

Radioimmuno-detection of cancer of gastrointestinal tract and liver metastasis with I-131 anti-CEA and I-131 anti-CA19-9 monoclonal antibody cocktail (IMACIS-1)

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We evaluated the intravenous infusion of a cocktail of I-131 anti-CEA and anti-CA19-9 monoclonal antibody F(ab')₂ (IMACIS-1) in patients with gastrointestinal neoplasm and liver metastases in order to assess its efficacy in detecting the presence of cancer. Seven patients with primary or recurrent gastrointestinal cancer in whom liver metastases were also detected were studied. Accumulation of radioactivity in the primary tumor was seen in only one patient. Visualization of the liver metastases was achieved in all patients. Thus detection of liver metastasis was better than in primary or recurrent tumors. While tumor visualization was most often seen in the 3 day image, optimal visualization of the tumor was seen at 5–7 days. There was no correlation between the serum concentration of CEA or CA19-9 and the visualization of tumors. Serum kinetics of I-131 IMACIS-1 showed biexponential components with a 1st phase T_{1/2} of 5.0 hours and 2nd phase T_{1/2} of 34.7 hours. The mean whole body (I-131) half-life determined from the whole-body scans was 1.95 days. The mean urinary excretion of I-131 in 7 days was 85%. This value agreed closely with total radioactivity retention detected by scanning. This series of studies demonstrated the potential utility of a cocktail of antibodies consisting of an anti-CEA and an anti-CA19-9 monoclonal F(ab')₂.

Key words: radioimmuno-detection, monoclonal antibody, IMACIS-1

INTRODUCTION

RADIOIMMUNODETECTION (RAID) is a new field of nuclear medicine in which radioisotope (RI)-labeled antibodies are used to detect lesions.¹ In addition to employment for the diagnosis of cancer, radiolabeled monoclonal antibodies (Mabs) have also been used for detecting the sites of myocardial infarction,² thrombosis³ and inflammation.⁴ The development of these reagents for radioimmuno-therapy is also in progress.⁵ However, there are still many problems to be overcome⁵ in areas involving immunology, immunochemistry, antibody pharmacology, radiochemistry and host-tumor interactions. Most studies have utilized a single antibody. In order to overcome some of the problems associated with antigen heterogeneity one

potential approach is to target various antigens that are frequently expressed by a given tumor type.

IMACIS-1 is a I-131 labeled cocktail consisting of an anti-CEA Mab and an anti-CA19-9 Mab.⁶ In this study, we evaluated the intravenous infusion of IMACIS-1 in patients with gastrointestinal neoplasm and liver metastases in order to assess its efficacy in detecting the presence of cancer.

MATERIALS AND METHODS

Seven patients with gastrointestinal cancer in whom liver metastases were also detected by ultrasonography, computed tomography (CT) and abdominal angiography were studied. The mean age was 73.3 years, and the patients were 2 men and 5 women. The tumor was gastric cancer in 2 patients, colorectal cancer in 2 patients and pancreatic cancer in 3 patients. One of the gastric cancers was a recurrent tumor in an area of gastroduodenal anastomosis. Both of the patients with gastric cancer had Borrmann type 3 lesions, with a histological diagnosis of moderately

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Table 1 Data on seven subjectives studied

No.	Age/Sex	Primary carcinoma	Serum CEA levels (ng/ml)	Serum CA19-9 levels (U/ml)	Uptake of IMACIS-1	
					Primary	Liver metastasis
1.	74/F	Colon	779	5.0	—*	+
2.	62/F	Pancreas	77.7	24000	—	±
3.	82/M	Stomach	371	141	—**	+
4.	77/F	Colon	14.2	44.2	—	+
5.	63/F	Pancreas	10.9	1077	—	+
6.	71/F	Pancreas	1.6	129	+	+
7.	87/M	Stomach	1.1	6.8	—	±

normal CEA; < 3.0 ng/ml normal CA19-9; < 37 U/ml

*recurrent lesion in rectosigmoid colon.

