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In vivo localisation of radiolabelled antibodies to carcinoembryonic antigen in human colon carcinoma grafted into nude mice

SEVERAL attempts have been made to show the specific localisation *in vivo* of anti-tumour antibodies. Most of these studies, however, either in experimental animals[1,2] or in humans[3] were performed with antibodies obtained by adsorption and elution from poorly characterised crude tumour fractions.

Here we report the specific localisation of ^{131}I-labelled antibodies against carcinoembryonic antigen (CEA) in heterografts of human colon carcinomas growing in nude mice and show that they can be detected by external scintillation scanning. This model system enables us to test the ability of antibodies directed against a well-characterised human tumour-associated antigen to concentrate in human tumours without performing preliminary experiments in patients.

We have recently shown[4] that CEA as identified by Gold and Freedman[5] was present in heterografts of human colon carcinomas serially transplanted into nude mice (Balb/c Nu-Nu from G1. Bomholtgard Ltd., Ry, Denmark). CEA was demonstrated on cryostat tumour sections by indirect immunofluorescence using a highly specific rabbit anti-CEA antiserum[6]. The histology of the grafted tumour as well as the localisation of CEA in this tissue was identical to that observed in the primary tumour. Also, CEA extracted from tumour grafts by the perchloric acid method[7] was found by radioimmunoassay[8,9] to be immunologically identical to reference human CEA[6] and, moreover, to be present in similar concentrations in both heterotransplanted and primary tumours. Using a double antibody radioimmunoassay[10,11], it was possible to detect circulating CEA levels of 20-70 ng ml^{-1} in sera of nude mice bearing colon carcinoma tumours larger than 1 g. No CEA was detected in sera of nude mice bearing colon carcinomas smaller than 0.5 g or bearing other human tumours, including breast carcinomas, hypernephromas and melanomas.

Anti-CEA antibodies were isolated by adsorption-elution from a specific immuno-adsorbent prepared in the following way. Eight milligrammes of purified CEA[6] were polymerised with 200 mg of BSA at *p*H 4.8, using 2 ml of 1% glutaraldehyde solution[12]. Following homogenisation, the polymer was mixed with an equal volume of Sephadex G-25 and packed in a 1 cm diameter column. One ml of hyperimmune goat anti-CEA antiserum which exhibited little reactivity with the non-specific cross-reacting antigen (NCA)[13–15], was run through the column at a flow rate of 3 ml h^{-1}. The adsorbed antibodies were eluted with 3 M KSCN[16], dialysed against 0.01 M Tris-HCl buffer, *p*H 7.5, and concentrated to 1 ml. This antibody fraction contained 2 mg of protein and was able to bind 50% of 1 ng of CEA at a dilution of 1:70.000 when titrated in the radioimmunoassay.

A hundred microgrammes of the antibody fraction were labelled with 2 mCi of ^{131}I using the lactoperoxidase method[17], mixed with 1 ml of normal goat serum and filtered on a Sephadex G-200 column. The 7S antibody fraction was collected and used immediately for the localisation experiments. About 60–70% of the radioactive material in the 7S fraction was specifically bound to the CEA immunoadsorbent. For control experiments, normal goat 7S globulins, treated with 3 M KSCN, were labelled with ^{125}I and further purified on Sephadex G-200.

Volumes of 0.2 ml of the 7S fraction containing about 2 μg of radioactive antibody (specific activity: 8 μCi μg^{-1}) and

