Indium-Labeled Anti-Colorectal Carcinoma Monoclonal Antibody Accumulation in Non-Tumored Tissue in Patients with Colorectal Carcinoma

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Indium-111- (^{111}In) labeled murine monoclonal antibodies ZCE 025 (against carcinoembryonic antigen) and CYT-103 MAb B72.3 (against tumor-associated glycoprotein - 72) have been used to image patients with colorectal cancers with encouraging results. The objectives of this study were to assess the frequency and causes of ^{111}In MAb localization in tumor-free, benign tissues. Thus, scans of 75 patients who have undergone exploratory surgery following radioimmunoscintigraphy with ^{111}In-ZCE 025 (n = 37) or ^{111}In-CYT-103 (n = 38) were reviewed in conjunction with operative and histopathology reports. Localization in non-tumored tissues was seen in 10.8% and 13.1%, respectively, of patients receiving ZCE 025 and CYT-103. The most common sites involved were: degenerative joint disease, abdominal aneurysms, postoperative bowel adhesions, and local inflammatory changes secondary to surgery or external irradiation. Review of patients' medical history and results of concurrent diagnostic modalities is likely to lessen the false-positive rate of ^{111}In-labeled MAb scan interpretation.

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The use of indium-111- (^{111}In) labeled monoclonal antibodies (MAb) to evaluate colorectal carcinoma patients has been steadily increasing over the past decade, due to the relatively low rate of MAb-related adverse reactions, availability of these products on a more regular and sustained basis, and also because of their relative merits in the work-up of patients with primary, recurrent and occult colorectal carcinomas (*1–10*).

In a recently conducted multi-institutional clinical trial, ^{111}In-labeled anti-CEA murine MAb ZCE 025 (HybriCEAker™ Hybritech, Inc., San Diego, CA) detected 80% of lesions in 113 patients with colorectal carcinoma evaluated prior to surgery. In that patient population, ^{111}In-ZCE 025 immunoscintigraphy had a positive predictive value (percent of positive scans which were surgically confirmed) of 94%, and an accuracy of 76% (*10*). Phase I/II clinical trials with anti-TAG-72 (tumored associated glycoprotein) MAb B72.3 labeled site-specifically with ^{111}In (CYT-103, Cytogen Corp., Princeton, NJ) demonstrated similar results, detecting 74% of lesions in 127 patients (*2*). In another 102 presurgical patients with colorectal carcinomas, the sensitivity, specificity, and accuracy of ^{111}In CYT-103 immunoscintigraphy were 70%, 90%, and 72%, respectively (*11*). Drug-related adverse reactions were reported in 4% and 6% of patients who received single intravenous infusions of ZCE 025 (*10*) and CYT-103 (*11*), respectively. In vivo biodistribution, tumor uptake, and physiologic concentration of ZCE 025 and CYT-103 were also similar. Recognition of normal and abnormal scan patterns following their intravenous infusion becomes essential for the radiologist/nuclear medicine specialist interpreting HybriCEAker or CYT-103 scans. Of equal importance is recognizing the potential for false-positive scan interpretations in tumor-free patients.

In this article, we have identified the most common causes of ^{111}In-ZCE 025 and ^{111}In-CYT-103 accumulation in non-tumored sites in a group of colorectal carcinoma patients who had undergone exploratory surgery.

MATERIALS AND METHODS

Scan results of 37 patients who have undergone radioimmunoscintigraphy following anti-CEA MAb ZCE 025 (group 1) (Hybritech Inc., San Diego, CA) and 38 patients with anti-TAG-72 monoclonal antibody CYT-103 (group 2) (Cytogen Corp., Princeton, NJ) labeled with ^{111}In followed by exploratory laparotomy were reviewed. Patients in group 1 received ~ 5.5 mCi (203.5 MBq) of ^{111}In-labeled ZCE 025 MAb at doses of 10 (3 patients), 20 (6 patients) or 40 (28 patients) mg

