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Clinical Radioimmunodetection of Cancer with Radioactive Antibodies to Human Chorionic Gonadotropin

Abstract. *Injection of iodine-131-labeled goat immunoglobulin G antibody to human chorionic gonadotropin (hCG) into patients with hCG-secreting trophoblastic and germinal tumors permitted tumor detection and location by external gamma-ray scintigraphy. Excision of one of the metastatic tumors located by this method indicated a tumor/nontumor ratio of 39.29. The method appears to offer a new clinical tool for precisely locating hCG-producing tumors in the body, even when tumor identification by other clinical methods has failed.*

Human chorionic gonadotropin (hCG) was one of the first tumor markers used to monitor disease activity in patients with hCG-producing neoplasms, such as trophoblastic and germ-cell tumors (*1*). Recently it was shown that serum hCG levels were elevated in a group of patients with a diverse array of cancers (*2*). The finding that tumors producing carcinoembryonic antigen (CEA) can be detected and located in humans in vivo by administering a radioactive foreign antibody to CEA and performing total-body scanning with a gamma-ray scintillation camera (*3*) suggests that this method of cancer radioimmunodetection can also be applied to hCG-containing neoplasms. Indeed, Quinones *et al.* (*4*) demonstrated that human choriocarcinomas grown in hamsters could take up 2.8 times more radioactively labeled antibody to hCG than the animals' livers. We report here that hCG-producing tumors in man can be detected and located by radioimmunodetection after administration of ^{131}I-labeled goat antibody to hCG, revealing tumor sites not demonstrated by other diagnostic techniques.

Hyperimmune goat antiserum was prepared with purified urinary hCG. The antiserum to hCG was absorbed with human urinary protein, using an automated chromatography system with a solid-phase (Sepharose 4B, Pharmacia) immunoadsorbent column (*5*). The immunoglobulin G (IgG) fraction of the absorbed antiserum was chromatographically purified with *O*-(diethylaminoethyl)cellulose and then concentrated to 7.35 mg of protein per milliliter by ultrafiltration through a membrane (Amicon PM30). The purity and immunoreactivity of the IgG were determined by immunodiffusion and immunoelectrophoresis against hCG and against donkey antibody to goat serum and IgG. The titer of the goat IgG antibody to hCG, as determined by radioimmunoassay with half of maximum binding used as the endpoint, was 5×10^5.

The goat antibody to hCG was labeled with ^{131}I (Amersham/Searle) by the chloramine-T method (*6*). The labeled antibody (specific activity, 5 to 10 Ci per gram of IgG protein) was diluted with 1 percent human serum albumin (Hyland) in physiological saline and filtered with a 0.22-μm sterile filter. Chromatographic analysis with a Sephadex G-200 gel indicated that 90 to 95 percent of the ^{131}I-labeled antibody cochromatographed with the IgG. Individual lots of ^{131}I-labeled goat IgG antibody to hCG were tested by an independent testing laboratory (Scientific Associates) and determined to be sterile, pyrogen-free, and nontoxic in short-term tests.

Subjects were patients with an hCG-secreting tumor or a tumor thought to have areas of hCG production. Their informed consent was first obtained. Also, before injecting the labeled antibody, we tested the subjects for anaphylactic hypersensitivity to goat IgG. To reduce thyroid uptake of ^{131}I, potassium iodide solution (Lugol's solution, Purepack Pharmaceutical) was administered (five drops twice daily, orally) to the patients as soon as they entered the study and until completion of scanning.

The ^{131}I-labeled IgG antibody to hCG was administered intravenously (2 to 3 μg per kilogram of body weight; total radiation per patient, 1.20 to 1.80 mCi) in 20 ml of sterile 0.9 percent NaCl over a period of 10 to 15 minutes. To subtract blood-pool activity and any free iodine activity secreted in the stomach and urinary bladder, 0.5 mCi of $^{99m}TcO_4^-$ (E. R. Squibb & Sons) and 0.5 mCi of ^{99m}Tc-labeled human serum albumin (Union Carbide) were injected intravenously 30 and 5 minutes before imaging, respectively. Images of the anterior, posterior, and lateral chest and abdomen were made with a gamma-ray scintillation camera (LFOV, Searle) at various intervals after application of the radioactive antibody, but usually after 24 and 48 hours. Scans

