Tumor Localization in Patients by Radiolabeled Monoclonal Antibodies against Colon Carcinoma

Jean-Pierre Mach, Jean-Francois Chatal, Jean-Denis Lumbroso, Franz Buchegger, Michel Forni, Jurg Ritschard, Christian Berche, Jean-Yves Douillard, Stephan Carrel, Meenhard Herlyn, Zenon Steplewski, and Hilary Koprowski

INSERM and Centre René Gauducheau, Nantes, France [J-F. C., J-Y. D.]; Gustave Roussy Institute and Clinical Radiobiology Institute, F-94800 Villejuif, France [J-D. L.]; Clinique Médicale and Division of Nuclear Medicine, Department of Medicine, University of Geneva, Geneva, Switzerland; U2.11 Zenon Steplewski, and Hilary Koprowski

ABSTRACT

A radiolabeled monoclonal antibody (MAb) that has been shown to react specifically in vitro and ex vivo to human colorectal carcinoma and to inhibit growth of human carcinomas grafted in nude mice was administered to 52 colorectal carcinoma patients and 15 patients with other types of cancer. Of 63 colorectal carcinoma tumor sites studied, 34 showed significant accumulation of antibody by external photoscanning and tomoscintigraphy, whereas none of the 20 sites of other cancer types gave positive results. One-third of the patients received F(ab')2 fragments of the MAb, which gave a slightly higher percentage (61%) of positive results than did intact MAb (51%). A few patients scheduled for tumor resection were given injections simultaneously of 131I-labeled MAb and 125I-labeled normal immunoglobulin G. Antibody concentration in resected tumors was 3.6 to 6.3 times higher than the average antibody concentration in adjacent normal tissues (1.5, 3.4, and 9.4 as compared with normal mucosa, serosa, and fat, respectively), and the specificity indices, calculated by differential radioactivity analysis, ranged from 2.1 to 5.1. The results show the potential value and limitations of this particular MAb for tumor detection by immunoscintigraphy.

INTRODUCTION

The use of antibodies against tumor antigens to deliver chemotherapeutic drugs (16), toxins (5, 13), or radioisotopes (34) to malignant cells is now being considered as an alternative to active immunotherapy or immunostimulation, which has been largely unrewarding (40). A useful step toward this aim has been the production of MAbs3 that react with a single epitope of known tumor markers, such as CEA (2, 14) or α-fetoprotein (1, 41), and MAbs that define new antigens associated to various degrees with a specific tumor type (7, 8, 22, 25, 26, 42). However, the tumor specificity of these new MAbs has until recently been demonstrated only in vitro, whereas the more relevant test for any antibody considered for passive immunotherapy should be in vivo tumor localization. This can be performed in nude mice bearing xenografts of human cancers or in patients by injecting small amounts of purified radiolabeled antibodies, which are subsequently detected by photoscanning techniques or by direct measurement of radioactivity in surgically resected tumors. Using these methods, encouraging results have been obtained with purified polyclonal antibodies (15, 29, 30) and MAbs against CEA (4, 6, 18, 19, 28). We describe here the tumor localization in patients of a radiolabeled MAb against an antigen the expression of which is restricted to colorectal carcinoma cells (22). This particular MAb was selected among others with similar specificity (25), because its localization in tumor xenografts has been demonstrated (20); it inhibits the growth of human colon carcinoma transplants in nude mice (21); it localizes in tumors ex vivo (37); and it detects an antigen not shed by tumor cells in tissue culture (39). Tumor localization in patients was determined in all cases by external photoscanning. In addition, tomoscintigraphy (single-photon emission computerized tomography) (3, 27, 31) or direct measurement of the radioactivity in surgically resected tumor and normal tissue fragments was sometimes used to study the specificity of antibody localization in tumors.