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intravenously. Characteristically, more than 85% of ^{111}In was protein bound by instant thin-layer chromatography in ammonium methanol solution. Patients in group 2 received 4.7 mCi (173.9 MBq) ^{111}In-labeled CYT-103 MAb at doses of 0.5, 1, 2, or 20 mg. Thin-layer chromatography in 0.9% sodium chloride solution equilibrated with DTPA indicated that 96% of ^{111}In radioactivity was protein bound. The relative immunoreactive fractions on ^{111}In-ZCE 025 and ^{111}In-CYT-103, measured were 57.2%, and 55.9%, respectively, by a solid-phase radioimmunoassay kit (Rhomed, Alburquerque, NM) (*12*). Spot views of the head, chest, abdomen, and pelvis in the anterior and posterior projections were obtained on a large field of view camera (GE 500 Maxicamera, GE 400T Milwaukee, WI or Siemens ZLC, Hoffman Estates, IL) equipped with a medium-energy collimator.

Indium-111-ZCE 025 Immunoscintigraphy. Each view was acquired for 750,000 counts on Days 2–3 postinfusion, and for 7.5 min/view on Days 5–7 (average of 350,000 counts over lower abdomen and pelvis). SPECT of the liver was performed at 2–3 days, while SPECT of the abdomen/pelvis was performed on Days 5–7. Data were acquired in digital format in a 256 × 256 matrix.

Indium-111-CYT-103 Immunoscintigraphy. Counts per view (10^6) were obtained during the first imaging session (Days 2–3). During the second imaging session (Days 4–7), acquisition was completed when 1,000,000 counts/view or 10 min were reached (average of 375,000 counts over lower abdomen/pelvis). Data were acquired in a 128 × 128 matrix. SPECT imaging included the liver (Days 2–3) and lower abdomen/pelvis (Days 4–7). Tomographic images were reconstructed using a filtered backprojection algorithm.

A numerical rating method, based on ROC curve analysis (*13*) was used to interpret ^{111}In-MAb scans. The five categories used were as follows: 1 = definitely or almost definitely negative, 2 = probably negative, 3 = possibly positive, 4 = probably positive, and 5 = definitely or almost definitely positive. Then, numerical rating values assigned to each abnormality on MAb scanning were compared to surgical and histopathologic results and with results of concurrent radiographic tests.

RESULTS

Early images following ^{111}In-ZCE 025 and ^{111}In-CYT-103 showed moderate cardiac blood-pool radioactivity, which cleared by 72 hr postinjection. Radioactivity in normal liver/spleen, bone marrow tissues and gonads was seen in all patients, and did not decrease with time. Nontumor uptake of ^{111}In-ZCE 025 and ^{111}In-CYT-103 were similar. In the early (48–72 hr) images, radioactivity localization in the urinary bladder was seen in 7 of 38 patients (18%) who received ^{111}In-CYT-103 and in 6 of 37 patients (16.2%) who received ^{111}In-ZCE 025. A relationship between the presence of

