Positive Anticalcitonin Immunoscintigraphy in Patients with Medullary Thyroid Carcinoma

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ABSTRACT

A cocktail of three monoclonal F(ab')2 fragments against three distinct epitopes of calcitonin or PDN 21 was labeled with either 111In or 131I. These F(ab')2 fragments, a control 125I-F(ab')2 fragment and 99mTc-pertechnetate were injected into four patients suffering from medullary thyroid carcinoma. Scintigraphy data were processed by energy factor analysis for an optimal separation of images corresponding to each isotope. The best tumor detection was obtained 1-3 days after injection of the 111In-F(ab')2 cocktail which clearly labeled the thyroid tumors in the four patients (smallest tumor detected, 0.6 cm) as well as lymph node and bone metastases. In the liver, positive detection was only successful with the 131I-labeled cocktail. These results were confirmed by counting rates of resected specimens which provided average specificity indices ranging from 3.3 to 13.1. Anticalcitonin antibodies could be particularly useful for immunoscintigraphy detection of residual or recurrent medullary thyroid carcinoma in patients with elevated calcitonin serum level.

INTRODUCTION

Owing to its histological origin (parafollicular C cells), MTC2 is associated with an enhanced production and secretion of CT (1, 2). Basal or pentagastrin-stimulated measurement of this hormone in the serum of MTC patients is the most specific and sensitive diagnostic method. CEA secretion is less constant and less specific, but its evolution is also of diagnostic and prognostic value (3, 4).

No efficient and specific method is currently available for the localization of metastases or recurrent disease when CT and/or CEA levels remain elevated or enhanced after initial therapy. Indeed, several nonspecific radiopharmaceuticals such as 131I-labeled metaiodobenzylguanidine (5-7), 99mTc-labeled dimercaptosuccinate (7-9), thallium-201 (10), 99mTc-labeled diphenylphosphonate compounds (11) have been investigated, but none has been proven diagnostically useful in MTC.

IS is potentially more specific. With 125I-labeled anti-CEA MAbs, significant but inconsistent results were obtained by our group in MTC (12, 13): five out of the 11 investigated patients had a positive scan. Positive immunoscmms were also reported with anti-CT polyclonal (5, 14) or monoclonal (15) antibodies but only in animal models. As far as we know, anti-CT IS has not yet been reported in humans, except in one negative case (16).

This study was undertaken in order: (a) to evaluate the feasibility of anticalcitonin immunoscintigraphy in patients with MTC; (b) to assess the specificity of the MAb localization by calculating specificity indices on surgical samples; (c) to optimize the detection efficacy in different sites by using a cocktail of MAbs labeled with either indium or iodine. This multisite approach was facilitated by the EFA data processing.

PATIENTS AND METHODS

Patients

Informed consent was obtained from four patients, selected as follows: (a) presence of operable, histologically confirmed MTC, with enhanced serum CT level; (b) presence of at least one tumor larger than 1.5 cm in diameter, as demonstrated by other techniques: ultrasonography (thyroid, liver), CT scan (liver, pelvis), X-ray, and methylene diphosphonate scintigraphy (bones), MRI (thyroid); and (c) absence of previous injection of mouse MAB. The clinical status of each patient has been summarized in Table 1.

Production and Characterization of MAbs

Four mouse monoclonal IgG1s were selected. Three of them recognize distinct epitopes of calcitonin and procalcitonin (17). CT03 and CT06 bind to an epitope located in the 26-32 region and in the 11-17 region of human calcitonin, respectively, with an affinity constant of 2.0 × 109/M (CT03), and 1.1 × 109/M (CT06) for CT. CT03 was found to be specific for the native form of CT (17). KC01 recognizes an epitope located in the 1-11 region of katalcin (PDN 21) with an affinity constant of 1.5 × 109/M (18).

AF08, specific for human AFP, was chosen as a negative control (19). Its affinity constant for AFP is 1.1 × 1010/M.

Antibodies were produced in nude mice ascites and purified by protein A chromatography (Pharmacia), according to standard protocol described elsewhere (19). The antibody-containing fraction was determined by RIA, using 125I-labeled CT (Amersham) or PDN-21 (20) and precipitation by polyethylene glycol 6000.

