Localization of $^{131}$I-labeled Antibodies in Human Renal Cell Carcinomas and in a Mouse Hepatoma and Correlation with Tumor Detection by Photoscanning


Abstract

Xenogeneic anti-human renal cell carcinoma globulins (ARCG’s), which had been rendered tumor specific by absorptions with normal human tissues, reacted on immunofluorescence with smears and sections of 21 of 22 human renal cell carcinomas tested, but not with any adult, normal human tissues, including kidney. The i.v. administration of $^{131}$I-labeled ARCG followed by photoscanning visualized 13 of 15 primary renal cell carcinomas and metastases in 7 of 8 patients. Immunofluorescence and autoradiography of sections and dissociated cells revealed more ARCG and radioactivity bound to tumor tissue than to adjacent normal tissue. However, assay of tissue digests showed more radioactivity in primary carcinoma than in adjacent renal tissue in five patients, similar amounts in both areas in two patients, and less radioactivity in tumor in another five cases. Correlation of scanning with the extent of tumor localization of $^{131}$I showed that all tumors which contained equal or greater radioactivity than did normal kidney, and three of five containing less radioactivity, were successfully imaged.

The s.c. transplants of the mouse H6 hepatoma were visualized by $^{131}$I-labeled anti-tumor globulin prepared by a method identical to that for ARCG. If anti-tumor globulin was further purified, a 2-fold increase in the tumor:blood ratio of radioactivity was obtained, but only minor improvement in the quality of scans. $^{131}$I-labeled normal rabbit globulin failed to visualize tumors, and $^{67}$Ga citrate scanning could not discriminate between hepatoma transplants and Freund’s complete adjuvant-induced granulomas.

The tumor specificity of $^{131}$I-labeled anti-tumor globulins as imaging agents is supported by: (a) the failure of normal rabbit globulin and $^{99m}$Tc-sulfur colloid to image the hepatoma and renal carcinoma, respectively; (b) the immunofluorescence and autoradiography evidence that i.v.-administered $^{131}$I-ARCG localized in and bound firmly to tumor tissue, but not to adjacent renal tissue; and (c) the fact that the tumor which did not bind $^{131}$I-ARCG could not be visualized. It appears that the success of $^{131}$I-labeled anti-tumor globulins as imaging agents is associated with their specific tumor localization but can be aided by such nonspecific factors as increased and more permeable tumor vasculature.

Introduction

Most of the currently available radiopharmaceuticals used for tumor imaging lack specificity, and the success of such imaging depends upon nonspecific secondary changes in neoplasms, i.e., altered microvasculature and nonspecific trapping of labels by tumor cells (8, 15). Following the observation of Pressman and Keighley (16), that labeled antibodies did in fact localize in a target tissue in vivo, several groups have now successfully used radioiodine-labeled antibodies for imaging human tumors (3, 10). We have recently reported successful imaging of primary renal cell carcinomas and their metastases (1, 2) using a xenogeneic antitumor globulin which reacted on immunofluorescence assay with renal carcinoma cells from most of the patients examined but not with any normal human tissues, including adult and fetal human kidney (5). We report here on the detection of primary and metastatic renal cell carcinoma by external photoscanning in patients given $^{131}$I-labeled ARCG. Photoscanning results have been compared with the results of assay of radioactivity in samples of tumor, adjacent renal tissue, and serum.

Anti-tumor globulin preparations used for imaging in clinical studies have varied in purity. Therefore, we have compared a specific rabbit anti-mouse H6 hepatoma globulin before and after affinity purification with respect to tumor localization in hepatoma-bearing mice. The effectiveness of $^{131}$I-labeled anti-tumor globulin for radionuclide imaging of this tumor has also been compared with that of $^{67}$Ga citrate which at present is the most widely used tumor-localizing radiopharmaceutical (14).

Materials and Methods

Patients. Patients with suspected metastatic lesions were admitted into this study on the basis of history of previous nephrectomy for histologically confirmed renal cell carcinoma. Those with primary renal cell carcinoma were undergoing preparation for nephrectomy for a unilateral lesion confirmed by pyelography and angiography. Prior to $^{131}$I-anti-tumor globulin scanning, all patients were also scanned with $^{99m}$Tc-sulfur colloid. The details of the procedure, the hazards of administration of radioactivity and foreign proteins, and the possible benefit of detection of metastatic lesions were explained to all patients. They were entered only after they gave their valid consent. Female patients of reproductive age were admitted only after establishing that they were not pregnant at the time.

The abbreviations used are: ARCG, anti-renal carcinoma globulin; AHG, anti-hepatoma globulin; AHG-P, affinity-purified anti-hepatoma globulin; NRG, normal rabbit globulin.
of investigation. Patients were tested prior to entry for hypersensitivity to goat or to rabbit immunoglobulins.

Mice. Inbred A/J mice (12- to 16-week-old females) were obtained from The Jackson Laboratory (Bar Harbor, Maine).

Tumors. Surgically excised specimens of human renal cell carcinoma were received in sterile Hanks' balanced salt solution (4°) from patients after the administration of 131I-labeled ARG. The size and weight of both the uninvolved kidney and the tumor and the extent of necrotic and hemorrhagic changes were noted. Whenever available, representative aliquots were obtained from perirenal fibrofatty tissue, normal kidney tissue, and primary or metastatic nonnecrotic tumor, as well as from necrotic and gross hemorrhagic areas of tumors. Blocks of tissue were fixed in 10% buffered formalin for histological examination.

The H6 hepatoma arose spontaneously in the A/J mouse at The Jackson Laboratory, which was the source of our tumor, and is maintained by serial s.c. transplantation in syngeneic mice.