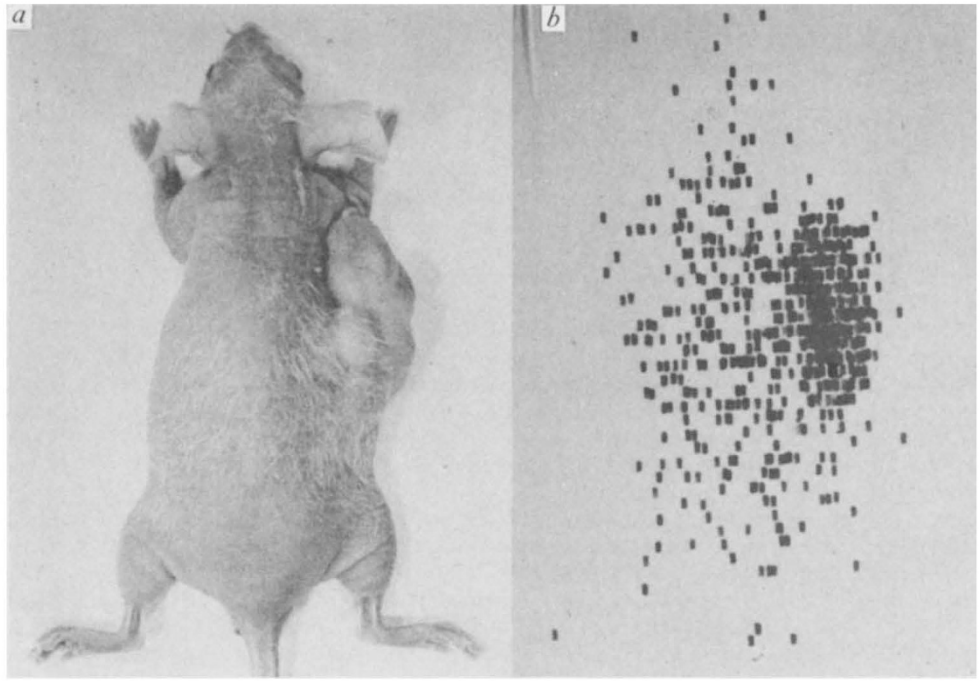

FIG. 1 *a*, Nude mouse bearing a heterograft of human colon carcinoma is shown in the scanning position. *b*, The total-body scan from the same mouse obtained 3 d after injection of 2 μg ^{131}I labelled anti-carcinoembryonic antibodies (dose of radioactivity injected = 16 μCi).

TABLE 1 Comparison of antibody and normal 7S concentration in tumour liver and muscles

Type and weight of tumours	Protein Injected	Tumour % per g*	Liver % per g*	Muscle % per g*	Tumour: liver ratio†	Specificity index†	Tumour: muscle ratio†	Specificity index‡
Exp. I	Antibody	17.3	4.9	2.0	3.5	4.1	8.7	4.5
Co 115 1.620 g	N 7 S	4.6	5.3	2.4	0.9		1.9	
Exp II	Antibody	24.5	4.4	1.2	5.6	2.7	20.4	2.4
Co 115 1.960 g	N 7 S	13.4	6.4	1.6	2.1		8.4	
Exp. III	Antibody	23.4	2.6	2.8	9.0	3.9	8.4	4.1
Co 115 2.200 g	N 7 S	13.2	5.7	6.5	2.3		2.0	
Exp. IV	Antibody	24.3	6.9	7.2	3.5	3.5	3.4	3.5
Co 115 0.575 g	N 7 S	12.4	12.4	12.8	1.0		1.0	
Exp. V	Antibody	15.0	10.0	1.8	1.5	1.9	8.3	1.8
Co 111 0.495 g	N 7 S	7.6	9.4	1.6	0.8		4.8	
Exp. VI	Antibody	12.9	6.3	1.7	2.0	2.3	7.6	2.1
Co 112 0.405 g	N 7 S	8.5	9.9	2.3	0.9		3.7	
Exp. VII	Antibody	7.4	3.9	1.8	1.9	1.2	4.1	1.0
El 4 0.510 g	N 7 S	8.9	5.5	2.1	1.6		4.2	

* Concentration of either antibody or normal 7S globulin (N 7S) expressed as % of the total specific radioactivity per g of tissue.
† Tumour: liver or tumour: muscle ratio are obtained by dividing the tumour concentration of either antibody or normal 7S globulin by the organ concentration of each of these proteins.
‡ Specificity indices are obtained by dividing the tumour: normal organ concentration ratio of antibody by that of normal 7S globulin.

200 μg of normal goat 7S globulins were injected intravenously into nude mice bearing subcutaneous grafts of human colon carcinomas. At different time intervals ranging from 2 h up to 3 d after injection, mice were scanned with a 3 inch crystal photoscanner (Picker Magnascanner 500). During the scanning procedure, the mice, anaesthetised by intraperitoneal injection of phenobarbital, were immobilised in the prone position as shown in Fig. 1*a*. After 2–6 h, the radioactivity was homogeneously distributed throughout the mice with no apparent localisation in the tumours. No difference could be detected between tumour-bearing mice and normal controls. After 1 d, however, the radioactivity began to localise in the tumour area and gave an optimal detectable contrast by day 3 (Fig. 1*b*). At that time, the mice were killed and scanned again after separating the tumour from the body. This last scanning confirmed that the major radioactive site was related to the tumour and not to the adjacent liver.