Preparation of F(ab')2 Fragments

Digestion was performed at 37°C in 0.1 M, pH 4.0, sodium acetate buffer, after addition of pepsin [Worthington; enzyme/substrate ratio, 0.01-0.03 (w/w)] to the antibody solution (3-10 mg/ml; Biorad protein assay). Reaction was stopped by pH neutralization when HPLC chromatograms demonstrated an optimized yield in F(ab')2, e.g., the almost complete disappearance of intact IgG without significant degradation in Fab' and little peptides (incubation time: usually 3-6 h).

Fragments of the digested mixtures were purified by using a one-step method combining gel filtration (Sephacryl S200 column) and ion-exchange chromatography (DEAE-Sephacel) (Pharmacia). Chemical purity of purified F(ab')2 was tested by both HPLC and SDS-electrophoresis (10% acrylamide-bisacrylamide, 0.1% sodium dodecyl sulfate gels) and found to be higher than 95%.

Radiolabeling of Fragments

With Iodine. As a first methodological step, we labeled separately the four MAB fragments with 125I, by the iodogen method (21): each
F(ab')

was incubated in 0.1 M, pH 7.4, phosphate buffer for 20 min at 20°C with 500 µg iodogen (Pierce) and about 370 MBq (10 mCi) 125I-Na (Cis-Biointustries) per milligram F(ab').

For injection to Patients 2–4, the cocktail of specific F(ab')

(CT03, CT06, KC01; 330 µg of each one, SMAbs) and the control AF08 F(ab')

(500 µg, CMAb) were labeled, respectively, with 131I [about 74 MBq (2 mCi)] and 125I [about 9 MBq (250 µCi)]. For Patient 1, the same procedure was performed but the anti-CT cocktail (1 mg) was labeled with 9 MBq (250 µCi) 125I and AF08 F(ab')

(500 µg) with 185 MBq (5 mCi) 131I. Purification was then performed by ion-exchange chromatography (Dowex AG 1 × 8, Biorad).

With Indium, F(ab')

fragments were labeled with 111In by the DTPA method (22). DTPA double anhydride was added separately to each F(ab')

solution (protein concentration 5 mg/ml) in pH 8.0, 0.1 M NaHCO3 buffer (molar ratio DTPA/F(ab')

= 5). After 10 min at 20°C, unreacted DTPA (~40%) was removed by gel filtration (Sephadex G100, Pharmacia). Concentrated F(ab')

-DTPA conjugates (Amicon G-100, Pharmacia) were conserved frozen at -30°C for several months, without significant damage. After thawing, noncovalently bound DTPA was removed by dialysis against 1 m NaCl solution. Each F(ab')

-DTPA conjugate (preliminary assay) or the F(ab')

-DTPA cocktail (330 µg of CT03, CT06, and KC01 for injection to the patients) was then incubated with 74–111 InCl3 per milligram MAb for at least 30 min at 20°C. No further purification was necessary (yield: >98%).

Quality Control of Radiolabeled Products. Radiochemical purity of purified iodinated antibodies was controlled by paper electrophoresis (Whatman 3MM): migration was performed for 20 min in 15 V/cm electrical field using 0.07 m, pH 8.5, barbital solution as buffer. For indium-labeled F(ab')

, radiochemical purity was controlled by chromatography on Gelman ITLC plates in 0.1 M, pH 4.0, sodium citrate buffer. More than 95% of the radioactivity (indium or iodine) was associated with purified antibodies, as demonstrated with a scanning integrator counter.

Immunoreactivity of each DTPA-conjugated F(ab')

was compared to free F(ab')

and to whole antibody by RIA, using 125I-labeled (Amersham) or 131I-PDN-21 (20). Unconjugated F(ab')

were 2–12 times more immunoreactive than whole MAbs. However, DTPA conjugation decreased the immunoreactivity by a 2–4 factor.

After labeling with radioisotopes, immunoreactivity was further controlled by enzyme-linked immunosorbent assay (ELISA), and found to be 75–100% of that obtained before labeling. Radiolabeled antibodies were then filtered on 0.22 µm Millex-HA filters (Millipore) and tested for sterility and apyrogenicity (Limulus test).

Injection of Radiolabeled F(ab')

Thirty min prior to each MAb injection, patients were premedicated with 25 mg promethazine i.m. Lugol's iodide solution (30 drops/day) and potassium perchlorate (1 g at the first day, next 2 × 250 mg/day) were given p.o. for 7 days, starting 6 h before the first injection of radiiodinated antibody.