Suspensions of cells from tumor and adjacent normal kidney tissue were prepared by mechanical dispersion in EDTA-containing Ca- and Mg-free Hanks' balanced salt solution. Viable cells were separated on a Ficoll-Hypaque gradient (13). The cells and 5-µm cryostat sections of tissues were then washed in 3 changes of phosphate-buffered saline (0.01 M sodium phosphate, pH 7.1, containing 0.145 M sodium chloride) at 4° before immunofluorescence or autoradiography.

Production and Specificity of Antitumor Sera. The detailed procedure for the production of anti-human renal cell carcinoma serum and the establishment of its specificity have been reported recently (5). In brief, 5 pairs of rabbits and 2 goats were given 2 i.m. injections of viable human renal carcinoma cells (4 x 10^8 cells/animal/injection) mixed with Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.) and then were given i.m. injections of tumor homogenate, twice a week for 5 weeks. Each pair of rabbits and each goat received tumor tissue from one patient. The immune sera were heat inactivated and then were absorbed repeatedly, first with Group AB, Rh+ RBC and then with homogenates of normal human liver, lung, heart, spleen, and kidneys. The sera were regarded as tumor specific when they would react with the immunizing tumor cells but not with other normal adult human tissues including the patients' peripheral blood leukocytes and cultures of fibroblasts derived from the patients' perirenal connective tissue. Immune sera pooled from the 2 goats and corresponding sera pooled from a pair of rabbits were used in this study. After being rendered tumor specific by the absorptions, both the goat and rabbit immune sera reacted with smears and sections of 21 of the 22 human renal cell carcinomas tested and of all 5 culture lines of human renal carcinoma cells maintained in our laboratory. These tumor-specific sera reacted neither with human fetal kidney nor with a number of different histological types of human malignant tumors other than renal cell carcinomas.

Our method of production of xenogeneic sera against the mouse H6 hepatoma was virtually identical with the method for the production of anti-renal carcinoma sera. In brief, each rabbit was given an i.m. injection of a total of 10^8 trypa blue-impermeable tumor cells mixed with Freund's complete adjuvant. A second set of these injections (including adjuvant) was given on Day 7. Starting on Day 14, 10^8 cells (without adjuvant) per animal were injected twice a week for 5 additional weeks. Ten days after the last injection, 5 ml of blood were obtained from each rabbit. Animals were bled terminally when they showed titers ≥1/64 of specific anti-hepatoma activity by immunofluorescence after appropriate absorptions as described below. The resulting sera were inactivated at 56° for 30 min and were absorbed repeatedly with washed homogenates of lung, kidney, heart, spleen, and liver from normal adult A/J mice until the serum reacted with sections and suspensions of H6 cells but neither with cryostat sections of normal adult A/J mouse tissues (including liver) nor with cryostat sections and suspensions of several other mouse tumors, e.g., the C1300 neuroblastoma of A/J mice, the B16 melanoma and EL4 lymphoma of the C57BL/6J mice, several spontaneous thymic lymphomas of the AKR mice, and transplant passages of a spontaneous renal adenocarcinoma of the BALB/c mice. Globulin fractions were obtained from the anti-hepatoma serum and normal rabbit sera by fractionation with 33% saturated ammonium sulfate. Anti-hepatoma activity was contained mainly in the 7S fraction.

Scanning Agents. Globulin fractions from the anti-renal carcinoma serum or the antithepatoma serum were obtained by fractionation with 33% saturated ammonium sulfate. An aliquot of AHG was further purified (AHG-P) by adsorption and elution from formalinized H6 cells following a batch method (9). Varying quantities of carrier- and reductant-free 131I and 125I (New England Nuclear, Boston, Mass.) were bound to globulins by the chloramine-T method (7). Reactivity of radiolabeled anti-tumor globulins with appropriate tumor cells was confirmed by immunofluorescence as well as by autoradiography using Eastman-Kodak NTB3 emulsion. 67Ga citrate was purchased from Mallinkrodt Chemical Works, Tuxedo, N. Y.

Preparation for Scanning. To prevent uptake of dissociated 131I by the thyroid, all patients and mice were given Lugol's iodine in drinking water starting at least 3 days before administering imaging agents. Patients were given i.v. injections of various amounts of 131I and the carrier ARCG (as specified in Tables 1 and 2) in 50 ml of 0.9% NaCl solution over a period of approximately 30 min. All the patients except M. W. and C. F. received ARCG derived from the pooled goat sera or from a single serum (P. C.).

Groups of 4 adult A/J mice were given injections of 2 x 10^7 H6 cells/mouse and 0.5 ml of Freund's complete adjuvant into the right and left flanks, respectively. Twelve days later, each mouse received, through the tail vein, 70 µCi of 131I bound to approximately 40 µg of NRG, AHG, or AHG-P. Each member of a fourth group of hepatoma- and adjuvant-injected mice were given 100 µCi of 67Ga citrate i.v.

Immunofluorescence Assay. Cytoplasmic immunofluorescence was performed either on 5-µm cryostat sections of snap-frozen (~176°) tumors and other human and mouse tissues, or on smears of acetone-fixed tumor cells. The sandwich method with fluorescein-labeled goat anti-rabbit or rabbit anti-goat globulins was used with appropriate controls (5). Membrane immunofluorescence was performed on viable tumor cells with the appropriate conjugate and normal serum controls described by us (5). Autologous peripheral blood lymphocytes and fibroblasts served as additional controls to confirm the specificity of the anti-renal carcinoma sera.