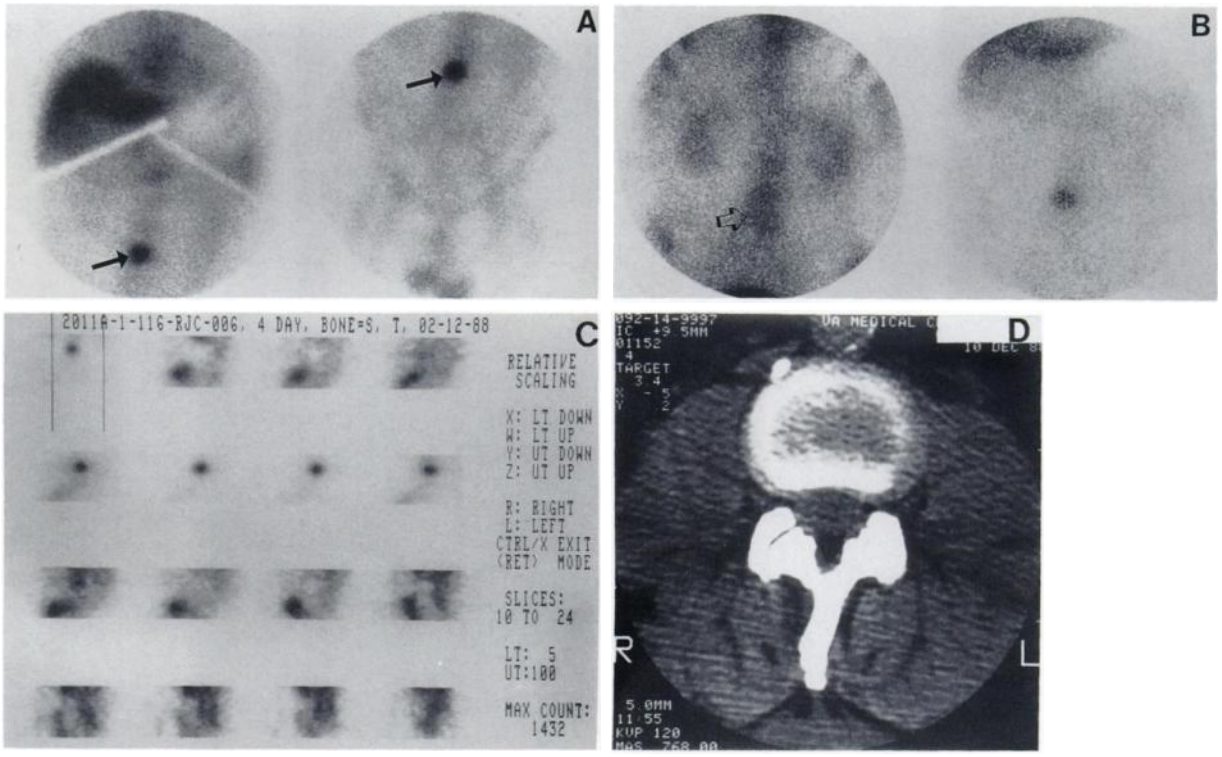


FIGURE 1
(A) Anterior planar views of Patient 3, 96 hr postinfusion of ^{111}In-labeled MAb, showing a focus of intense radiolocalization at the level of the 3rd and 4th lumbar vertebrae (arrow). (B) Technetium-99m-MDP bone scan (left hand panel) and ^{111}In-labeled MAb scan (right hand panel) obtained during the same imaging session. An area of low grade radioactivity overlying the L_3-L_4 region is seen on the bone scan (open arrow), which is disproportionately less intense than the MAb scan. (C) Indium-111-MAb SPECT in the sagittal projections. The focus of increased MAb uptake appears to be confined to the lumbar spine. (D) CT of lumbo-sacral-spine studies showing moderate degenerative hypertrophic changes in the facet joint between L_3-L_4.

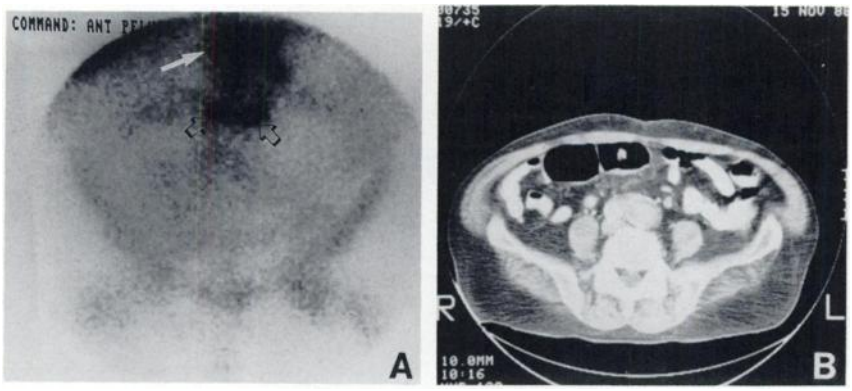


FIGURE 2
(A) Anterior view of the pelvis of patient 96 hr postinfusion of ^{111}In-MAb. Radiolocalizations in the midline (straight arrow) and, in paravertebral regions (open arrows) are demonstrated. Uptake was considered abnormal, and not inconsistent with lymph node metastases from the patient's rectal carcinoma. (B) CT of the pelvis with contrast medium, showing aneurysmal dilation of the left common iliac artery with thrombus formation.