**recurrent lesion in area of gastroduodenal anastomosis.

differentiated tubular adenocarcinoma. One colorectal cancer was located in the ascending colon and the other was a recurrent tumor in the rectosigmoid; both of these tumors were well differentiated adenocarcinomas. One of the pancreatic cancers was located in the body of the pancreas and the other 2 occupied both the body and the tail. These lesions were tubular adenocarcinomas. The serum CEA concentration ranged from 1.1 to 779 ng/ml, and the CA19-9 concentration ranged from 5.0 to 24,000 U/ml (Table 1).

IMACIS-1 is a cocktail of the IgG F(ab')₂ fragments of mouse anti-CEA and CA19-9 Mabs. The antibodies were labeled with I-131 by CIS Co. (France) by the iodogen method and were purified with ion exchange resin and shipped frozen. These methods have previously been shown to result in an immunoreactivity of approximately 77% (CEA) and 85% (CA19-9).⁶ The components of IMACIS-1 consisted of 1 mg of I-131 labeled anti-CEA Mab F(ab')₂, 1 mg of I-131 labeled anti-CA19-9 Mab F(ab')₂, 9 mg of human serum albumin, and 2 ml of phosphate-buffered 0.15 M NaCl (PBS, pH 7.0). The antibodies were labeled separately and then mixed together in equal amounts. The preparation was thawed 1 hour before use. As a quality control, IMACIS-1 was precipitated with 10% trichloroacetic acid (TCA) and the protein-bound I-131 level was determined. A mean of 98% of the radioactivity was antibody bound. Approximately 111 MBq (3 mCi) of I-131 IMACIS-1 was diluted with 100 ml of saline and infused intravenously over 30 minutes.

In order to determine cross reactivity with normal blood components IMACIS-1 (1.0 μ l) was added to 2 ml of peripheral blood, and incubated for 1 hour at room temperature. The sample was then washed with PBS (pH 7.40) containing 1% bovine serum albumin (BSA), and the radioactivity in the blood cells fraction was measured.

Potassium iodide was administered at 600 mg/day for 10 days starting 3 days before IMACIS-1 infusion to prevent the accumulation of free I-131 in the thyroid. A gamma camera (SNC500R or LFOV; Siemens) fitted with a medium-energy collimator was used to obtain

whole-body scans and spot images. The images were acquired digitally with a 20% window over the 364 keV photopeak of I-131. Images were obtained immediately, 24 hours, and 3, 5, and 7 days after administration.

Images were interpreted by the primary author as well as independently by two the other authors who were blinded only with respect to the results of other imaging methods. There was no significant interobserver difference in interpretation. Scans were interpreted as positive if there were focal and persistent areas of increased tracer concentration in the liver. Extrahepatic areas of increased radiotracer concentration were interpreted as positive only if the uptake was very intense and persistent. Scans were classified as "well concentrated" (++), "moderately concentrated" (+), "doubtful" (\pm) and "negative" (—).

The clearance from serum was determined by counting serial aliquots of serum obtained at 30 minutes, and 1, 2, 4, 6, 8, 12, 24, 48, 72 hours and 96 hours after infusion and counting them in a gamma counter and fitting the data to a biexponential curve. The protein bound fractions in these serial samples were determined by precipitation with 10% TCA. Urinary excretion of I-131 was determined by totalling urine collected each 24 hours, including samples up to 8 days post infusion. In addition, blood cell bound radioactivity was determined in a gamma counter after washing serial aliquots of peripheral blood twice with PBS containing 1% BSA. Following antibody administration, serial CEA and CA19-9 concentrations were determined for 5 days with CEARIA beads (Dinabot) and a Centocor CA19-9 RIA kit, respectively. In order to avoid interference with the immunoassay by the injected radioactivity (I-131), the serum samples were permitted to decay prior to the assay.