Scanning and Assay of Radioactivity. Patients and mice were scanned with a Nuclear Chicago Pho-Dot rectilinear scan-
ner or an Anger-type scintillation camera (1, 2) at 2 and 6 hr after the injection of imaging agents and then every 24 hr. At 120 hr, 0.5 ml blood per mouse was obtained under anesthesia by cardiac puncture from all mice given injections of $^{131}$I-labeled globulin preparations. Then the axillary vessels were cut, blood was flushed out of organs by injecting 50 ml of warm phosphate-buffered saline into the heart, and internal organs were removed. Patients emptied their bladder just before the injection of $^{131}$I-ARCG, after which samples were taken from each 24-hr collection of urine. Whenever possible, samples of blood were obtained immediately after the injection and every 12 hr thereafter. For assay of radioactivity, aliquots of tissue were washed in phosphate-buffered saline, blotted dry, weighed, and individually digested in concentrated sulfuric acid. $^{131}$I activity was determined in duplicate aliquots using a well-type gamma counter (Packard Instrument Co., Downers Grove, III.). Whenever adequate tissue samples were available, radioactivity was assayed using at least 2 different dilutions of each tissue digest. Appropriate windows in analysis of tissues from the patients who had received $^{131}$I-labeled ARCG and $^{125}$I-labeled NRG were used for separate assay of $^{131}$I and $^{125}$I activities (18). Eastman Kodak NTB3 emulsion was used for autoradiography.

Results

Patients with Histologically Proven Metastatic Renal Cell Carcinoma. The age and sex of the patients, the site and size of the metastases, the amount of ARCG and $^{131}$I injected, and the earliest evidence of tumor localization by scanning are presented in Table 1. The first 8 patients listed had metastases established by radiological and histological examinations. Diagnoses were further confirmed by $^{99m}$Tc diphosphonate scans in patients with skeletal lesions (Fig. 1). The lesions could be localized by external scanning in 7 of these 8 patients. The ARCG given to Patient P. C. was obtained by immunizing a goat with the patient’s primary tumor only. $^{131}$I localized in Patient P. C.’s metastasis as early as 2 hr after the administration of $^{131}$I-ARCG (Fig. 2). There was also considerable radioactivity in his liver and spleen which decreased appreciably at 48 hr, but none was detectable in a Bacillus Calmette-Guérin-induced granuloma in the thigh. The amount of $^{131}$I activity in the metastasis appeared to persist undiminished at least for 96 hr, so that scans obtained at 48 hr or thereafter revealed better definition of this metastasis than did the earlier scans. This patient was given $^{131}$I-ARCG and scanned several times subsequently to monitor the lesion during chemotherapy, and it was successfully localized each time. The pattern of localization in the liver and spleen at various times after the administration of $^{131}$I-ARCG remained unchanged.

The second patient, V. C., was scanned twice. The first scan showed diffuse $^{131}$I activity over the abdomen. Subsequent laparotomy revealed multiple small metastatic deposits of renal carcinoma (histologically confirmed) disseminated throughout the peritoneal cavity. A second scan using the identical ARCG preparation revealed localization in the abdomen at 2 hr and in the mediastinum and the lungs at 24 hr. The patient had radiologically confirmed pulmonary and mediastinal metastases at this stage.

Patient H. O. had a primary tumor in the right kidney (silent lesion) and a large metastasis involving the sternum and anterior mediastinum at the time of scanning (Fig. 1). Both the primary and metastatic lesions were visualized by the $^{131}$I-ARCG scan. The primary lesions of Patients F. H. and H. W. were visualized by scans prior to nephrectomy, and metastases were identified in scans 4 and 4.5 months, respectively, after removal of the kidney.

Patient J. J.’s scalp lesions showed localization of $^{131}$I-ARCG; however, there was no localization in 2 suspected metastatic lesions in the lungs revealed by skiaigraphy of the chest. Review of this patient’s lung skiagrams prior to the development of the renal carcinoma indicated that these were tuberculous granulomas.

Tumor tissue (but no tumor-free tissue adjacent to the lesions) was available from 6 of these 8 patients subsequent to the administration of $^{131}$I-ARCG. Immunofluorescence and autoradiography revealed tumor cell-bound goat globulin and radioactivity in all the tumor tissues. Tumor tissue and blood obtained at the time of excision were available for assaying radioactivity from 3 patients. The serum:tumor ratio of radioactivity in Patient J. J. at 120 hr after the administration of $^{131}$I-ARCG was 6.7, while in Patients E. V. and H. W. the ratios were 7.6 and 16, respectively, at 72 hr. Patient H. W.’s metastasis could not be localized by external scanning in spite of its s.c. location. The lesion consisted mostly of liquefied necrotic tissue. The nature of the lesions in the last 2 patients in Table 1 was not histologically confirmed.

Patient F. S. underwent nephrectomy for a renal cell carcinoma of the left kidney in May 1973. In January 1975, a metastasis in the vagina was excised. $^{131}$I-ARCG scanning in May 1975 showed suspicious localization in the mediastinum, especially on the left side. However, no abnormality could be detected by skiaography and tomography of the chest. The patient was put on a schedule of active immunotherapy using injections of Vibrio comma neumaminidase-treated autologous tumor cells and Bacillus Calmette-Guérin. Follow-up X-ray examination revealed a metastatic lesion in the left hilar area early in 1978.

Patient E. B. had pain in the left loin in November 1976. An i.v. pyelogram revealed a mass in the left kidney suggestive of a renal neoplasm. Angiography suggested a renal carcinoma without invasion of the renal vein. There was no X-ray evidence of metastasis but a $^{99m}$Tc diphosphonate scan revealed an increase in the sixth rib and in T11, L4, and L5 vertebrae. $^{131}$I-ARCG scanning did not show any preferential localization in the kidney tumor, ribs, or vertebrae. No nephrectomy was performed in view of the patient’s advanced age, but this individual is surviving to date without progression of any of the lesions.