Injections of radiolabeled F(ab')

were performed i.v. following different schedules (Table 1). All patients underwent surgery 7 or 8 days (D7 or D8) after the first MAb injection.

Scintigraphy Data Acquisition

Scintigraphy data were collected every day using a 38.5-cm field of view gamma camera (Acticamera, CGR, France) with on-line energy correction. Data acquisitions were performed in double-word list mode, in order to record spatial and temporal coordinates and energy of each event. A pili collimator was used for thyroid study. For imaging of other regions, either high or low energy parallel collimator was used, depending on the presence or the absence of 131I at the moment of scanning.

A total of 500–1000 kilocounts were acquired with suitable energy windows to take into account the different photopeaks of present radionuclides. Practically, 50% energy windows were used, except for performed acquisitions in the presence of 111In and 131I (100% window). Acquisition time ranged between 3 and 10 min with parallel hole collimator and between 10 and 30 min for pinhole acquisitions. Each Patient's head was kept immobile by using two cushions.

Image Processing

After acquisition, data were reorganized into N 64 × 64 frames, each one corresponding to an energy window of 5 keV. The subsequent analysis was similar to previously described Factor Analysis of Dynamic Structures (FADS) (23, 24), except that the time dimension was replaced by the energy dimension (24). This method was called EFA.

EFA processing included three steps: data scaling, orthogonal analysis (principal component analysis) and oblique analysis, as developed previously for FADS (23, 25). This latter step corresponded to the processing of factors and their associated factor images by means of an iterative algorithm taking into account the constraint of nonnegativity. Energy factor analysis enabled the extraction from the original data sampling of n + 1 factor estimates (usually 2 to 4) of underlying spectra and of their associated factor images. These n + 1 spectra and images corresponded respectively to the n present isotopes and to pure scattered radiations. This latter spectrum characterized regions where the radioactivity followed a pure scattered component.

Scintigraphic images were produced using the linear spectrum analysis as the primary processing method. Typical images generated by this method are shown in Figure 1.
ANTICALCITONIN IMMUNOSCAN OF MEDULLARY THYROID CARCINOMA

Fig. 1. Right lateral acquisition of Patient 4's neck region, performed 48 h after infusion of $^{111}$In-SMAbs and 1 h after administration of $^{99m}$Tc-pertechnetate. Energy band = 205 ± 100 keV. Three-factor EFA processing. A, unprocessed acquisition; B–D, images corresponding to the three factors; B, $^{99m}$Tc image with the thyroid and the salivary glands (arrows); C, $^{111}$In image with hot spots corresponding to tumors (arrows) (the cervical bone marrow is also weakly labeled); D, image corresponding to scattered radiations; E, spectra corresponding to the three factors: $^{99m}$Tc (— —), $^{111}$In (---), scattered radiations (-----). These three factors correspond respectively to 33, 53, and 14% of the whole acquired information.

between these EFA estimated and measured pure spectra.

In MTC patients, EFA was very efficient for the separation of the present isotopes, even when their energy was very close. For example, $^{111}$In and $^{131}$I, whose closest photopeaks differ from only 12 keV, were completely separated (Fig. 1).

Investigations on Surgical Samples

Freshly resected specimens of both tumors and normal tissues were washed, weighted (0.2–1 g) and fixed in aqueous Bouin's solution. Each sample was conditioned in similar polystyrene tubes and Bouin's solution was added to obtain an equal final volume. The amount of each isotope in these samples was determined in a gamma counter by measuring the radioactivity in three energy windows corresponding to each isotope. In order to take into account the interference of a gamma-emitter with each channel, samples of pure isotopes were also counted in similar conditions. The specific activity (cpm/g) of resected tissues was measured for each isotope. SI were also calculated with iodinated antibodies, according to the Pressman's formula (26):

$$\text{SI}_{\text{specific}} = \frac{\text{specific antibodies in tumors} + \text{specific antibodies in normal tissue}}{\text{specific antibodies in tumors} + \text{specific antibodies in normal tissue}}$$

After paraffin embedding and slicing, both classical hematoxylin-eosin-saffron and immunoperoxidase staining was performed. In this latter case, the three specific F(ab')$_2$ fragments were tested separately: sections were treated with H$_2$O$_2$, washed with PBS and successively incubated with 1/10 diluted goat serum and the tested fragment or with PBS. After washing with PBS, goat F(ab')$_2$ specific for mouse F(ab')$_2$ fragments (Cappel) diluted 1/50 in normal human serum (1/25 in PBS) were added for 30 min. After three more washings, diaminobenzidine (Polysciences) development was achieved (27).