Human anti-mouse IgG (HAMA) levels were determined by an enzyme-linked immunosorbent assay (ELISA). Microtiter plates (Coster, USA) were coated with mouse anti-CA19-9 F(ab')₂ and anti-CEA F(ab')₂ monoclonal antibodies. The wells were then filled with 50 μ l of the patient's serum diluted 1 : 100 in PBS-BSA 0.1% for 2 hr at room temperature. After washing, 50 μ l of goat anti-human IgG F(ab')₂ conjugated with peroxidase

substrate was reacted with the serum according to the ELISA procedure.⁷ Optical density (OD) was determined at 495 nm for each well in an ELISA reader.

RESULTS

Accumulation of radioactivity in the primary tumor was seen in only one patient. Visualization of the liver metastasis was grade + in 5 patients and grade ± in 2 patients. Thus detection of liver metastasis was better than in primary tumors. While tumor visualization was often seen in the 3 day image, most commonly optimal visualization of the tumor was possible at 5–7 days. There was no correlation between the serum concentrations of CEA and CA19-9 and the visualization of tumors (Table 1).

The serum kinetics of I-131 IMACIS-1 showed biexponential components with a 1st phase $T_{1/2}$ of 5.0 hours and 2nd phase $T_{1/2}$ of 34.7 hours. The mean whole body (I-131) half-life determined from the whole-body

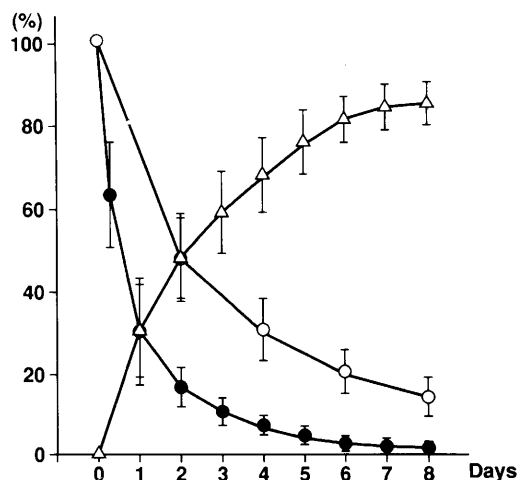


Fig. 1 Serum retention of IMACIS-1, whole body retention of I-131 and excretion of I-131 in urine. Serum retention of IMACIS-1; (●-●), I-131 whole body retention; (○-○), excretion of I-131 in urine; (△-△)

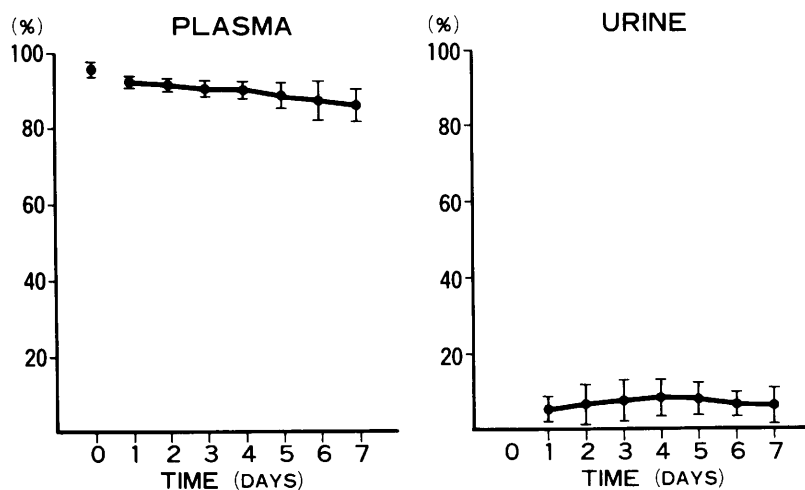


Fig. 2 Percent TCA-precipitable counts in plasma and urine.