Patients with Primary Renal Cell Carcinoma. Descriptions of these patients, the tumors, the scans, and the amount of $^{131}$I activity assayed in various tissues are presented in Table 2. Fifteen individuals were scanned with $^{131}$I-ARCG, and lesions could be localized in 13 of them. Adequate tumor and adjacent normal renal tissue were available for assay from 12 patients. Immunofluorescence and autoradiography revealed tumor cell-bound ARCG and $^{131}$I activity (Figs. 3 to 5) in the tumor tissue from all of them except Patient D. C. In all cases, comparatively little ARCG or radioactivity could be seen in the washed sections and in the suspensions of normal kidney tissue (Figs. 5 and 6). In 5 of the 12 patients (G. H., J. S., B. V., M. W., and B. H.) whose tumors and normal renal tissues could be assayed
for $^{131}$I activity, tumor tissue contained higher radioactivity levels than did the adjacent normal tissue. These kidneys were excised at 24 hr (B. V., M. W.), 48 hr (J. S., B. H.), and 144 hr (G. H.) after the injection of $^{131}$I-ARCG. There was no difference in $^{131}$I activity between the normal and the neoplastic tissues in 2 cases (F. H. and H. O.); in the remaining 5 patients, the activity was lower in tumor tissue than in the normal kidney. The tumors could not be localized by external scanning in 2 of

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Tumor site</th>
<th>Tumor size (cm)</th>
<th>ARCG (mg)</th>
<th>$^{131}$I (mCi)</th>
<th>Time (hr) between injection and positive scan</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. C.</td>
<td>40</td>
<td>M</td>
<td>Right pedicle, 5th lumbar vertebra.</td>
<td>5 x 3 x 3</td>
<td>100</td>
<td>4.2</td>
<td>2</td>
<td>ARCG obtained from a goat immunized with patients' primary tumor.</td>
</tr>
<tr>
<td>V. C.</td>
<td>57</td>
<td>M</td>
<td>1st: disseminated in peritoneum. 2nd: also in the mediastinum and lungs.</td>
<td>Innumerable small metastases observed at laparotomy.</td>
<td>1st: 100 2nd: 100</td>
<td>1st: 2 2nd: 4.0</td>
<td>1st: 2 2nd: abdomen after 2 hr. Mediastinum and lungs after 24 hr.</td>
<td>1st and 2nd scans 3 months apart.</td>
</tr>
<tr>
<td>B. D.</td>
<td>55</td>
<td>F</td>
<td>Anterior mediastinum.</td>
<td>2 x 2 x 2</td>
<td>100</td>
<td>3.5</td>
<td>6</td>
<td>No tissue available after scan.</td>
</tr>
<tr>
<td>F. H.</td>
<td>45</td>
<td>M</td>
<td>Sternum and both lungs.</td>
<td>2 x 2 x 1 (sternum)</td>
<td>50</td>
<td>4.6</td>
<td>24</td>
<td>No tissue available after scan. Primary tumor visualized by $^{131}$I-ARCG prior to nephrectomy, 4 mos. before this scan.</td>
</tr>
<tr>
<td>H. O.</td>
<td>56</td>
<td>M</td>
<td>Sternum and anterior mediastinum.</td>
<td>10 x 8 x 2</td>
<td>50</td>
<td>2.7</td>
<td>24</td>
<td>A primary renal carcinoma visualized by this $^{131}$I-ARCG scan.</td>
</tr>
<tr>
<td>J. J.</td>
<td>79</td>
<td>M</td>
<td>Scalp.</td>
<td>2 x 2 x 2; 2.5 x 2 x 2; 3 x 2.5 x 2</td>
<td>100</td>
<td>4.0</td>
<td>6 (scalp)</td>
<td>Review of lung X-ray pictures prior to development of renal carcinoma revealed lung lesions to be old tubercular granulomas. Radioactivity in tumor tissue excised 120 hr after injection = 56.77 x $10^{-9}$ Ci/g. Radioactivity in serum at time of excision = 380.4 x $10^{-9}$ Ci/ml.</td>
</tr>
<tr>
<td>E. V.</td>
<td>35</td>
<td>F</td>
<td>Right tibia.</td>
<td>5 x 3 x 1</td>
<td>50</td>
<td>2.7</td>
<td>6</td>
<td>Radioactivity in tumor tissue excised 72 hr after injection = 7.51 x $10^{-9}$ Ci/g and in serum = 57.95 x $10^{-9}$ Ci/ml.</td>
</tr>
<tr>
<td>H. W.</td>
<td>59</td>
<td>M</td>
<td>Neck (s.c.)</td>
<td>3 x 3 x 2</td>
<td>100</td>
<td>4.6</td>
<td>No localization up to 72 hr.</td>
<td>Radioactivity in tumor excised 72 hr after injection = 18.36 x $10^{-9}$ Ci/g and in serum = 294.2 x $10^{-9}$ Ci/ml. Primary tumor visualized 4.5 mos. earlier using $^{131}$I-ARCG.</td>
</tr>
<tr>
<td>F. S.</td>
<td>52</td>
<td>F</td>
<td>No clinically detectable metastasis.</td>
<td></td>
<td>10</td>
<td>1.0</td>
<td>Localization in the mediastinum and the left hilar area at 24 hr.</td>
<td>Scan positive on May 5, 1975. Patient on active immunotherapy with V. comma neuraminidase-treated autologous tumor cells and B. Calmette-Guérin. Lesions detected in left hilar area in October 1978; findings: left hilar mass, left hilar nodes enlarged, one focus mid-zone in left lung.</td>
</tr>
<tr>
<td>E. B.</td>
<td>82</td>
<td>M</td>
<td>$^{99m}$Tc scan shows 6th rib, vertebrae; thoracic 11, lumbar, 4 and 5 (?metastasis). X-ray negative.</td>
<td>Suspected primary tumor replacing most of left kidney.</td>
<td>100</td>
<td>4.0</td>
<td>No localization either in kidney or suspected metastases until 120 hr.</td>
<td>Angiography and i.v. pyelography revealed large renal carcinoma without vascular invasion. Patient refused surgery and shows no progression of disease for last 2 yr.</td>
</tr>
</tbody>
</table>