MATERIALS AND METHODS

Preparation of Radiolabeled MAbs, F(ab')2, and Control IgG. Hybridoma cell line 1083-17-1A (hereafter designated 17-1A) produces MAbs that react specifically with human colorectal cancer cells (22). MAb 17-1A (IgG2a class) was purified from ascites fluid of a Staphylococcus aureus protein A-Sepharose column (38) (Pharmacia, Uppsala, Sweden). Control mouse IgG was purified by ion-exchange chromatography as described previously (6) from BALB/c mouse serum and from ascites from mice given injections i.p. of P3x63Ag8 myeloma cells (24). The F(ab')2 fragments were obtained from purified MAb 17-1A or P3x63Ag8 IgG1 by pepsin digestion (33). Purified MAb 17-1A or its F(ab')2 fragments were labeled with 131I, and normal IgG or its F(ab')2 with 125I using the chloramine T method or using Iodo-Beads (Pierce Chemical, Rockford, Ill.). Both labeling procedures were performed at 4°C. Unbound iodine was removed by filtration on a Sephadex G-200 or G-25 column equilibrated in pyrogen-free 0.15 M NaCl. The resulting specific activities were 4 to 10 μCi of 131I per μg of antibody and 1 to 2 μCi of 125I per μg of normal IgG. The labeled immunoglobulins were sterilized by filtration through a 0.22-μm Millipore filter and tested for sterility and pyrogenicity before injection.

Immunoreactivity and Specificity of Radiolabeled Antibodies. Radiolabeled MAb 17-1A or its F(ab')2 fragments were regularly tested for binding activity in vitro to 3 human colon carcinoma cell lines (LOVO, HT29, and Co115) and to 3 unrelated human cancer lines (melanoma IgR3, glioma LN 229, and endometrial carcinoma End 1) (8). In each case, 5 × 10⁶ cells were incubated with shaking for 3 hr at 4°C with 40 nCi of 131I-labeled MAb 17-1A and 40 nCi of 125I-labeled normal IgG diluted in 0.15 M phosphate-buffered saline (NaCl [8.0 g/liter]-KCI [0.20 g/liter]-KH₂PO₄ [0.20 g/liter]-Na₂HPO₄ [1.15 g/liter]-CaCl₂ [anhydrous, 0.10 g/liter]-MgCl₂·6H₂O [0.10 g/liter]) containing bovine serum albumin

Received December 28, 1982; accepted July 27, 1983.

1 Supported in part by Grants CA-10815, CA-21124, and RR-05540 from the NIH and by the W. W. Smith Charitable Trust. A preliminary report of these results was presented at the Third Congress of Nuclear Medicine (9, 32). 2 To whom requests for reprints should be addressed.

3 The abbreviations used are: MAb, monoclonal antibody; CEA, carcinoembryonic antigen.

Received April 12, 2017. © 1983 American Association for Cancer Research. cancerres.aacrjournals.org Downloaded from cancerres.aacrjournals.org on April 12, 2017. © 1983 American Association for Cancer Research.
The cells were washed, and radioactivity was measured in a dual-channel γ counter. As shown in Chart 1, 16 to 28% of the 131I-labeled antibody was bound to the colon carcinoma cells, whereas less than 1% was bound to the cells from the other cancer lines. Less than 1% of the 125I-labeled normal IgG was bound to the colon carcinoma and the other cell lines. MAb 17-1A or its F(ab′)2 fragments were also tested for their tumor localization in nude mice grafted with CEA-producing human colon carcinoma and found to give excellent results (20).

Patients. Informed consent was obtained from 52 patients with a diagnosis of colorectal adenocarcinoma and no personal or familial history of allergy, according to the recommendation of our ethics committee. In most cases, the clinical diagnosis, made by conventional radiological methods and/or computerized axial tomography scan, was confirmed by histological examination. Table 1 gives the tumor site studied, the serum CEA level (ng/ml) at the time of scanning, and the center where testing was done for each patient. Table 2 gives the tumor weight and Dukes' staging for 3 resected tumors. In addition, 15 patients (1 mg/ml). The cells were washed, and radioactivity was measured in a dual-channel γ counter. As shown in Chart 1, 16 to 28% of the 131I-labeled antibody was bound to the colon carcinoma cells, whereas less than 1% was bound to the cells from the other cancer lines. Less than 1% of the 125I-labeled normal IgG was bound to the colon carcinoma and the other cell lines. MAb 17-1A or its F(ab′)2 fragments were also tested for their tumor localization in nude mice grafted with CEA-producing human colon carcinoma and found to give excellent results (20).