RESULTS

Imaging (Table 2, Figs. 1–4). With $^{111}$In-SMAbs, all palpable thyroid tumors were detected with a good contrast in the four patients. Small-sized tumors (0.6–1 cm) were visualized in Patients 2 and 3 (Figs. 2A and 2B). Previously unsuspected isthmus involvement was also diagnosed in Patient 2 and confirmed by histology. In the few localizations where a weak (Patient 1’s right lobe) or only peripheral (Patient 4, Fig. 2B) uptake had been observed, the resected nodules were found to be either cystic or necrotic. However, a variable physiological uptake of $^{111}$In by the bone marrow was also witnessed. In spite of the thyroid blockade, $^{131}$I-SMAbs (and $^{123}$I-CMAb) were...
Anticalcitonin Immunoscintigraphy Results

<table>
<thead>
<tr>
<th>SMAbs labeled with:</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid tumors</td>
<td>$^{111}$In</td>
<td>$^{131}$I</td>
<td>$^{111}$In</td>
<td>$^{131}$I</td>
</tr>
<tr>
<td>Left</td>
<td>++</td>
<td>ND</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Right</td>
<td>+</td>
<td>ND</td>
<td>--</td>
<td>++</td>
</tr>
<tr>
<td>Palpable neck lymph nodes</td>
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<td></td>
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<tr>
<td>Left</td>
<td>0</td>
<td>ND</td>
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<tr>
<td>Right</td>
<td>0</td>
<td>ND</td>
<td>+</td>
<td>++</td>
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<td>Liver metastases</td>
<td>--</td>
<td>ND</td>
<td>++</td>
<td>0</td>
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<td>Bone metastases</td>
<td>0</td>
<td>ND</td>
<td>++</td>
<td>0</td>
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<tr>
<td>Mediastinal masses</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

* ND, not done; --, no detection (false negative result); ±, doubtful localization; +, significant localization; ++, very clear localization; 0, no tumor (true negative result).

In the three patients with liver metastases (Patients 1, 2, and 4), $^{111}$In-SMAbs did not provide any interpretable image. But with $^{131}$I-SMAbs, we detected most of Patient 2’s liver metastases with a diameter ranging from 1.5 to 5 cm (Fig. 4B). In Patient 4, the large excess of $^{111}$In prevented us from obtaining any accurate image of the liver tumors with $^{131}$I-SMAbs. In the unique patient into whom $^{125}$I-CMAb had been injected (Patient 1), no tumoral mass was seen.

Bone metastases were visualized in Patient 2 (tibia, Fig. 4C) and Patient 4 (skull) with both isotopes but better with $^{111}$In-SMAbs.

Investigations on Surgical Samples. SMAbs were taken up preferentially by all tumor tissues in the four patients and with both isotopes when compared to skin, fat, connective tissue, muscle or an average of these tissues (defined as the background).

When expressing the results in percents of the administered dose per gram of tumor, the obtained ratio, with $^{111}$In-SMAbs, ranged between $0.3 \times 10^{-2}$% and $3 \times 10^{-2}$%, 7 or 8 days after injection. As far as dosimetry is concerned, thyroid irradiation with $^{131}$I was lower than 2 rads/mCi whereas liver irradiation was held in the 1–3 rads/mCi limits with $^{111}$In. Exposure of other organs was far lower, whatever the isotope used.

Preferential SMAbs uptake by thyroid tumors was confirmed by $^{111}$In countings (tumor/thyroid: 1.7–12.4). A certain degree of SMAbs specific uptake by non-tumoral thyroid was also demonstrated by comparing the $^{131}$I-SMAbs and the $^{125}$I-CMAb countings in the thyroid and in the surrounding tissues (SI = 1.4 to 2.2).

In the lymph nodes, $^{111}$In was almost similarly taken up by normal and tumor-involved tissues; but $^{131}$I, conjugated to the same antibodies, was clearly more concentrated in tumoral lymph nodes in contrast to the $^{125}$I-labeled control antibodies (Table 3).

From SI, significant differences appeared between Patients 1 and 4 (SI comprised between 3 and 4), and Patients 2 and 3 (SI more variable but frequently higher than 10). This has to be correlated with the fact that Patients 1 and 4 had a significantly higher CT level than the two others whereas the four patients received the same dose of antibody.