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### Table 2
Description of tumors and scanning of patients with primary renal cell carcinoma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Symptoms and signs&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tumor site</th>
<th>Tumor size</th>
<th>Extent of necrosis and hemorrhage&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Time (hr) scan is positive</th>
<th>Time (hr) between injection and excision</th>
<th>ARCG (mg)</th>
<th>Radioactivity in specimens (x 10&lt;sup&gt;4&lt;/sup&gt; Ci/g or ml)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. M.</td>
<td>66</td>
<td>F</td>
<td>Pain in left flank.</td>
<td>Left lower pole.</td>
<td>9 x 7 x 4 cm</td>
<td>+</td>
<td>24</td>
<td>72</td>
<td>100</td>
<td>4.0</td>
<td>Not counted.</td>
</tr>
<tr>
<td>B. D.</td>
<td>49</td>
<td>M</td>
<td>Pain in right flank.</td>
<td>Right middle.</td>
<td>7 cm</td>
<td>0</td>
<td>24</td>
<td>72</td>
<td>100</td>
<td>4.0</td>
<td>Not counted.</td>
</tr>
<tr>
<td>P. G.</td>
<td>45</td>
<td>M</td>
<td>Hematuria and colic.</td>
<td>Left middle.</td>
<td>5 cm (intrarenal, i.e. not bulging)</td>
<td>+</td>
<td>1st scan: 24</td>
<td>1st scan: 10</td>
<td>1st scan: 1.5</td>
<td>Tr&lt;sup&gt;c&lt;/sup&gt; (n = 1)&lt;sup&gt;f&lt;/sup&gt; = 8.86; NK not counted.</td>
<td>Interval between scans was 5 wk.</td>
</tr>
<tr>
<td>G. H.</td>
<td>54</td>
<td>M</td>
<td>Painless hematuria.</td>
<td>Right lower 1/3.</td>
<td>7 x 6 x 4.2 cm</td>
<td>+</td>
<td>24</td>
<td>144</td>
<td>100</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>B. V.</td>
<td>46</td>
<td>M</td>
<td>Pain in right flank.</td>
<td>Right upper 1/3.</td>
<td>6 cm</td>
<td>+</td>
<td>24</td>
<td>24</td>
<td>100</td>
<td>4.2</td>
<td>Tr (n = 1) = 107.61; NK (n = 1) = 84.15; necrotic Tr (n = 1) = 72.5.</td>
</tr>
<tr>
<td>M. W.</td>
<td>75</td>
<td>F</td>
<td>Renal colic.</td>
<td>Left middle 25% cystic.</td>
<td>7 x 4 x 4 cm</td>
<td>+</td>
<td>6</td>
<td>24</td>
<td>50</td>
<td>4.3</td>
<td>Tr (n = 1) = 181.62; NK (n = 2) = 77.55; necrotic Tr with hemorrhage (n = 2) = 47.15.</td>
</tr>
<tr>
<td>B. H.</td>
<td>32</td>
<td>F</td>
<td>Pain in left flank and hematuria.</td>
<td>Left lower pole.</td>
<td>6 x 5 x 2 cm Cystic tumor (65 ml fluid)</td>
<td>+</td>
<td>6</td>
<td>48</td>
<td>100</td>
<td>3.2</td>
<td>Solid Tr (n = 1) = 23.16; Tr cystic fluid (n = 2) = 19.42; NK (n = 1) = 10.75.</td>
</tr>
<tr>
<td>J. S.</td>
<td>60</td>
<td>F</td>
<td>Hematuria.</td>
<td>Left midzone.</td>
<td>10 x 9 x 6 cm 1/3 cystic</td>
<td>+</td>
<td>24</td>
<td>48</td>
<td>100</td>
<td>3.0</td>
<td>Tr (n = 8) = 94.18 ± 10.03; NK (n = 4) = 24.59 ± 4.79; serum (n = 4) = 436.4 ± 18.86.</td>
</tr>
<tr>
<td>F. H.</td>
<td>45</td>
<td>M</td>
<td>Fatigue and malaise.</td>
<td>Left lower pole.</td>
<td>5 x 3.5 x 3 cm 75% of kidney replaced.</td>
<td>+</td>
<td>24</td>
<td>96</td>
<td>50</td>
<td>4.6</td>
<td>Clot (n = 2) = 39.98 ± 2.09; Tr (n = 4) = 10.13 ± 2.39; NK (n = 2) = 12.95 ± 0.26; PFF (n = 2) = 3.94 ± 0.26; serum (n = 4) = 45.8 ± 0.39.</td>
</tr>
<tr>
<td>Patient</td>
<td>Age</td>
<td>Gender</td>
<td>Diagnosis</td>
<td>Localization</td>
<td>Spherical diameter</td>
<td>Radioactivity</td>
<td>Radioactivity Comments</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>H. O.</td>
<td>56</td>
<td>M</td>
<td>Primary asymptomatic. Prominuding sternal metastasis.</td>
<td>Primary in right middle.</td>
<td>Spherical diameter 4.5 cm (primary)</td>
<td>+ 6 192 50</td>
<td>Tr (n = 4) = 3.89 ± 0.31; NK (n = 2) = 5.3 ± 0.00; serum (n = 2) = 29.12 ± 0.93.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. W.</td>
<td>59</td>
<td>M</td>
<td>Pain in left flank and hematuria.</td>
<td>Left upper 1/2.</td>
<td>2 connected masses: 8 x 6.5 x 5 cm, 8 x 6 x 5 cm Cystic +</td>
<td>++ 6 48 100</td>
<td>Tr (n = 8) = 17.31 ± 4.0; NK (n = 8) = 26.73 ± 6.54; PFF (n = 4) = 7.65 ± 0.68; serum (n = 4) = 85.87 ± 2.25; blood clot in Tr (n = 4) = 12.99 ± 1.38; blood clot from peripheral blood (n = 4) = 13.49 ± 2.57.</td>
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<tr>
<td>H. T.</td>
<td>67</td>
<td>M</td>
<td>Tumor discovered at laparotomy for bowel obstruction.</td>
<td>Left upper pole. Small separate cyst in lower pole.</td>
<td>4 cu cm; cyst diameter: 1.5 cm</td>
<td>++ 24 72 100</td>
<td>Tr (n = 4) = 48.31 ± 6.86; NK (n = 2) = 53.40 ± 1.63.</td>
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<tr>
<td>C. F.</td>
<td>37</td>
<td>M</td>
<td>Pain in left flank.</td>
<td>Left kidney 95% involved.</td>
<td>12 x 8 x 6 cm</td>
<td>++ + 24 48</td>
<td>Tr (n = 4) = 13.1: 6.2; 125I: 10</td>
<td>Radioactivity in Tr &lt; NK ARCG (131I): p = 0.00006; NRG (125I): p = 0.00055. TR/NK ratio: ARCG = 0.60, NRG = 0.58.</td>
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<tr>
<td>M. M.</td>
<td>26</td>
<td>M</td>
<td>Hematuria.</td>
<td>Right, at junction of upper 1/2 and lower 1/2.</td>
<td>2 cu cm</td>
<td>++ Negative until surgery 48 50</td>
<td>Tr (n = 1) = 24.71; NK (n = 1) = 27.43.</td>
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<tr>
<td>D. C.</td>
<td>75</td>
<td>M</td>
<td>Asymptomatic.</td>
<td>Right, in lower pole. Cyst in upper pole of left kidney.</td>
<td>4 cu cm; cyst diameter: 1 cm</td>
<td>0 Negative until surgery 72 100</td>
<td>Tr (n = 4) = 13.04 ± 0.74; NK (n = 6) = 24.37 ± 3.63; benign cyst (n = 4) = 21.68 ± 3.77; PFF (n = 4) = 6.79 ± 1.53.</td>
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* The tumors in all 15 patients were correctly diagnosed by i.v. pyelography and angiography.