Patients. Informed consent was obtained from 52 patients with a diagnosis of colorectal adenocarcinoma and no personal or familial history of allergy, according to the recommendation of our ethics committee. In most cases, the clinical diagnosis, made by conventional radiological methods and/or computerized axial tomography scan, was confirmed by histological examination. Table 1 gives the tumor site studied, the serum CEA level (ng/ml) at the time of scanning, and the center where testing was done for each patient. Table 2 gives the tumor weight and Dukes' staging for 3 resected tumors. In addition, 15 patients

Columns, mean of the percentage of binding obtained with 6 different labeled preparations of MAb 17-1A tested in duplicate before injection in the patients; bars, S.D.

RESULTS

Sensitivity of Tumor Detection. The results of tumor detection obtained in the 3 centers are summarized in Table 1. In the 23 patients tested in Geneva by external photoscanning and the subtraction method, 4 tumor sites of 12 gave positive results after injection of intact MAb 17-1A, and 8 tumor sites of 13 studied were positive after injection of F(ab′)2 fragments of MAb 17-1A. In the 21 patients tested in Nantes by the external photoscanning and subtraction method (with special emphasis on late scanings up to 11 days after injection), 18 tumor sites of 30 tested gave positive results using intact 17-1A only. Eight patients were tested in Villejuif by tomoscintigraphy. One of 3 patients given injections of intact MAb 17-1A gave positive results, and 3 of 5 given injections of F(ab′)2 fragments from MAb 17-1A gave positive results (Table 1). Furthermore, 20 tumor sites (in 15 patients) of cancers other than colorectal carcinoma were tested in Nantes with intact MAb 17-1A, and all of them gave negative photoscanning results (not shown).

PHOTOSCANNING AND SUBTRACTION TECHNOLOGY. Fig. 1 shows the results of the tumor detection in 2 patients by photoscanning and subtraction technology. Figs. 1, A to C, shows the results obtained with Patient G9, who had a carcinoma of the upper part of the sigmoid diagnosed by barium enema. Fig. 1A shows the distribution of total 131I radioactivity in the abdomen and pelvis 48 hr after injection of 0.5 mg of intact MAb 17-1A labeled with 1.2 mCi of 131I. A distinct radioactive spot (arrow) corresponds to the tumor area. The radioactivity is also concentrated in liver and urinary bladder areas. Fig. 1B shows the radioactivity due to 99mTc-labeled albumin and free 99mTcO4− injected before
scanning. There is no uptake in the tumor area. Fig. 1C shows the 131I activity after computerized subtraction of the 99mTc activity. The major radioactive spot (arrow) corresponds to the tumor; the minor spot in the upper right corner may be due to nonspecific accumulation of antibody in the spleen. A carcinoma from the upper sigmoid (diameter, 6 cm; estimated volume, 40 cu cm) was surgically resected in this patient 18 days after antibody injection. Several small liver metastases (diameter, <1 cm) were also found that were not detected by scanning. Thus, the patient was classified as "++" for the primary carcinoma and "-" for the liver metastases (Table 1).