After complete dissection of the animal, each organ and pieces of the body were weighed and placed in well-type scintillation counter. For the 24 g mouse seen in Fig. 1, it was found that 40% of the total radioactivity recovered was concentrated within the 1.8 g tumour. Several other scanning pictures showing clear tumour localisation were obtained with different mice bearing variously sized heterografts, all derived from the same colon carcinoma (Co 115). The smallest tumour nodule detectable by scanning weighed 200 mg. Grafts of two other human colon carcinomas (Co 111 and Co 112), with more differentiated histology and less abundant stroma and vascularisation[4] than Co 115, showed a lower specific concentration of antibodies (see Table 1) which were not detectable by scanning.

To take into account the possible non-specific accumulation of proteins in the extravascular space and necrotic regions of the tumour[18], the antibody localisation was studied by simultaneous injection of ^{131}I-labelled antibodies and ^{125}I-labelled normal goat 7S globulins (N 7S). In two experiments, the labels were reversed. The average values of four representative experiments performed in mice bearing Co 115 tumour are presented in Fig. 2. The results are expressed in percentage of the total radioactivity recovered per g of each tissue studied. The concentration of antibodies in the tumour was more than two-fold greater than N 7S whereas in normal tissues the antibodies had lower values than the control protein.

In Table 1 the individual results from seven different experiments are summarised. Concentration values of labelled antibodies and N 7S observed in tumour, liver and muscle

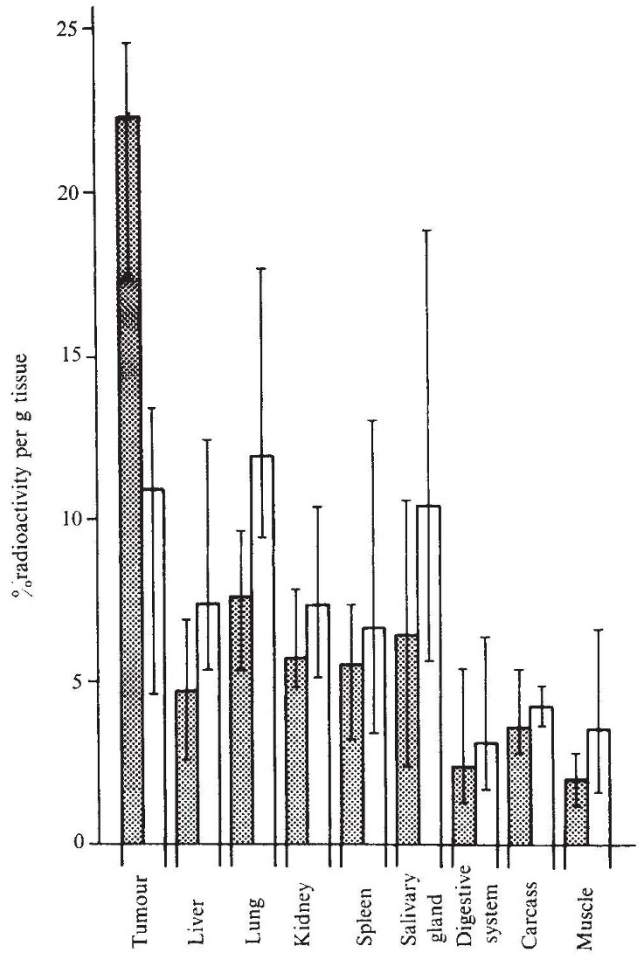

FIG. 2 Distribution of goat anti-CEA antibodies and normal goat 7S globulins in nude mice bearing heterografts of human colon carcinoma (Co 115). Three days after simultaneous injection of both proteins each labelled with a different iodine radioisotope (as described in the text), the tumour and different tissues were weighed and then measured in a dual channel well-type scintillation counter. Bar graphs represent the average value of four different experiments (% of total specific radioactivity recovered per g of tissue) for antibodies (shaded area) and for normal goat 7S globulin (open area). The solid lines represent the range of values.

can be compared. For both radioactive preparations, the ratio of tumour:normal organ concentration was calculated. A specificity index was obtained by dividing the concentration ratio of antibodies by that of N 7S. This index was significantly positive in all colon carinomas tested, including Co 111 and Co 112 (experiments V and VI), which were not detectable by scanning. In contrast, when the same experiments was performed in C57Bl/6 mice bearing a transplanted EL4 solid lymphoma (experiment VII), the specificity indices were not significant.