bladder radioactivity and dose of MAb injected was apparent, although this could not be statistically significant. In patients studied with ^{111}In-CYT-103, bladder radioactivity was seen in 1/7, 1/7, 2/7, and 3/7 patients who received 0.5, 1.0, 2.0 and 20.0 mg CYT-103, respectively. Similarly, in five of six patients who received 20.0 mg ^{111}In-ZCE 025, bladder radioactivity was demonstrated. During the second imaging session, bladder radioactivity was absent in all but one patient, who was found to have acute cystitis.

Positive MAb accumulation in histopathologic tumor-free tissues were seen in 5 of 38 patients (13.1%) who received ^{111}In-CYT-103 independent of MAb dose, and in 4 of 37 patients (10.8%) who received ^{111}In-ZCE 025. Degenerative changes of the lumbar vertebrae (Fig. 1) aneurysms of the abdominal aorta (Fig. 2), and adhesions between adjacent loops of the bowel were frequently the sites of false-positive MAb accumulation. The anatomic distribution of these areas and the methods of confirmation are listed in Table 1.

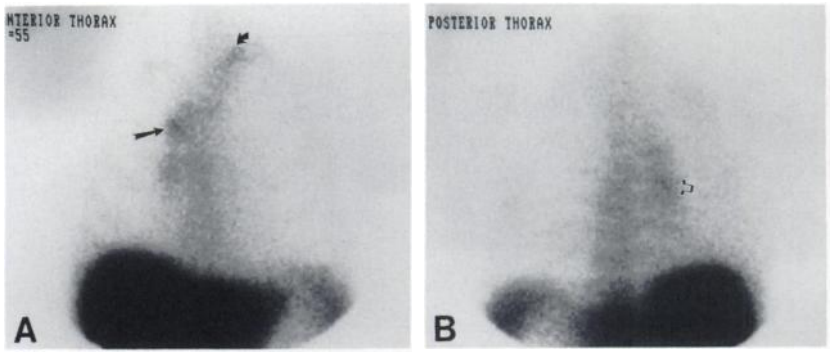
Radiolabeled MAb accumulation in the superior vena cava was seen in one patient who had a right central venous catheter (Fig. 3). In another patient, radioactivity accumulation along a surgical incision in the abdomen was noted seven days post-surgery.

Prominent bowel radioactivity was observed in several patients at various times. Bowel radioactivity usually decreased or disappeared following administration of evacuants prior to the next imaging session. In one patient, there was no change in bowel radioactivity following the administration of bowel evacuants and visualization of the patient's primary tumor in the rectosigmoid region was not possible. In four patients, the liver exhibited multiple focal areas of diminished or absent radioactivity which were caused by prominent rib indentations. In another four patients, prominent accumulation of radioactivity at the colostomy site and in colostomy bags were identified.

TABLE 1
Indium-111-Labeled MAb Localization in Non-Tumored Tissue

Patient No.	^{111}In-MAb results	Site of MAb uptake	Confirmation
1	Abdominal uptake, periaortic region	Abdominal aneurysm	CT, no tumor found intraoperatively
2	Abdominal uptake, periaortic region	Degenerative spondylolysis	CT, no tumor found intraoperatively
3	Single focus of uptake at L_3-L_4 vertebrae	Degenerative changes in facet joint of L_3	CT, no tumor found intraoperatively
4	Abdominal uptake, periaortic region	Abdominal aneurysm	CT
5	Abdominal uptake, right lower quadrant	Adhesions between loops of bowel	Surgery
6	Abdominal uptake in right paraumbilical region	Herniation of small bowel through anterior abdominal wall	Surgery
7	Pelvic uptake	Fibrocollagenous tissue with inflammatory changes, post-irradiation	Surgery
8	Single focus, left paravertebral region	Degenerative spondylolysis of L_3	Surgery
9	Diffuse uptake in urinary bladder	Acute cystitis	Cystoscopy and culture

FIGURE 3
Anterior (A) and posterior (B) views of the chest of Patient 23, 5 days post-^{111}In-MAb infusion. Radiolocalization in the right parasternal area corresponding to the site of previous right central venous catheter is seen (arrows). Inferiorly, another focus of radioactivity corresponding to the inferior aspect of the superior vena cava is also seen (open arrows). Uptake in the left supraclavicular areas (curved arrow) corresponds to the left subclavian venous catheter port.