Fig. 1, D to F, shows the images obtained with Patient G15 who had a palpable tumor mass of about 6 cm in diameter over the urinary bladder compressing the inner side of the sigmoid, as determined by barium enema. Results of tumor biopsy suggested the diagnosis of colon adenocarcinoma, but the primary tumor was not found. Fig. 1D shows the distribution of total 131I radioactivity in the abdomen and pelvis 48 hr after injection of 0.3 mg of 17-1A F(ab')2 fragments labeled with 1.5 mCi of 131I. A distinct uptake of radioactivity (arrow) can be seen in the area above the urinary bladder. Fig. 1E shows the 99mTc radioactivity, obtained as described for Patient G9, with no accumulation of radioactivity in the tumor area. Fig. 1F shows the 131I activity after subtraction of the 99mTc activity. The radioactivity is concentrated in the area corresponding to the tumor (arrow), with some radioactivity remaining in the urinary bladder and some activity due to the technical artifact called "edgepacking," along the upper border of the scan.

Kinetics of Antibody Localization. Interestingly, the relative concentration of MAB 17-1A in the tumor as compared to normal tissues increased with time up to 10 days after injection. The kinetics of antibody accumulation in the tumor is illustrated in Fig. 2. Patient N9 had a large hepatic metastasis from a rectal carcinoma, demonstrated by a defect (arrows) in the sulfur colloid scintigraphy shown in Fig. 2A. He was given an injection of 0.3 mg of intact 17-1A labeled with 1.5 mCi of 131I. At Day 1 after injection, the photoscan of the upper abdomen (Fig. 2B) shows 131I radioactivity in the tumor area that corresponds to the defect in Fig. 2A, but the concentration of radioactivity in the tumor does not exceed that detected in normal heart (H) and in stomach (S). On Day 4 (Fig. 2C), contrast in the tumor area is improved, but activity is still similar in the stomach area, whereas on Day 11 (Fig. 2D), the remaining 131I radioactivity is concentrated mainly over the tumor metastasis. No subtraction was necessary for this demonstration.

Tomoscintigraphy. Tomoscintigraphy was used to further demonstrate the tumor localization of MAB 17-1A using a protocol described for the detection of 131I-labeled MAB against CEA (4). Fig. 3 shows the results obtained with Patient V4, who had a large metastasis in the left lobe of the liver after resection of a sigmoid carcinoma. The patient was given injections of 0.3 mg of 17-1A F(ab')2 fragments labeled with 1.7 mCi of 131I at Day 0 and of 2 mCi of 99mTc-labeled sulfur colloid 30 min before scintigraphy. Fig. 3A shows the reconstructed static image of 131I radioactivity in the upper abdomen obtained on Day 2 after antibody injection. A significant accumulation of radioactivity is