Table 1. Photoscanning results for four patients with benign or malignant tumors. (+) Denotes tumor identified; (−), tumor not identified; and (0), tumor excised. The activity concentration ratio (T/NT) represents ^{131}I counts in tumor compared to an equivalent area of the same organ or body region, and the activity count ratio [T/NT(S)] denotes the same relation after subtraction of the ^{99m}Tc image from the ^{131}I picture, both computations made 24 hours after injection of labeled goat IgG antibody to hCG. Numbers in parentheses indicate number of sites positive for tumor. N.A., not available.

Patient no.	^{131}I Dose (mCi)	Primary diagnosis	Serum hCG (ng/ml)	Radioimmunodetection findings		T/NT	T/NT(S)
				Primary site	Secondary sites		
200	1.20	Embryonal carcinoma	0	0	−, Lungs (5)	1.00 to 1.20	1.20 to 2.30
228	1.74	Hydatidiform mole (preoperative)	34,700	+*		2.87*	8.65*
228	1.74	Hydatidiform mole (postoperative)	N.A.	0		1.40	1.07
230	1.80	Teratocarcinoma	117	0	+, Pelvis (1)	2.20	38.30
231	1.25	Embryonal carcinoma with choriocarcinoma	1,721	0	+, Lungs (2)	1.02 to 1.28	7.68 to 11.34
					+, Abdomen (1)	2.20	14.19

*One hour after the injection of labeled antibodies (preoperative).

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were obtained of the ^{131}I-labeled antibody distribution (at 364 keV with a 20 percent window) and stored in a digital minicomputer. The background activity levels of the ^{131}I and ^{99m}Tc were equalized, the radioactivity of the latter (140 keV) subtracted from that of the former, and the difference between the two displayed in eight hues ranging from dark blue (lowest radioactivity) to white (highest radioactivity) (see Figs. 1 and 2). The radioactivity remaining after computer-assisted subtraction represented areas of excess ^{131}I over ^{99m}Tc—presumably reflecting antibody-antigen sites. Descriptions of this method of radioimmunodetection have appeared in studies in which radioactive antibody to CEA rather than hCG was utilized (*3*).

Table 1 gives the results of hCG radioimmunodetection testing in four patients with germ-cell and trophoblastic tumors. Patient 200 had five lung metastases detectable by chest x-ray, but none could be visualized by immune scintigraphy with radioactive antibody to hCG. The patient's blood hCG level was not elevated, so we presume that this was not an hCG-secreting tumor. However, in previous studies performed on this patient, radioactive antibody to alpha-fetoprotein (AFP) successfully visualized all five lung metastases, whereas normal goat IgG labeled with ^{131}I did not. (*7*). The remaining three patients had hCG-secreting tumors, as indicated by serum hCG elevations, and hCG immune scintigraphy did identify their location. Patient 228 had a hydatidiform mole that was located by radioimmunodetection prior to surgery; there was an absence of abdominal radioactivity after total excision of the hCG-producing tumor (Fig. 1). A comparison of pre- and postoperative T/NT (tumor/non-tumor activity concentration ratio) and T/NT(S) (tumor/non-tumor activity count ratio) values confirms the increased accretion of radioactivity recorded by the imaging camera in this benign tumor. Similar positive imaging results were obtained in the last two patients, who had hCG-secreting germ cell tumors of the testes. The pelvic and abdominal cancers imaged in these two patients were confirmed surgically, while the lung metastases in patient 231 were first detected by chest x-ray (see Fig. 2) and then confirmed by partial lung resection. A sample of the cancer tissue removed from the right lung was minced and counted in a scintillation counter; the tumor tissue activity level was 5.50 nCi/g, compared with 0.14 nCi/g for adjacent normal lung tissue. This represents a T/NT ratio of 39.29. The abdominal tumor metastasis disclosed by radioimmunodetection (Fig. 2) and later confirmed surgically was undetectable by abdominal computerized tomography, intravenous pyelography, and inferior venacavography. Thus in this particular case radioimmunodetection succeeded in identifying tumors not identified by other diagnostic measures—as we indeed experienced with CEA radioimmunodetection (*3*). Conversely, sites that were negative for tumor by immune scintigraphy were likewise presented as normal by other methods.