DISCUSSION

In this report, we described the commonest causes of ^{111}In-labeled MAb accumulation in non-tumored lesions in a group of presurgical colorectal carcinoma patients. Focal and intense MAb localization in degenerative vertebral joint disease were seen in three patients and were indistinguishable from isolated bone or lymph node metastasis. Indium-111-labeled MAb localization in abdominal aneurysms was usually less intense and linear in shape, extending vertically along the vertebral column, thus mimicking the uptake seen in paravertebral lymph node metastases. In two of the three patients with degenerative joint disease, and in the two patients with aneurysmal dilation of the abdominal aorta, concurrent computer tomography scans demonstrated these abnormalities and provided an explanation regarding these false-positive MAb areas.

The causes of ^{111}In-labeled MAb accumulation in degenerative joint disease, aneurysms, post-irradiation changes or other inflammatory conditions such as cystitis are not known, but could represent nonspecific accumulation of immunoglobulin at these sites. The recent report by Rubin et al. (*14*), using radiolabeled, nonspecific polyclonal immunoglobulin in the detection of focal inflammation offers some insight into the possible causes of ^{111}In-labeled tumor specific MAbs at sites other than tumors. The mechanism of accumulation could be due to (a) leakage of antibodies into tissues; (b) increased vascular permeability of the capillary bed at the site of inflammation; and (c) retention of the immunoglobulins at the site of inflammation via Fc receptors on inflammatory cells (*14*). Indium-111-MAb localization confined to a vessel following central venous line placement or at the site of recent surgical incision could be due to the same mechanisms.

Radioactivity accumulation in the urinary bladder usually seen within the first 24–48 hr postinjection could be due to the presence of free ^{111}In, ^{111}In-DTPA or a low molecular weight metabolite of the ^{111}In-labeled MAb. If persistent, repeat imagings following bladder evacuation will help distinguish bladder activity from tumor. Alternatively, single-photon emission computed tomography of the pelvis could be used to differentiate bladder activity from that in sigmoid or rectal tumors which are more posteriorly located.

The presence of ^{111}In activity in the bowel showed marked differences between individual patients and could represent either metabolism of the ^{111}In-labeled conjugate through the bile or interaction between the radiolabeled MAb and antigen in normal colonic tissue. We have recently demonstrated the presence of CEA and TAG-72 in normal colonic tissue (*4,6*). Persistent radiolabeled MAb accumulation in the bowel secondary to adhesions or in herniation through the anterior abdominal wall in patients with previous colorectal surgeries will be difficult to distinguish from localized bowel tumors.

In conclusion, we have identified several benign conditions which could lead to false-positive interpretations of ^{111}In-labeled anti-CEA (ZCE 025) and anti-TAG-72 (CYT 103) MAbs in sites not involved by tumors in colorectal carcinoma patients. Our study showed false-positive ratios of 10.8% (ZCE 025) and 13.1% (CYT 103), which were associated with the presence of degenerative joint disease, abdominal aneurysms, bowel adhesions and local inflammatory changes secondary to surgery or irradiation. Critical review of the patient's history concerning concomitant medical conditions, as well as reviewing results of other diagnostic X-ray modalities will decrease the occurrence of false-positive ^{111}In-MAb image interpretations in colorectal carcinoma patients. Reader familiarization with the biodistribution spectrum for each antibody within each patient's state of disease (work-up for primary or suspected recurrences) is also likely to lessen the potential for false-positive scan interpretation.

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