### Table 1

<table>
<thead>
<tr>
<th>MAB 17-1A</th>
<th>++†</th>
<th>+</th>
<th>±</th>
<th>−</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact</strong></td>
<td>G9 sigmoid (68)</td>
<td>G1 sigmoid (36)</td>
<td>G8 transverse colon (52)</td>
<td>G9 liver (68)</td>
</tr>
<tr>
<td></td>
<td>G5 sigmoid (6)</td>
<td>G3 sigmoid (9)</td>
<td>G6 right colon (12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N6 left colon (36)</td>
<td>N1 liver (300)</td>
<td>N3 left colon LR (300)</td>
<td>N11 cutaneous M (17)</td>
</tr>
<tr>
<td></td>
<td>(3 sites)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N9 liver M (280)</td>
<td>N2 liver M (275)</td>
<td>N12 liver (1180)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N15 liver M (490)</td>
<td>N4 rectum (ND)*</td>
<td>N24 liver M (49)</td>
<td>N13 rectum (19)</td>
<td></td>
</tr>
<tr>
<td>N24 liver M (49)</td>
<td>N5 sigmoid LR</td>
<td>N31 rectum LR (83)</td>
<td>N16 sigmoid (ND)</td>
<td></td>
</tr>
<tr>
<td>N46 lung M</td>
<td>N9 liver M (280)</td>
<td>N35 lung M (ND)</td>
<td>N29 left colon (15)</td>
<td></td>
</tr>
<tr>
<td>(&gt;3000)</td>
<td>N10 ocemum (6)</td>
<td>N2 liver M (790)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N45 liver M</td>
<td>N24 liver M (49)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;3000)</td>
<td>N45 sigmoid LR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N45 liver M</td>
<td>(300)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>and cutaneous M</td>
<td>N45 liver M (3000)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V3 sigmoid (3.5)</td>
<td>V2 sigmoid (10)</td>
<td>V1 sigmoid (1.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>F(ab')2</strong></td>
<td>G12 right colon (60)</td>
<td>G16 transverse colon (7)</td>
<td>G13 sigmoid (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G15 left pelvis M (110)</td>
<td>G20 liver M (295)</td>
<td>G14 sigmoid (7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G19 right colon (74)</td>
<td>G21 sigmoid LD (760)</td>
<td>G17 sigmoid (7)</td>
<td>G21 liver M (790)</td>
</tr>
<tr>
<td></td>
<td>V4 liver M (100)</td>
<td>G22 sigmoid (9)</td>
<td>G18 cecum (4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V6 rectum LR (29)</td>
<td>G23 sigmoid (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>V8 sigmoid LR (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The tumor sites studied are grouped according to their positivity in the scintigraphic analysis: ++, good contrast and tumor clearly detectable; +, sufficient contrast to detect tumor; ±, insufficient contrast to detect tumor; −, no contrast.
† The letter before the patient number indicates the nuclear medicine center: G, Geneva; N, Nantes; and V, Villejuif.
* The extent of the tumor is designated: I, primary; M, metastasis; LD, local dissemination; LR, local recurrence.
* Numbers in parentheses, serum CEA levels at the time of scanning.
* ND, not done.
detectable in the suspected tumor area, but contrast is poor. Fig. 3B shows a similar reconstructed image detecting $^{99m}$Tc sulfur colloid and showing a defect in the area of antibody accumulation (arrow). The horizontal lines indicate the level in which 2 transverse sections were scanned to better localize the antibody concentration and the sulfur colloid defect. Fig. 3, C and D, shows the upper transverse section for $^{131}$I and $^{99m}$Tc radioactivity, respectively. A major $^{131}$I radioactive spot (large arrow) located in the anterior part of the left lobe of the liver is detectable and corresponds to the $^{99m}$Tc defect. A few small $^{131}$I spots (small arrow) also correspond to small $^{99m}$Tc defects. These results strongly suggest the presence of a major metastasis in the left lobe of the liver and may indicate the presence of a few smaller metastases in the right lobe.

Fig. 3, E and F, shows transverse sections, taken 2.5 cm below those in Fig. 3, C and D, which confirm the correlation of the areas of concentration of antibody and sulfur colloid defect. Lower transverse sections showed a decrease in antibody accumulation below the major metastasis and some radioactivity in the area corresponding to the right and left kidneys (data not shown). Nonspecific accumulation in the kidneys was often observed in the scans done on Day 1 or 2. The presence of the large left lobe metastasis was confirmed by a late (Day 8) static photoscan, which showed significant $^{131}$I accumulation in a single area (Fig. 3G, arrow), and by a conventional sulfur colloid scintigraphy, which showed a well-defined defect in the same area (Fig. 3H, arrow).

Differential Analysis of Antibody and Normal IgG Radioactivity in Resected Tumor and Adjacent Normal Tissues. To measure more precisely the specificity of tumor localization of the antibody, 3 patients scheduled for tumor resection were given simultaneous injections of equal amounts of MAb 17-1A and normal IgG labeled with $^{131}$I and $^{125}$I, respectively. (The injected $^{125}$I radioactivity, which is not detectable by photoscanning, was 5 times lower than that of $^{131}$I.) The surgically resected tumor and adjacent normal tissues were dissected into 0.5- to 1-g fragments of tumor, normal mucosa, normal serosa (external bowel wall separated from its mucosa), and normal fat. The radioactivity of the 2 isotopes present in the different fragments and in serum samples taken at the time of surgery was measured in a dual-channel $\gamma$ counter. The results expressed in nCi/g of tissue are shown in Chart 2. From these paired labeling results (29, 35), the relative tumor uptakes (radioactivity per g in tumor divided by radioactivity per g in normal tissues) could be calculated separately for both antibody and normal IgG. The specificity indices are the ratios of relative specific MAb uptake to relative normal IgG uptake (Table 2).