** a: 0, <25%; +, 25 to 50%; ++, 51 to 75%; +++, >75%.

° Tr, tumor; NK, normal kidney; PFF, perirenal fibrotic tissue.

* The number of samples assayed.
these 5 individuals. Tumor-free perirenal fibrofatty tissue could be obtained for $^{131}$I activity assay from 4 patients (G. H., F. H., H. W., and D. C.); in all 4, radioactivity was the lowest in that tissue.

It was possible to assay necrotic tumor tissue separately for $^{131}$I activity in 2 patients (M. W. and B. V.). In both, radioactivity in the necrotic part of the tumors was lower than in the nonnecrotic tumor and normal kidney. The $^{131}$I activity in partly necrotic tumor tissue from Patient G. H. was less than that in nonnecrotic tumor tissue, but more than that in normal kidney. In Patient B. H., more radioactivity was found in the solid part than in fluid in a cystic region of the tumor. The assay of $^{131}$I activity in a benign cyst (at the pole opposite to the carcinoma) in these 5 individuals. Tumor-free perirenal fibrofatty tissue could be obtained for $^{131}$I activity assay from 4 patients (G. H., F. H., H. W., and D. C.); in all 4, radioactivity was the lowest in that tissue.

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An additional patient (A. S., not included in Table 2) showed multiple nodular tumors in both kidneys at laparotomy, and his right kidney was removed in 1970. Local pathologists diagnosed the tumor as a multifocal renal cell carcinoma. A second opinion from an Armed Forces Institute of Pathology pathologist suggested the lesions to be multiple adenomas. There was no localization of $^{131}$I-ARC-G in the left kidney during a scan in 1974, and the patient has done well to date.

**Clearance of $^{131}$I-ARC-G from Serum.** Chart 1 shows patterns of clearance of $^{131}$I-ARC-G from the serum of 6 patients. Approximately 50% of $^{131}$I was cleared from the blood 24 hr after administration. Approximately 30 to 35% and 10 to 25% of the administered $^{131}$I activity persisted at 48 and 120 hr, respectively. The pattern of clearance of radioactivity from the serum of other patients given $^{131}$I-ARC-G was similar. (In one patient not included in this study, there was accidental leakage of $^{131}$I-ARC-G into s.c. tissue resulting in a different pattern of clearance from the serum.) Chart 2 compares the rate of clearance of $^{131}$I activity from serum with its rate of excretion in Patient G. H. About 50% of the total administered dose was excreted in the urine by 48 hr and approximately 65% by 120 hr. A comparable pattern of urinary excretion of $^{131}$I was observed in 3 other patients who were adequately studied. Most of the clearance of radioactivity from the serum could be accounted for by its loss through urinary excretion.

**Paired Labeling.** In one patient (D. C.), it was possible to administer $^{131}$I-labeled ARCG and $^{125}$I-labeled NRG (i.e., paired labeling). Unfortunately, this was 1 of the 2 patients whose tumor could not be localized by external scanning, and assay of radioactivity revealed less activity in tumor tissue with no immunofluorescence or autoradiographic evidence of binding of ARCG to tumor cells. The $^{125}$I/$^{131}$I activity ratio was the same both in tumor and in the adjacent renal tissue. The pattern of disappearance of $^{131}$I and $^{125}$I from the patient’s serum was identical (Chart 3).

**Optimal Amounts of ARCG and $^{131}$I for Scanning.** The amount of ARCG used for scans varied from 10 to 100 mg, and the amount of $^{131}$I varied from 1.0 to 6.4 mCi per patient. $^{131}$I in amounts exceeding 3 mCi/patient produced scans with pronounced background activity. No effect of the amount of carrier ARCG on the quality of the scans could be detected.