These initial observations support our view that tumor-associated markers other than CEA, even when located within the cancer cell, can serve as targets for tumor-seeking labeled antibodies. The clinician may find in the blood an elevated level of a marker substance such as CEA, AFP, or hCG before a tumor is clinically detectable (*8*). This raises the problem of locating the site of the tumor

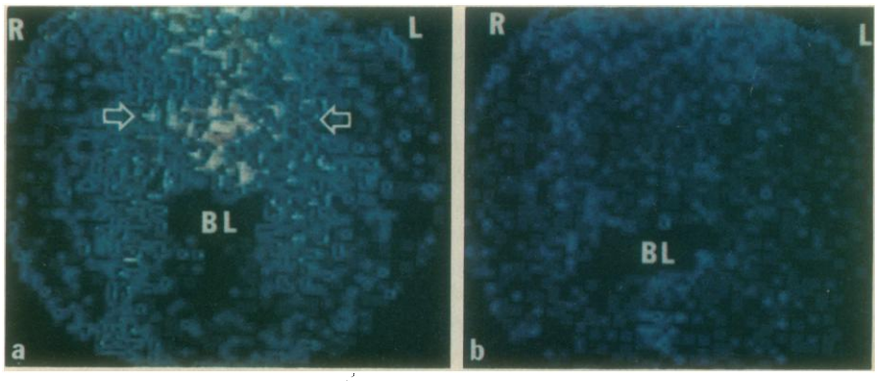

Fig. 1. (a) Preoperative gamma-ray scintigram of the anterior pelvis of patient 200 1 hour after intravenous injection of 1.74 mCi of ^{131}I-labeled goat IgG antibody to hCG. The patient's hydatidiform mole is clearly imaged (arrows). (b) Postoperative immune scintigram showing pelvis devoid of radioactivity. These pictures represent the images obtained after subtraction of the ^{99m}Tc preparation from the ^{131}I-labeled antibody. *BL*, urinary bladder.

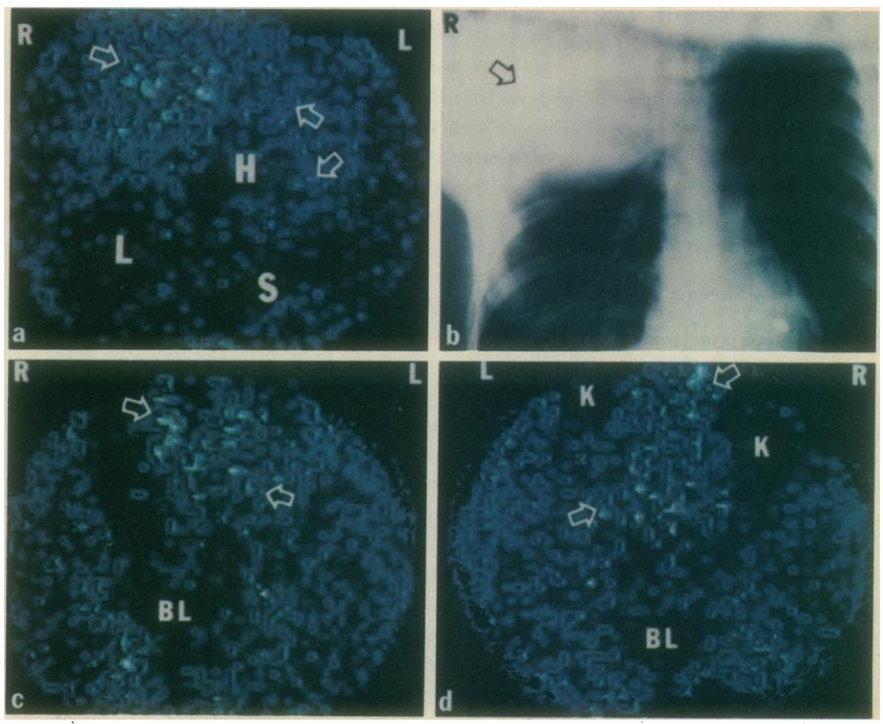

Fig. 2. (a) Immune scintigram of anterior chest of patient 231, who had a history of embryonal carcinoma with choriocarcinomal elements. Metastases are present in the right upper lobe and left lower lobe of the lungs (arrows). *H*, heart; *L*, liver; *S*, spleen. (b) Chest x-ray revealing massive density in right upper lung (arrow), corresponding to major focus of radioactivity seen in (a). (c) Anterior abdominal view showing abnormal radioactivity in mid-abdomen (arrows). (d) Posterior abdominal view showing increased radioactivity in retroperitoneal area of mid-abdomen. This retroperitoneal tumor, confirmed at surgery, could not be identified by other detection methods. *K*, kidney.