The relative uptakes of antibody in tumor are low when compared with the dissected normal mucosa, but they are much higher than those of normal IgG. The uptake of antibody to normal mucosa is also lower than that of normal IgG. The uptake of antibody to normal serosa and fat is significantly higher than that of normal IgG. The specificity indices are the ratios of relative specific MAb uptake to relative normal IgG uptake (Table 2).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Day after injection</th>
<th>Tumor Site</th>
<th>Wt (g)</th>
<th>Comparison with normal tissue</th>
<th>Antibody uptake</th>
<th>Normal IgG uptake</th>
<th>Specificity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>5</td>
<td>Sigmoid (Dukes' C)</td>
<td>16</td>
<td>Mucosa</td>
<td>2.02</td>
<td>0.98</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serosa</td>
<td>3.58</td>
<td>1.00</td>
<td>3.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fat</td>
<td>13.40</td>
<td>3.42</td>
<td>3.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Av</td>
<td>6.33</td>
<td>1.80</td>
<td>3.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serum*</td>
<td>0.84</td>
<td>0.19</td>
<td>4.42</td>
</tr>
<tr>
<td>G5</td>
<td>3</td>
<td>Sigmoid (Dukes' A)</td>
<td>13</td>
<td>Mucosa</td>
<td>1.10</td>
<td>0.53</td>
<td>2.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serosa</td>
<td>3.68</td>
<td>0.57</td>
<td>6.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fat</td>
<td>7.34</td>
<td>1.11</td>
<td>6.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Av</td>
<td>4.04</td>
<td>0.74</td>
<td>5.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serum*</td>
<td>0.63</td>
<td>0.09</td>
<td>7.0</td>
</tr>
<tr>
<td>G6</td>
<td>3</td>
<td>Right colon (Dukes' C)</td>
<td>12</td>
<td>Mucosa</td>
<td>1.2</td>
<td>1.11</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serosa</td>
<td>2.86</td>
<td>1.05</td>
<td>2.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fat</td>
<td>6.7</td>
<td>2.56</td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Av</td>
<td>3.59</td>
<td>1.57</td>
<td>2.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serum*</td>
<td>0.58</td>
<td>0.21</td>
<td>2.76</td>
</tr>
</tbody>
</table>

* nCi of $^{131}$I per g of tumor divided by nCi of $^{131}$I per g comparison of normal tissue.

** nCi of $^{125}$I per g of tumor divided by nCi of $^{125}$I per g comparison of normal tissue.

^ Antibody uptake divided by normal IgG uptake.

' Average value for normal mucosa, serosa, and fat.

" Serum at the time of surgery.
higher compared with normal serosa or fat. The specificity indices are the most significant values, since they include an internal control of normal IgG which can correct for any nonspecific accumulation of foreign proteins in necrotic tumor tissues. The average values for the 3 patients studied were 1.7 for the accumulation of foreign proteins in necrotic tumor tissues. The most significant values, since they include an internal control of normal serosa, 4.4 with normal mucosa, and 4.7 with normal serosa, 4.4 with normal mucosa, and 4.7 with normal serosa, 4.4 with normal mucosa.