**Optimal Time for Scanning.** Some of the scans revealed tumor-specific localization of $^{131}$I-ARC-G as early as 2 or 6 hr after its administration, but others showed localization only at 24 hr. All scans which showed tumor localization were positive at 24 hr. There was a decrease in background activity and an improvement in contrast with time in all scans. This correlates well with the pattern of clearance of radioactivity from serum.

**Toxicity and Untoward Reactions.** Pyrogenic reactions were observed in 2 patients (P. C. and D. C.) in the early phase of this study. Stringent aseptic procedures and testing for pyrogenicity of preparations before use have now successfully eliminated this reaction. No evidence of renal toxicity of ARCG was seen in any patient; i.e., there was no increase in urinary albumin, blood urea nitrogen, or creatinine. Complete blood and platelet counts performed twice a week failed to reveal any evidence of hematological toxicity.

**Comparison of Results with Other Methods of Diagnosis.** All the histologically established metastatic lesions could be detected by X-ray examinations or by $^{99m}$Tc diphosphonate scans. No lesion could be detected in Patient F. S. by sigmography, tomography, bronchoscopy, and mediastinoscopy immediately after scanning. Whether $^{131}$I-ARC-G scanning did indeed detect a small quiescent lesion remains conjectural considering the long interval (i.e., almost 3 years) between the positive scan and the detection of a lesion by routine follow-up skigraphy. All 15 primary renal cell carcinomas were correctly diagnosed by i.v. pyelography and angiography. Ultrasound also correctly localized 13 of these tumors; only in Patients D. C. and M. M. were the results equivocal. $^{99m}$Tc-sulfur colloid could not localize any of the primary or metastatic lesions. The failure of $^{131}$I-ARC-G to localize in 2 primary lesions and the suspected metastatic lesions in the ribs and vertebrae of Patient E. B. are interesting. Sigmagraphy and $^{131}$I-ARC-G scanning failed to confirm the lesions detected by $^{99m}$Tc. Furthermore, the lack of progression of the disease suggests that the renal lesion might be relatively benign. This is comparable to the lack of localization of $^{131}$I-ARC-G in the left kidney of Patient A. S. whose lesions are likely to be multiple adenomas.

**Localization of $^{131}$I-Immunoglobulins and $^{67}$Ga Citrate in H6 Hepatoma Transplants in Mice.** Most of the radioactivity detected 2 hr after i.v. injection of $^{131}$I-NRG was localized in the thoracic and abdominal cavities. Some radioactivity was also present in the hepatoma but not in the granuloma induced by Freund’s complete adjuvant in the left flank. At 24 hr, there was also considerable activity in the bladder (i.e., urine). This scanning pattern persisted in all the $^{131}$I-NRG-injected mice until their death.

The whole-body scans of the mice given injections of $^{131}$I-AHG or $^{131}$I-AHG-P were essentially the same (Figs. 7 and 8). These patterns did not differ from that in the mice given injections of $^{131}$I-NRG at 2 hr; i.e., radioactivity was diffusely spread throughout the body with localization evident in the thoracic and abdominal cavities. Some localization was also seen in the tumor but not in the granuloma. At 24 hr, radioactivity in the thoracic and abdominal cavities had decreased and was approximately the same as in the tumor in both groups (Figs. 7 and 8). Little activity was detected in the granuloma. Thereafter, there occurred a gradual decrease of radioactivity
in the 2 cavities but not in the tumor, so that at 120 hr radioactivity was most marked in the tumor with marginally better contrast in the group given AHG-P. When $^{67}$Ga citrate was used for scanning (Fig. 8), radioactivity was diffusely scattered over the body at 2 hr. At 24 hr, most of the radioactivity was in the abdominal cavity, the granuloma, and the tumor. This pattern of localization persisted in all the mice until 120 hr when the observation ended.

Table 3 shows the distribution of $^{131}$I in the different organs of mice killed 120 hr after the injection of $^{131}$I-labeled immunoglobulins. The tumor:blood ratio for $^{131}$I was 2.03 in the group given injections of $^{131}$I-AHG-P, approximately 1 in the group given injections of $^{131}$I-AHG, but only 0.55 in the group given injections of $^{131}$I-NRG. The tumor and liver had the highest radioactivity in mice given injections of $^{131}$I-AHG-P. In mice given injections of $^{131}$I-AHG, the tumor, liver, and blood had the highest activity; whereas in the mice given injections of $^{131}$I-NRG, blood had by far the highest radioactivity, followed by the tumor. There was no localization of $^{131}$I-AHG-P or of $^{131}$I-AHG in the granulomas.

Discussion

The aim in linking radionuclides to antitumor antibodies is to bestow on them the specificity which they otherwise lack. The specific role of anti-tumor globulin preparations for successfully imaging the tumors was demonstrated in this study by the following. (a) $^{131}$I linked to NRG could not image the mouse hepatoma nor could a $^{99m}$Tc-sulfur colloid image the human renal carcinomas. This shows that nonspecific vascular factors themselves did not lead to localization of these agents in amounts adequate for imaging. (b) A tumor which did not bind the administered ARCG in situ and was histologically relatively benign (D. C.) was not visualized by scanning. Other tumors (A. S. and E. B.) that followed a relatively benign clinical course also could not be localized by scanning. (c) The result of immunofluorescence and autoradiography of dissociated tumor cells and of sections containing tumor and adjacent normal tissue established that i.v.-administered ARCG’s indeed localized in and were bound firmly to the target tumor tissue but not to adjacent tumor-free tissue. Assay of radioactivity bound to different subcellular fractions of tumor cells, as suggested by Day (4), might further elucidate the specificity of localization of $^{131}$I-ARCW.

It is interesting to note that $^{131}$I-labeled anti-tumor globulins localized neither in the Bacillus Calmette-Guérin-induced and tuberculous granulomas of Patients P. C. and J. J. nor in the Freund’s complete adjuvant-induced granulomas in A/J mice. On the other hand, $^{67}$Ga citrate could not discriminate between the granulomas and the hepatoma transplants. Further, because of its excretion through the gastrointestinal tract (14), $^{67}$Ga citrate produced scans of inferior contrast compared to $^{131}$I-labeled AHG.