scans was 1.95 days. The mean urinary excretion of I-131 in 7 days was 85%. This value agreed closely with the whole body radioactivity retention assured by scanning (Fig. 1). Most of the I-131 activity in serum was protein bound although the plasma protein radioactivity decreased slightly as time passed. In the urine, more than 80% of the I-131 was present in the non-precipitable fraction (Fig. 2). *In vitro* determination of the binding of I-131 IMACIS-1 to the patients peripheral blood, preceding intravenous antibody administration, showed it to be 0.12% (range: 0.08 to 0.14%) of the activity to be cell bound. Determination of the *in vivo* cell bound fraction of radioactivity also was approximately 0.12% (range: 0.08 to 0.15%) of the activity to be associated with the cellular elements. Baseline CEA and CA19-9 are shown in Table 1. Serial CEA and CA19-9 levels were determined after the infusion of IMACIS-1. When the initial serum concentrations of these antigens were relatively low, they tended to decrease after the intravenous infusion of IMACIS-1 (Fig. 3). There was no clear relationship between the plasma RI half-life and the serum CEA and CA19-9 concentrations.

Before and at one month after the administration of IMACIS-1, HAMA IgG (OD 495 nm) was 0.084 ± 0.015 and 0.092 ± 0.025 , respectively. There was no difference in HAMA IgG before and after treatment with IMACIS-1.

Case presentation

Case 4, 77 years old, female, had a sigmoid carcinoma and metastatic tumor in the left lobe of the liver. Serum CEA and CA19-9 were 14.2 ng/ml and 44.2 U/ml, respectively. On the anterior view of Tc-99m phytate scan there was a defect in the left lobe of the liver. X-CT scan showed a low density area (LDA) in left lobe. An accumulation of I-131 IMACIS-1 in a metastatic lesion was seen at 6 days after injection, but no obvious uptake was seen in the primary lesion (Fig. 4).

Case 1, 74 years old, female, had a recurrent colon

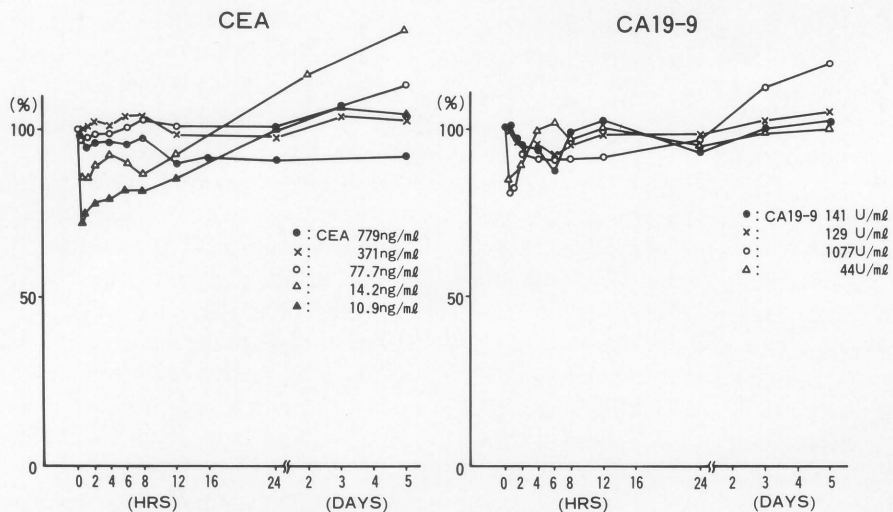


Fig. 3 Monitoring of serum CEA and CA19-9 concentrations.