Immunohistochemistry was not sensitive enough to detect the antibodies taken up in vivo. But the in vitro recognition of CT by the three specific F(ab')2 fragments (CT03, CT06, and KC01) was demonstrated after separate addition of the MAbs to serial tumor sections. In these conditions, the intense and specific labeling of the cytoplasm of MTC cells was similar with $^{131}$I-SMAbs but their visualization was better with the $^{111}$In-SMAbs.

Fig. 3. A, anterior view of the thyroid region performed 24 h after injection of $^{111}$In-SMAbs; $^{111}$In-image obtained by EFA processing (two factors); tumor sites in both lobes (arrows) and a part of the cervical column (in the latter site, uptake of free released $^{111}$In-DTPA by the bone marrow) are visualized. B, MRI anterior image of the neck region (repetition time 1500 msec, echo time: 40 msec, first echo). Tumors are indicated with arrows.

Involved lymph nodes (Patients 2 and 4, Fig. 2B) and a large upper mediastinum mass (Patient 4) were detected with the $^{131}$I-SMAbs but their visualization was better with the $^{111}$In-SMAbs.

Unable to label positively any thyroid tumor in any patient.

In involved lymph nodes (Patients 2 and 4, Fig. 2B) and a large upper mediastinum mass (Patient 4) were detected with the $^{131}$I-SMAbs but their visualization was better with the $^{111}$In-SMAbs.

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Fig. 4. Detection of Patient 2’s metastases. A and B, upper abdomen region scintigraphy performed 24 h after injection of SMAbs labeled with both $^{111}$In and $^{131}$I. A, $^{111}$In image. Normal liver strongly took up $^{111}$In whereas a large metastasis of the left lobe appeared as a defect (arrow). B, with $^{131}$I, this metastasis was clearly labeled as well as small metastases in the right lobe (arrows). H, heart. The presence of a large metastasis (4 x 5 cm) in the left lobe of the liver and several metastases in the right hepatic lobe (diameter, 1–2 cm) was confirmed by US. C, $^{111}$In image of lower limbs, 72 h after injection of SMAbs labeled with both $^{111}$In and $^{131}$I; three-factor EFA. The metastatic site in the upper third of the right tibia was strongly labeled by $^{111}$In (arrow). The isocontour corresponds to 5% of image maximum activity.

Table 3 Counting data from resected samples

<table>
<thead>
<tr>
<th>Patient</th>
<th>Days after injection</th>
<th>Thyroid tumors</th>
<th>Lymph node tumors</th>
<th>Liver tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NT* Bg Bl</td>
<td>NLN Bg Bl</td>
<td>Lv Bg Bl</td>
</tr>
<tr>
<td>Counting ratios with indium-labeled specific MAbs</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>D 8</td>
<td>1.9 6.7 2.7</td>
<td>ND 10.6 4.2</td>
<td>0.79 29.4 11.6</td>
</tr>
<tr>
<td>2</td>
<td>D 7</td>
<td>12.4 5.5 4.5</td>
<td>ND 4.3 3.5</td>
<td>0.43 13.6 0.97</td>
</tr>
<tr>
<td>3</td>
<td>D 8</td>
<td>2.3 6.7 5.3</td>
<td>0.7 7.4 5.8</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>4</td>
<td>D 7</td>
<td>1.7 2.6 1.8</td>
<td>1.2 2.6 1.9</td>
<td>ND ND ND</td>
</tr>
</tbody>
</table>

Counting ratios with iodine-labeled specific MAbs

| 1       | D 2                  | 0.17 8.6 0.92  | ND 1.7 0.18      | 4.6 4.3 0.47 |
| 2       | D 7                  | 0.21 146.0 44.0| ND 23.5 7.1      | 0.66 3.7 1.1 |
| 3       | D 3                  | 0.47 16.7 4.9  | 30.0 20.3 5.9    | ND ND ND    |
| 4       | D 5                  | 0.27 10.2 15.4 | 2.0 1.5 2.2      | ND ND ND    |