releasing the marker. Radioimmunodetection, in which relatively specific antibodies are directed against these tumor-associated products and computer-assisted enhancement is made of tumor/nontumor radioactivity count ratios by dual isotope subtraction, provides an opportunity for noninvasive detection of tumors in man. Moreover, the increased accretion of these antibodies in certain neoplasms suggests that they could serve as carriers of drugs or radiation for more selective anticancer therapy. The locating of tumors in mice by means of labeled monoclonal antibody (*9*) also suggests that this may be another approach toward developing very specific tumor-locating radioactive antibodies for clinical applications.

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Bioluminescence in Mesopelagic Squid: Diel Color Change During Counterillumination

Abstract. *Two species of mesopelagic squid greatly altered the color of their bioluminescence during counterillumination. The color change was triggered by changes in water temperature corresponding to those normally encountered by these vertically migrating animals. These squid can probably conceal themselves under the different colors of downwelling light that they encounter in their day and night habitats.*

Concealment is difficult for opaque animals living in the sunlit waters of the open ocean, since they are silhouetted against downwelling light. There is no complete solution to this problem for animals living in the upper few hundred meters of the ocean, where downwelling sunlight is intense. However, in the deeper, dimmer depths of the upper mesopelagic zone (*1*), animals can eliminate their silhouettes with bioluminescence. To effectively counterilluminate, the animal must regulate the intensity of its bioluminescence and accurately match the angular distribution and color of the downwelling daylight. Recent studies indicate that some mesopelagic animals have some of these abilities (*2*).

Similar problems with camouflage arise for mesopelagic animals at night. In Hawaiian waters, nearly half of these animals migrate into the upper 400 m at night, and many reach the upper 100 m (*3*), depths at which the photic environment is extremely complex. The intensity of detectable downwelling light near the surface varies over several orders of magnitude with the phase of the moon

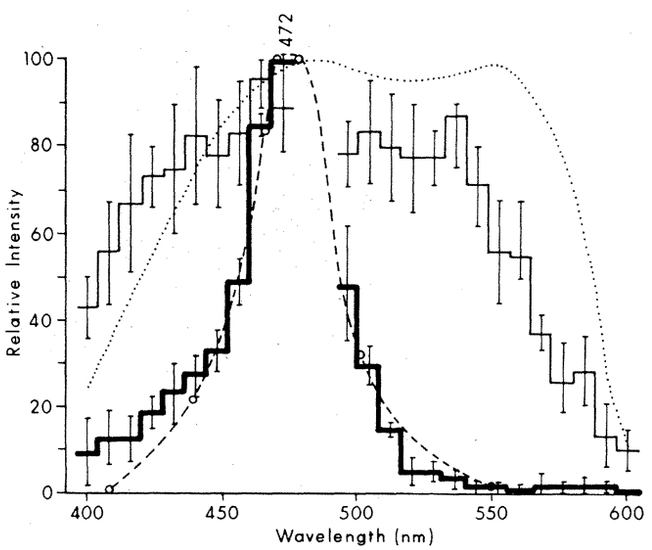

Fig. 1. Emission spectra of luminescence from an *Abraliopsis* sp. B individual, with superimposed spectral irradiance curves of moonlight and sunlight in the ocean. The thick solid line represents bioluminescence at 8°C; the thin solid line, bioluminescence at 23°C. The gap from 476 to 492 nm is the emission band region of the overhead light. Error bars are drawn at 95 percent confidence intervals (N = 6). The dashed line represents sunlight off Hawaii at 380 m (*7*); the dotted line, moonlight off Enewetak at 20 m (*9*).

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