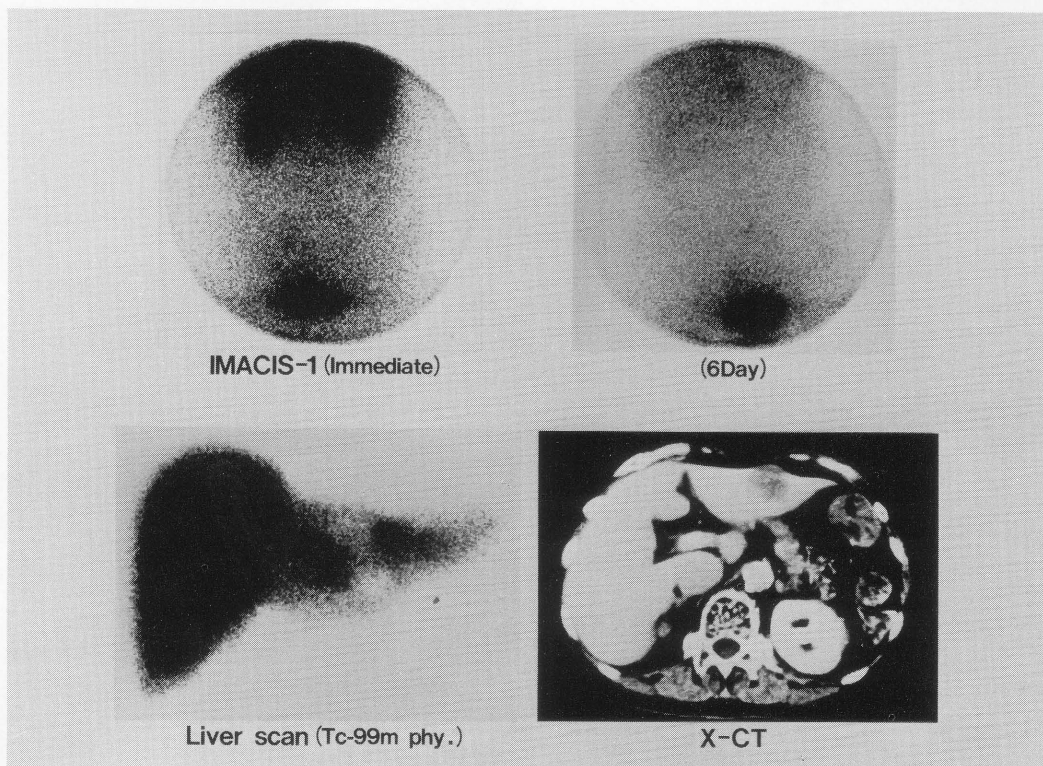


Fig. 4 Case 4, 77 years old, female, with sigmoid carcinoma and metastatic tumor in the left lobe of the liver. Serum CEA and CA19-9 were 14.2 ng/ml and 44.2 U/ml, respectively. An accumulation of I-131 in a metastatic lesion was seen at 6 days after injection, but no obvious uptake was seen in the primary lesion.

cancer which was in the rectosigmoid colon. Serum CEA was 779 ng/ml and CA19-9 was in the normal range. Tc-99m phytate study and X-CT scans showed that the multiple metastatic lesion occupied the liver. Uptake of IMACIS-1 showed in the metastatic lesion 7 days after the injection. No obvious uptake was seen in the primary lesion (Fig. 5).

Case 3, 82 years old, male, had recurrent gastric carcinoma. There was a recurrent lesion in the area of gastro-

duodenal anastomosis. Serum CEA and CA19-9 were 371 ng/ml and 141 U/ml, respectively. In the Tc-99m phytate study multiple large defects were revealed in the liver. There were LDAs in the liver on X-CT scans. In the IMACIS-1 study metastatic lesions in the liver showed cold areas in the early image and accumulation of I-131 in a tumor was seen at 7 days after infusion. However there was no accumulation of I-131 in the recurrent lesion (Fig. 6).