Evidence was obtained that the specificity of antibody localisation was decreased with the presence of necrotic tissue within the tumour. In experiment II, the tumour exhibited a significant degree of central necrosis. Despite a high absolute concentration of antibodies in this tumour, relatively low specificity indices were observed compared with those obtained for less necrotic tumours (experiments I, III and IV). Furthermore, when the radioactivity of a small non-necrotic fragment selected from the tumour of experiment II was measured, the tumour:liver ratio of antibody was 14.0 and the specificity index reached 8.6 (the highest value yet observed). In three other experiments, cells from disrupted tumour fragments were counted after several washes with buffered saline. It was found that about 55% of the antibody radioactivity remained bound to the cells compared with 15% of the N 7S radioactivity.

It should not be concluded from these experimental studies that the injection of patients with labelled heteroanti-CEA antibodies will, in any case, enable the scanning detection of CEA-containing cancers. The major difficulties in humans would be the relatively large amounts of circulating CEA[8,9,11], as well as the presence of small quantities of CEA in non-malignant tissues[6,19–21]. Further work is needed to assess whether the use of highly specific antibodies directed against CEA may open a new dimension in the field of tumour localisation by radioactive tracers.

We thank Professor H. Isliker for advice and suggestions, Dr B. Scazziga, Miss L. Cuendet and Miss R. Kollman, Thyroid Function Unit, Department of Medicine, University, of Lausanne, for assistance in radioactive scanning. This work was supported by grants from the Ludwig Institute for Cancer Research and the Swiss National Foundation for Scientific Research.

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Received November 8, 1973.

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Immunological detection of antigen(s) associated with rat colon carcinoma

Cancer of the colon and rectum is exceeded only by cancer of the lung as a cause of cancer death in the United States. Of the approximately 350,000 deaths from cancer in 1973, 47,400 were from cancer of the colon and rectum; of the approximately 665,000 cancers diagnosed in 1973, 79,000 were in the colon and rectum. Denmark, Great Britian, Ireland, Canada, New Zealand, Australia, Belgium and Austria have equally high or even slightly higher death rates from this disease and it is a significant cause of morbidity and mortality in most western countries. Improved surgical techniques and the development of new diagnostic methods have not significantly affected these mortality statistics[1].

A useful animal model for the study of human colon carcinoma is the rat bearing adenocarcinomas of the colon induced by 1,2-dimethylhydrazine (DMH)[2,3]. Six to 8 months after initiation of treatment, 60 to 100 per cent of rats have one or more colon tumours which cause bleeding, intussusception or obstruction. The tumours are visible through the thin wall of the colon and are readily accessible for surgical or other treatment. Methods for early detection of tumours are needed for (*a*) development and evaluation of preventive or therapeutic measures and (*b*) studies of tumour development under dietary or other regimens designed to retard or enhance colon carcinogenesis[3–5].

Human colon carcinomas produce a glycoprotein, carcinoembryonic antigen (CEA)[6], which is detectable in serum[7] or plasma[8]. Although CEA is not specific for colon tumours, detection and quantitation of circulating quantities may be useful in the diagnosis of colon and other cancers, for prognosis after surgical resection and for monitoring chemotherapy[9]. We have detected antigen(s) in DMH-induced rat colon carcinomas using antisera produced in rabbits (see below). Since the antigen(s) was found in the rat tumours but not in normal rat colon it may provide a rat model for CEA in human colon carcinoma.

Indentification of the tumour-associated antigen analogous to human CEA, in the rat model described above would facilitate study of (1) the relationship between the blood level of antigen and size or other anatomic characteristics of colon tumours, (2) response of tumours to treatment, and (3) experimental induction and development of tumours.

Twenty-eight male, 4-week-old Sprague-Dawley rats (Charles River Laboratories) were fed a semisynthetic diet for 3 months, given ten weekly intragastric doses of DMH (30 mg kg^{-1} in 0.9% NaCl) and killed when they developed signs of tumour in the intestine or ear duct. Then control rats were given 0.9% NaCl. At autopsy, colon tumours 3