Correlation of scanning with the extent of localization of $^{131}$I in tumor tissue samples showed that tumors which contained
equal or greater radioactivity than did normal kidney could be successfully imaged. Moreover, 3 of 5 tumors which contained less radioactivity than did normal kidney could also be visualized. This suggests that factors other than tumor-specific localization of 131I-ARCG (or 131I-AHG) had contributed also to the imaging. Thus, the success of 131I-labeled anti-tumor globulins as imaging agents is associated with their tumor-specific localization but is also considerably aided by such nonspecific factors as increased tumor vascularity (leading to an increase in blood volume in relation to surrounding tissues) and increased permeability of tumor vasculature. This may lead to considerable extravascular accumulation of labeled xenoglobulins as seen in the hepatoma-bearing mice given injections of 131I-NRG. Current subtraction methods (10) will eliminate the contribution of radioactivity from the blood pool but not the contribution from the extravasated nonspecific immunoglobulin in the tumor bed.

Our observation on the improvement in contrast and hence, detectability of lesions with time is consistent with previous reports (9, 11, 12, 17). This improvement may be explained by the much longer half-life of tissue-bound antibodies compared to circulating xenoglobulins (4).

Since 70% of the administered 131I was being excreted in urine in the first 48 hr and 90% by 120 hr, a large part of 131I in functioning renal tissue must be associated with its excretion. This conclusion is supported by the comparatively much lower radioactivity found in the perirenal tissue. Thus, 131I is not the ideal agent for imaging kidney tumors, and it is surprising that higher tumor localization was observed in 5 of the 12 renal carcinomas investigated.

The results in Table 2 showed much less localization of 131I in necrotic areas of tumors. In fact, 1 of the 2 primary renal carcinomas and the one metastasis which could not be detected by scanning had extensive necrotic changes. This appears to constitute a limitation on tumor detection. Other factors which might have contributed to the failure to visualize the 2 primary tumors were their small size and deep location (e.g., Patient D. C.’s tumor was buried in the parenchyma and did not protrude), as well as the high background activity due to a relatively large dose of 131I in Patient M. M. Also, it is uncertain whether Patient D. C.’s lesion was malignant (1) since, histologically, it did not show any pleomorphism, mitosis, or evidence of invasion, and dissociated tumor cells did not reveal any cell surface-bound ARCG. A prior 67Ga citrate scan had failed to localize this tumor.

Studies in mice with affinity-purified AHG showed only marginal improvement in the quality of the scans in spite of a doubling in the tumor: blood radioactivity ratio. The relative lack of improvement in scanning is not unexpected in view of the contribution of nonspecific localization predicted for these large tumors with their attendant vascular changes. On the other hand, vascular factors would be unlikely to play a major role in localization of 131I-AHG in small tumors and metastases. Thus, the benefit of highly purified antitumor antibodies will be best realized in detection of small metastases.

Acknowledgments

The authors are grateful to Drs. F. G. Mack, O. H. Millard, S. G. Lannon, and W. A. Ernst, Halifax Infirmary and the Victoria General Hospital, Halifax, Nova Scotia, Canada, for referring the renal cell carcinomas to us and to M. Mannen, H. Noldo, and D. Sadi for technical help.

References


Fig. 1. Schematic (A), a 131I-WBC scan (B), and a 99mTc scan (C) of Patient H. O. showing a metastatic lesion involving the sternum and anterior mediastinum (arrow).
Fig. 2. Skiagram (A) and $^{131}$I-ARCG scan (B) of a metastatic lesion in the fifth right lumbar vertebra of Patient P. C. (thin arrows). Thick arrow, radioactivity of urine in the bladder. L, left.

Fig. 3. Fluorescence photomicrograph of dissociated renal carcinoma cells (from Patient R. S.) treated with fluoresceinated rabbit anti-goat IgG. Diffuse cytoplasmic staining shows the administered goat IgG ($^{131}$I-ARCG) localized in the tumor cells. $\times$ 480.

Fig. 4. Fluorescence photomicrograph of a viable tumor cell from the renal cell carcinoma of Patient A. M. treated as in Fig. 3 showing binding of the administered goat IgG ($^{131}$I-ARCG) to the cell surface. $\times$ 800.

Fig. 5. Fluorescence photomicrograph of cryostat section containing tumor (arrow) and adjacent normal tissue (from Patient A. M.) treated with fluoresceinated rabbit anti-goat IgG. Considerable localization of goat IgG ($^{131}$I-ARCG) can be seen in the tumor tissue compared to adjacent renal tissue. $\times$ 190.

Fig. 6. Autoradiograph of a section containing renal cell carcinoma and adjacent tumor-free kidney from Patient A. M. who was given $^{131}$I-ARCG 72 hr prior to surgery. Considerable radioactivity is seen in the tumor (arrow) but not in the adjacent renal tissue. $\times$ 275.
Fig. 7. Scan showing preferential tumor localization of $^{131}$I-AHG in an A/J mouse bearing a H6 hepatoma in the right flank and a Freund’s complete adjuvant-induced granuloma in the left. Times (hr) indicate the interval between injection of label and scan.

Fig. 8. Scans showing the patterns of localization of $^{131}$I-AHG-P and $^{67}$Ga citrate in 2 different mice, each bearing a H6 hepatoma in the right flank and a Freund’s complete adjuvant-induced granuloma in the left. Times (hr) indicate the interval between injection of imaging agent and scan. Ab, antibody.
Localization of $^{131}$I-labeled Antibodies in Human Renal Cell Carcinomas and in a Mouse Hepatoma and Correlation with Tumor Detection by Photoscanning


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