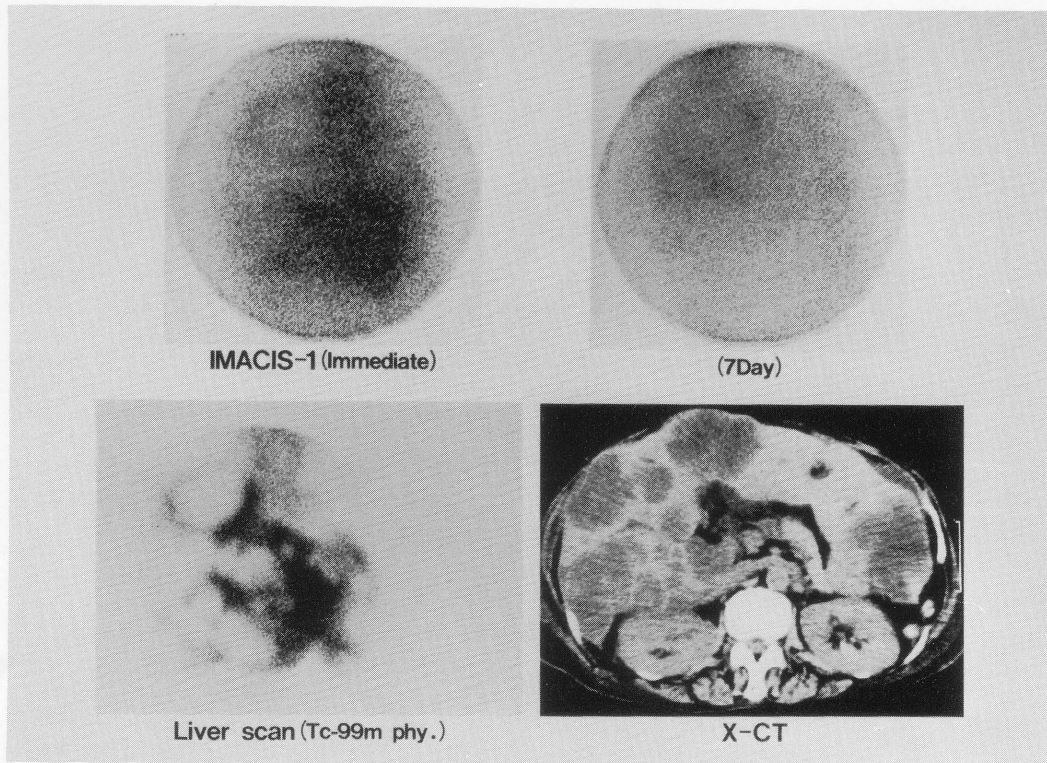


Fig. 5 Case 1, 74 years old, female, with recurrent colon cancer. Serum CEA was 779 ng/ml and CA19-9 was in the normal range. Multiple metastatic lesion occupied the liver. Uptake of IMACIS-1 showed in the metastatic lesion 7 days after injection.