**DISCUSSION**

In this study, we have demonstrated tumor localization in patients by a MAb directed against an antigen which is expressed only on colorectal carcinoma cells and which is not shed by tumor cells in vitro (39) and not detected in the circulation. These properties of the antigen recognized by MAb 17-1A may explain why the radiolabeled MAb could be detected by scintigraphy up to 11 days after injection and why this MAb gave positive results exclusively in gastrointestinal carcinomas. The percentage of positive tumor detection was higher when F(ab')2 fragments were used, and they appear to give less nonspecific accumulation of radioactivity in the reticuloendothelium. However, this last point has not yet been established by statistical evaluation. Tomoscintigraphy was found very useful in determining the 3-dimensional localization of radioactive uptake and its correspondence with suspected tumor sites as also shown recently for an anti-CEA MAb (4, 9). It was not possible to determine whether the immunoscintigraphic results correlated with tumor antigen expression in patients, since it is difficult to detect the antigen recognized by MAb 17-1A using immunoperoxidase techniques on fixed-tissue sections, and as yet, there is no quantitative assay for this antigen.

The specificity of tumor localization was confirmed by direct measurement of antibody radioactivity in resected tumors and adjacent normal tissues and by comparison with the radioactivity associated with a control normal IgG injected simultaneously. Radioactivity calculated per g of resected tumor and dissected normal tissues must be measured in a $\alpha$-counter for an objective evaluation of tumor localization of antibodies. Gross estimation of resected tumor radioactivity by scanning methods without weight determination, as done in a recent immunoscintigraphic study using MAb against milk fat globule antigen (11), cannot be substituted. Another recent publication has described the use of a MAb raised against human osteogenic sarcoma cells to detect colorectal carcinoma in patients (12).

In the present study, as well as in previous studies using $^{131}$I-labeled anti-CEA antibodies (28, 29, 32), the absolute amount of antibody radioactivity recovered in the tumors 3 to 5 days after injection was relatively low. This might be due to dehalogenation of the antibodies in the circulation. If this hypothesis is correct, one may assume that larger amounts of unlabeled antibodies are actually reaching the tumor. Alternative methods of antibody labeling using radiometal chelates are available (17, 23, 36) and may improve the sensitivity and specificity of immunoscintigraphy. In addition, the use of chelates permits the labeling of antibodies with radioisotopes that are most suitable for $\gamma$ camera imaging, such as $^{111}$In, as well as the labeling of the MAb with $\alpha$-emitting isotopes which offer the most localized and efficient destruction of the target tumor cells.

**ACKNOWLEDGMENTS**

We thank Professors A. Donath, A. Rohner, and A. F. Muler from the University of Geneva and Professors M. Tubiana and C. Parmentier from the Institut Gustave Roussy for advice and suggestions.

**REFERENCES**


Fig. 1. Photoscans of the abdomen and pelvis obtained with Patient G9 (A to C), who had a primary carcinoma of the upper sigmoid, and of Patient G15 (D to F), who had a metastatic adenocarcinoma located over the urinary bladder (arrows). A and D, total 131I radioactivity; B and E, 99mTc radioactivity; C and F, 131I minus 99mTc radioactivity.
Fig. 2. Photoscans of the upper abdomen obtained with Patient N9 who had a liver metastasis from a rectal carcinoma. A, $^{99m}$Tc radioactivity; B to D, scans detecting $^{131}$I radioactivity taken 1, 4, and 11 days, respectively, after injection of intact MAb 17-1A. H, heart; S, stomach. In B to D, the arcs delineate the liver.
Fig. 3. Photoscan and tomoscintigraphy comparing the uptake of 131I-labeled MAb 17-1A (A, C, E, G) and the defect of 99mTc colloid sulfur (B, D, F, H) in the liver metastases (arrows) from Patient V4. A and B, reconstructed static images taken 2 days after MAb injection. The 2 white horizontal lines, levels of the transverse sections presented below. C and D, upper transverse sections taken at identical levels. E and F, lower transverse sections taken at identical levels. G, static photo-scanning detecting 131I radioactivity taken at Day 8. H, static photoscanning detecting 99mTc colloid sulfur. On the tomographic sections, a.l., anterior left; in D and F, S, spleen.
Tumor Localization in Patients by Radiolabeled Monoclonal Antibodies against Colon Carcinoma

Jean-Pierre Mach, Jean-Francois Chatal, Jean-Denis Lumbroso, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/43/11/5593

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.