Specificity indices with iodinated specific and control MAbs

| 1       | D 2                  | 1.6 3.1 4.0    | ND 3.3 4.5       | 3.5 3.6 5.2 |
| 2       | D 4                  | 1.6 12.6 22.0  | ND 18.1 30.9     | 0.7 3.8 6.5 |
| 3       | D 3                  | 2.2 5.8 5.8    | 25.0 20.3 19.0   | ND ND ND    |
| 4       | D 5                  | 1.4 3.0 7.7    | 22.2 3.8 9.6     | ND ND ND    |

* NT, normal thyroid; NLN, normal lymph nodes; Lv, normal liver; Bg, background (average of muscle, connective tissue, adipous tissue); Bl, blood; ND, not done.
* According to Pressman et al. (26), see “Materials and Methods.”

the three MAbs. However, CT03-treated slices constantly displayed a slightly more colored background. Finally, the liver metastasis of Patient 2, whose in vivo uptake of $^{131}$I-labeled SMAbs was lower than in other tumor sites of the same patient (Table 3), was also less strongly labeled in vitro by the three antibody fragments.

**DISCUSSION**

Our results demonstrate by both positive imaging in patients and counting of surgical specimens that an anti-CT MAb cocktail specifically concentrates in tumors of MTC patients. The specificity indices observed are high and even unexpected and this deserves to be discussed:

(a) By using a cocktail containing three F(ab')2 fragments against three different epitopes of calcitonin and procalcitonin, we obtained a good contrast, probably by labeling more intensely a higher number of tumor cells. In another unpublished series of three metastatic MTC patients into whom only one $^{125}$I-labeled anti-CT MAb had been injected (CT08: IgG1, recognizing the same epitope as for CT06, with a slightly higher affinity (18), we failed to image any tumor. Three tentative explanations of these discrepant results are possible: firstly, the epitope accessible on the tumor cells is not the 11-17 sequence of calcitonin recognized by the antibodies CT08 and CT06 but another one recognized by CT03 and/or KC01; secondly, CT08, although being very similar to CT06, differs by some properties modifying its in vivo behavior; thirdly, scan positivity with the cocktail could be due to an accumulation of CT-anti-CT immune complexes in extracellular tumoral spaces. Whatever the mechanism, circulating antigens did not prevent MAb uptake by tumors, even when blood CT level was high enough to saturate all the injected MAbs. However, lower specificity indices were obtained in the two cases (1 and 4) with the highest CT circulating pool. This fact, although lacking statistical significance in only four patients, suggests also that larger amounts of MAbs could be injected in cases with high CT levels or that plasma CT lowering by either plasmapheresis or magnesium infusion (28) could be of interest.
(b) F(ab′)2; fragments were prepared to avoid Fc interactions but also to conserve bivalency. Indeed, F(ab′)2; fragments have often a higher affinity than the monovalent Fab and are more suitable for an eventual endocytosis. But the role of endocytosis is only hypothetic since Samaan et al. (15) showed by autoradiography that anti-CT MAbs injected into MTC-xenografted rats were concentrated on the outer surface of tumor cells. It is likely that CT, mainly contained in secretion vesicles, is shortly expressed on the cell membrane during the secretion process. Another explanation of scan positivity could have been the preferential uptake of MAbs by necrotic parts of tumors (5), where cell membrane lysis could account for an improved tracer accessibility to the antigen. This hypothesis was not confirmed by our study since necrotic tumors of Patients 1 and 4 (confirmed by MRI and histology) were labeled either weakly or only at the periphery.

(c) The different uptake distribution of iodine- or indium-labeled MAbs is probably a consequence of the imperfect stability of the isotope-MAb conjugate in vivo, since the nontumoral hot spots correspond for each isotope to the commonly observed pattern of the free tracer. The same problem has been frequently encountered in other models and is partly due to physiological detoxification processes.

The multistop approach of MTC, with EFA processing of scintigraphic data and counting of surgical samples, was undertaken to collect a maximum of information from the few available patients, for both specificity evaluation and isotope comparison. For detection of local recurrences after thyroidectomy or of some distant metastases, two main problems in MTC management, iodine, and particularly 123I, is probably better than 111In, which can lead to artefacts in the liver and the lymph nodes.

In conclusion, immunoscintigraphy of MTC with anti-calcitonin MAbs is an effective method, but its results are closely dependent on the protocol. The use of a cocktail containing complementary antibodies appears to be an important factor in the success of the study. In optimal conditions, the method is very specific and small lesions (0.6–2.5 cm) have been detected. As yet, the comparison is likely to be on tumors larger than 2.5 cm.

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REFERENCES

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