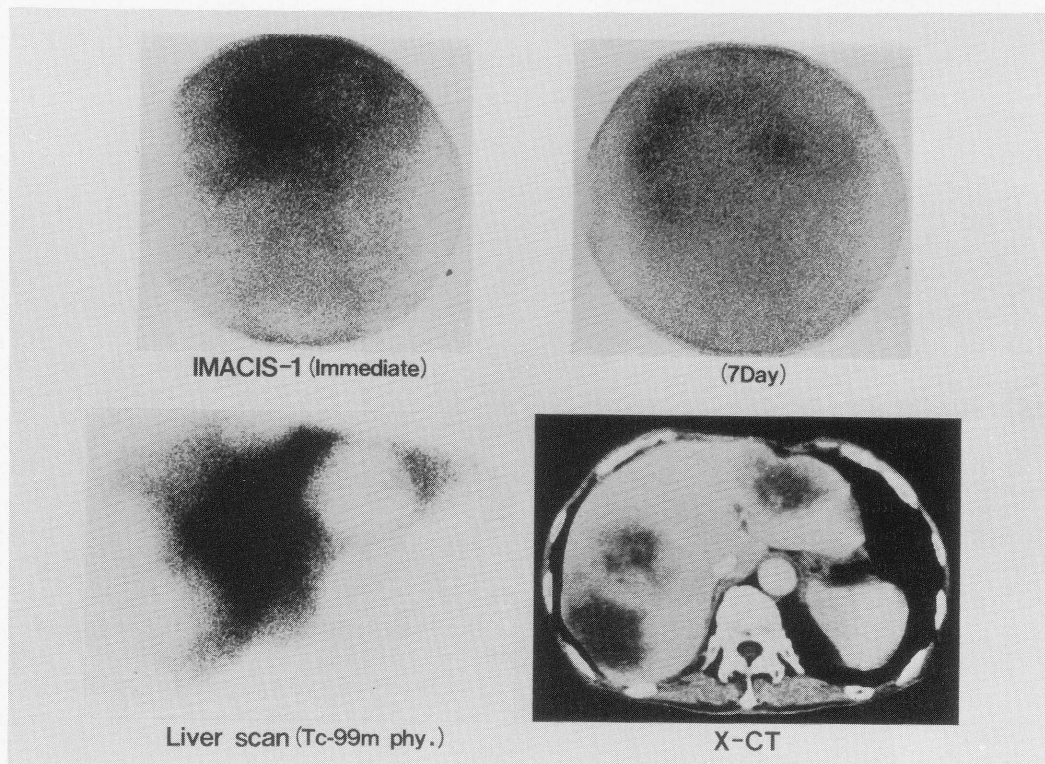


Fig. 6 Case 3, 82 years old, male, with recurrent gastric carcinoma. Serum CEA and CA19-9 was 371 ng/ml and 141 U/ml, respectively. Metastatic lesions in the liver showed cold areas in the early image and accumulation of IMACIS-1 in the tumor was seen at 7 days after infusion. However there was no accumulation of I-131 in the recurrent lesion.

DISCUSSION

Since the development of Mabs by Köhler and Milstein⁸ in 1975 their clinical applications in immunoassay and immunochemistry have been well established. At present studies are evaluating the use of Mabs as anti-tumor agents, taking advantage of their direct anti-tumor effects⁹ or their efficacy when conjugated to toxin,¹⁰ drugs¹¹ or isotopes.¹² One theoretical approach to improving the delivery of Mabs to tumors is to direct them at various antigens that may be expressed in the tumors, therefore potentially decreasing the problems of antigen heterogeneity.¹³

Most studies performed to date have been directed at a single antigen. To aim at two different antigens frequently present in gastrointestinal tumors¹⁴ we utilized a cocktail of two different antibodies.

IMACIS-1 showed good detection of metastatic liver disease in 5 of 7 patients having definite visualization of their tumor. Although the serum kinetics of I-131 IMACIS-1 resulted in faster clearance than others have reported with intact IgG^{15,16} optimal images were obtained at 5 to 7 days, although tumor visualization was obtained as early as 3 days. Optimal visualization occurred when background activity had decreased. It is possible that earlier localization and improved imaging may be observed by utilizing SPECT, which has better contrast resolution than planar imaging.¹⁷ This may be particularly useful for visualization of the primary lesions where only 1 of 7 were visualized possibly due to the lower lesion to background ratios in the abdomen. Since vascular distribution and blood supply of the tumor and tumor marker production differed among primary lesion in the digestive organs and metastatic lesion in the liver,^{18,19} metastatic tumor images with IMACIS-1 are superior to those for primary or recurrent tumor.

The CEA and CA19-9 levels in serum varied considerably but did not appear to affect clearance from the circulation nor did we detect a relationship between CEA and CA19-9 concentrations and tumor uptake. At low circulating levels of CEA or CA19-9 we saw a drop in the serum antigen. This may have been related to faster elimination of the circulating antigen once the antigens were bound by Mab or alternatively the presence of the injected Mab may have interfered with the assay.

Nonspecific cross-reacting antigen (NCA), a glycoprotein cross-reactive with CEA is expressed on the surface of normal granulocytes.²⁰ IMACIS-1 bound only 0.08–0.15% of the peripheral blood cells. This value was considered as nonspecific binding.

Our dose, consisting 1 mg of each preparation, is within the dose range used successfully by others for imaging.²¹ This low dose together with the lack of Fc fragment may be responsible for the lack of HAMA observed in our patients. As in many other studies with Mab, no side effects were observed in this group of patients. The

presence of a small amount of antibody binding to the patients' circulating cells was seen but did not result in any effects.

IMACIS-1 can be useful in detecting metastatic lesions in the liver, but primary lesions were not detected satisfactorily. This series of studies demonstrate the potential utility of a cocktail of antibodies consisting of an anti-CEA and anti-CA19-9 monoclonal F(ab')₂. More extensive studies directly comparing the imaging results for each individual antibody versus the cocktail of both Mabs should be performed to determine if there is any advantage in using the cocktail.

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