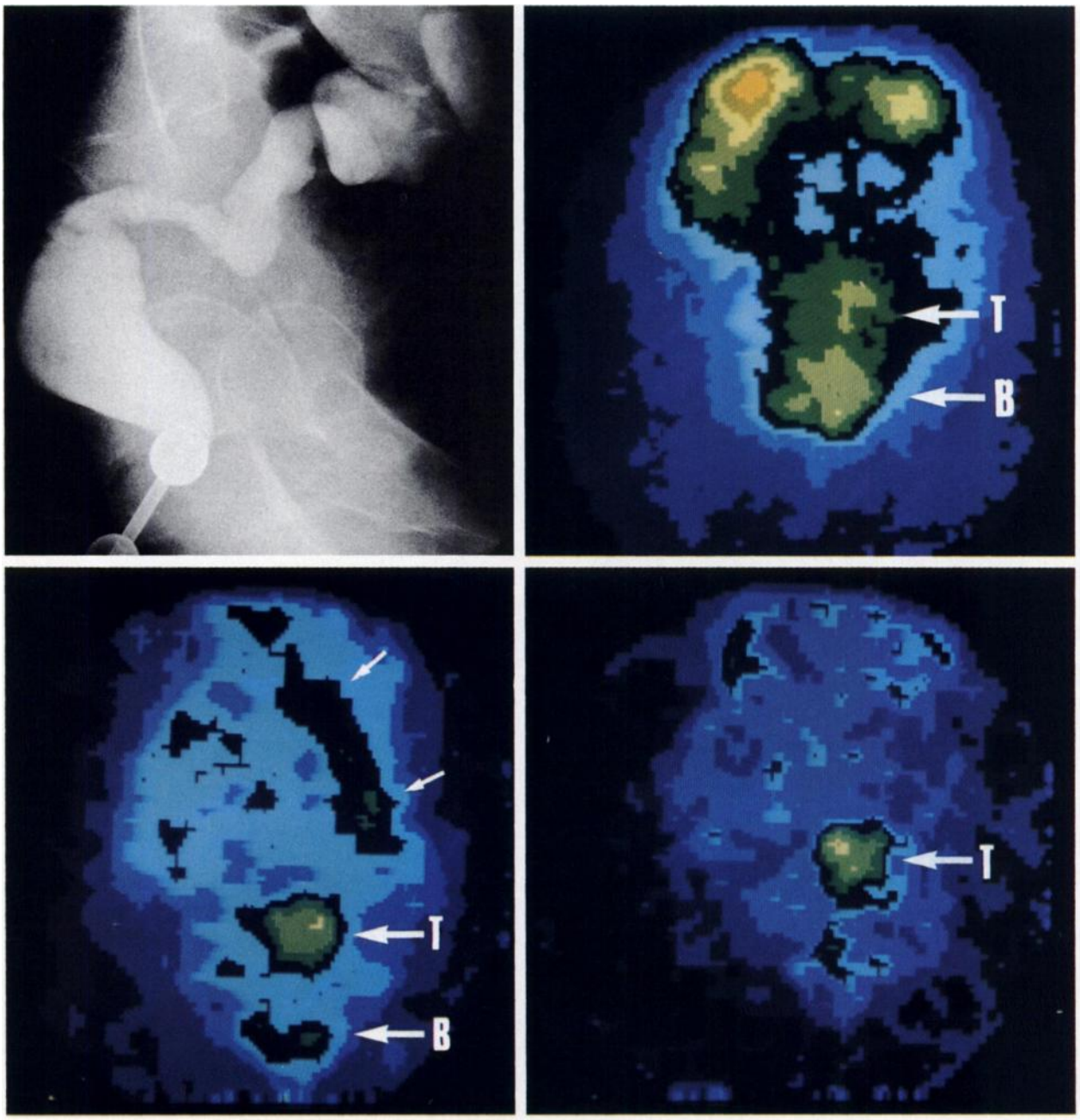

FIG. 3. Female patient, age 70, with local recurrence from previously resected sigmoid carcinoma. Barium enema (upper left) shows irregular narrowing of lumen of sigmoid. Posterior image (upper right) with I-131-labeled 19-9 $F(ab')_2$ shows poorly contrasted tumor uptake 24 hr after radioantibody injection; this would require computerized subtraction for interpretation. Radioactivity sites in upper part of image correspond to liver on right and stomach on left. Bladder localization relative to tumor uptake was determined by prior injection of Tc-99m DTPA. Same tumor at 5 days (lower left) and (lower right) at 7 days after injection of tracer, showing clearly contrasted tumor uptake. Radioactivity in liver and stomach has disappeared. Nonspecific radioactive spots (arrows) tend to change in contrast and location in late images (between 5 and 7 days). T = tumor; B = bladder; L = liver; S = stomach.

facts because of differences in autoattenuation of the I-131 and Tc-99m energies.

The problem of the specificity of scintigraphic images deserves special attention, particularly in a prospective application that might involve surgery. Though all nonglandular tumors and tumors outside the digestive tract were negative with antibody 17-1A, these results must be supplemented and confirmed by enlarging the spectrum of tumors studied. By performing the recordings late after antibody injection, we can avoid the problem of nonspecific accumulation areas that cause confusion in the interpretation of early images. Repeated studies are also desirable. Changes in the shape, the anatomical location, and the contrast of an area of doubtful uptake from one recording to the next (see Fig. 3, C and D) increase the probability that the image re-

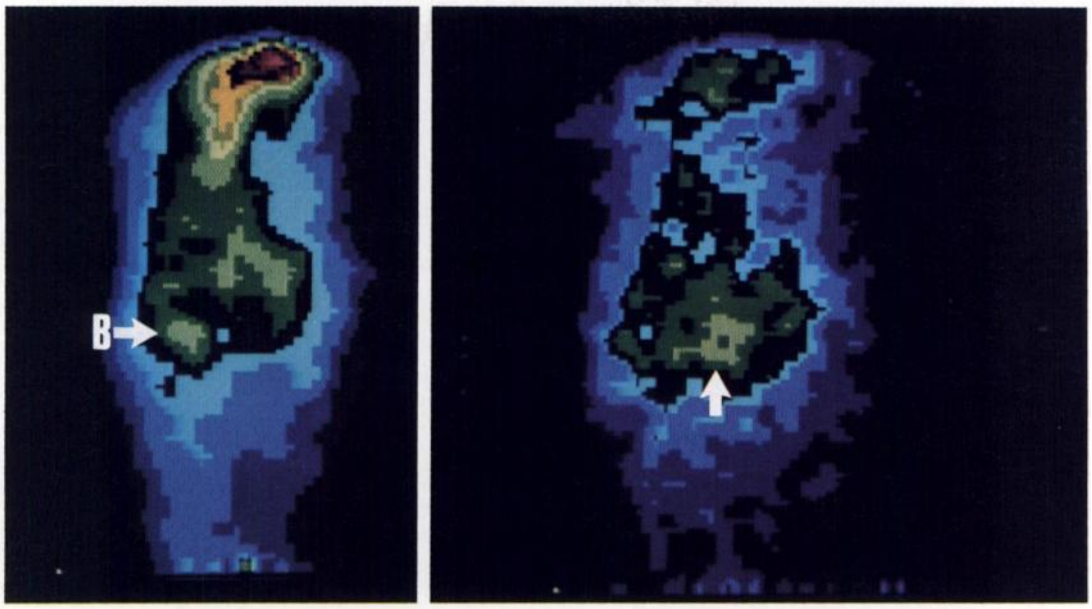

FIG. 4. Female patient, age 35, with pelvic pain and increased serum CEA, raising suspicion of recurrence of previously resected rectal carcinoma. Left lateral views: Image with Tc-99m-tagged RBCs and DTPA (left) shows anatomic location of bladder and radioactivity in aorta and iliac vessels (arrows). I-131-labeled 19-9 F(ab')$_2$ image (right) demonstrates area of increased tracer uptake behind bladder (arrow) at 5 days after tracer injection. Second-look surgery confirmed perivisceral local recurrence. B = bladder.

sults from nonspecific accumulation of radioactivity. In this study, the specificity of antibody accumulation in tumors has been demonstrated in two ways. After simultaneous injection of I-131-labeled 17-1A or 19-9 antibody together with an I-125-labeled irrelevant immunoglobulin of the same class, localization indices were higher than 2.5 for tumors in positive cases and were approximately 1 for normal tissues. Such values indicate similar distributions of the specific antibody and the irrelevant immunoglobulin in normal tissues and a preferential accumulation of the specific antibody in the tumor. A good correlation between the results of immunoscintigraphy with 19-9 antibody and those of the immunohistochemical study performed on excised sections further demonstrates the specificity of antibody accumulation in the tumor. Indeed, all the tumors that were seen immunoscintigraphically with the I-131-labeled 19-9 antibody expressed the corresponding antigen in immunoperoxidase staining, and no immunohistologically negative case was positive by immunoscintigraphy. Nevertheless, since nine tumors undetected by immunoscintigraphy with 19-9 and 17-1A antibodies (Table 2) expressed either 19-9 or CEA in immunoperoxidase assay, the combined use of several antibodies that recognize different antigens seems a reasonable approach toward increasing the chances of tumor detection. Our preliminary results in the use of antibodies 17-1A, 19-9, and anti-CEA in pairs are quite encouraging, with positive images obtained in ten out of 13 sites explored.

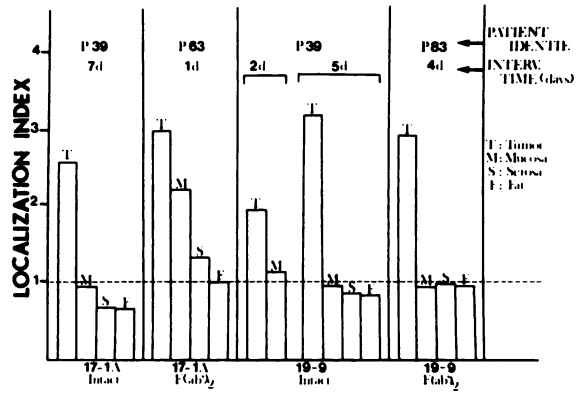

FIG. 5. Accumulation of 17-1A and 19-9 monoclonal antibodies [intact and F(ab')$_2$ fragments] specifically in tumors of four patients with colorectal carcinoma. Dotted line indicates value of localization index corresponding to similar distribution of specific I-131-labeled antibody and irrelevant I-125-labeled immunoglobulin. Above this value there is preferential accumulation of I-131-labeled antibody.

The results presented in this study must now be confirmed in a larger series of patients. Detection sensitivity might still be improved once all the problems of conjugating bifunctional chelating agents, and of labeling with metallic radionuclides such as indium-111 or gallium-67, are solved. It should then be possible to administer greater activities than with I-131 and to take full advantage of single photon emission tomography.

FOOTNOTES

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