Monoclonal antibodies to detect human tumours: an experimental approach

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SUMMARY The use of monoclonal antibodies which can be raised to antigens of choice offers a selective and specific approach for the detection of tumours both *in vivo* and at a cellular level in biopsy specimens. We demonstrate that a monoclonal antibody raised to human teratoma will localise in a teratoma, growing as a xenograft in immune-suppressed mice.

One of the major problems facing oncologists, both clinical and laboratory-based, is the detection of metastatic disease, in particular, minimal residual disease (micrometastases), when they may be more amenable to various therapeutic regimens. While numerous technological advances in physical diagnostic methods have been made, neither they nor the biochemical or immunological assay of tumour markers in the serum or urine are able to detect micrometastases from bronchial, colorectal, or mammary carcinomas.¹

An alternative approach may reside in the use of immunological methods to detect tumour cells either *in vivo* or in biopsy specimens. This latter approach, which involves the use of immunocytochemistry, may be of profound significance for histopathologists in relation to their role in tumour detection in the future. This is well illustrated by Sloane and his associates,² who recently reported that it was possible to increase the histological detection rate of bone marrow micrometastases from mammary carcinoma when marrow aspirates were stained immunocytochemically for an antigen (EMA).

Another approach to the detection of metastases involves the *in vivo* use of radiolabelled antibodies. Success has recently been achieved by some workers³⁻⁵ although others have expressed doubt about the clinical applicability of this approach.⁶

Most of those studies have used radioantibodies to the ubiquitous carcinoembryonic antigen (CEA). Monoclonal antibodies⁷ offer the possibility of deriving reagents with a higher degree of tissue and/or tumour specificity. Their potential value, however, needs to be assessed in model systems before clinical studies are attempted. We present our initial results

using a monoclonal antibody (LICR-LON-HT/13) raised to human teratoma cells and assess its potential role for tumour localisation using a human malignant teratoma xenograft as a model system.

Material and methods

Tissue culture cells from an undifferentiated human malignant teratoma (embryonal carcinoma) (MTU) growing as a xenograft (HX 39) in immune-deprived mice⁸ were used to immunise mice in order to raise monoclonal hybrids. Twenty clones making antibody against this teratoma were isolated,⁹ and one of them, an IgG (LICR-LON-HT/13), was used for the purpose of radiolocalisation. The antibody was purified from ascitic fluid by ammonium sulphate precipitation followed by Sephadex G200 column chromatography.

The *in vivo* localisation experiments were performed in immune suppressed/CBA/lac mice bearing HX 39 human teratoma xenografts.⁸ These subcutaneously, serially transplanted tumours have been shown to maintain most of their histological and ultrastructural characteristics (unpublished observation) (Figs 1 and 2). This tumour is similar histologically to the lesion removed from the patient (Fig. 1). It secretes neither human chorionic gonadotrophin nor alpha fetoprotein but continues to express placental alkaline phosphatase.

Antibodies were labelled with ¹²⁵I or ¹³¹I by the chloramine-T method.¹⁰ One millicurie ¹²⁵I was reacted with 100 µg of monoclonal antibody, and free iodine was removed by gel filtration chromatography with a G25 Sephadex column. After iodination the HX 39 cells bound to the antibody

(a)

(b)

Fig. 1 *Malignant teratoma. The tumour cells form a cystic pattern with marked adenopapillary projections. Nuclear pleomorphism, areas of individual cell necrosis, and mitotic activity are marked. The appearances have a strong resemblance to those of some yolk sac carcinomas. In vivo the tumour was intermittently associated with elevated serum AFP levels. (Haematoxylin and eosin, a $\times 100$; b $\times 170$).*

Fig. 2 *Xenograft malignant teratoma. There is a considerable degree of resemblance between the xenograft and the original tumour (Fig. 1). However, the xenograft has larger, more pleomorphic tumour cells, which form many more solid than small cystic areas. Mitotic activity is even more marked. (H and E, a $\times 100$; b $\times 175$).*

specifically, showing that no loss of radioactivity had occurred. As a control for non-specific accumulation of proteins in tumours and tissues, normal mouse IgG was iodinated with ^{131}I to an equivalent specific activity.

Tumours used for this study had been implanted subcutaneously at varying periods of time between 10 and 21 days before the start of the localisation experiment. ^{125}I -monoclonal antibody and ^{131}I -mouse IgG were injected simultaneously intravenously into the tail vein of tumour-bearing and tumour-free animals, each receiving a total of 10-20 μCi of radioactive protein. The animals were subsequently killed by exsanguination between 2 and 96 hours after antibody injection. The tumours and organs were removed, and their radioactivity was assessed in a gamma-counter programmed for double isotope measurement.

Results are expressed as:

(a) localisation index, ie, the ratio of specific (^{125}I) to non-specific (^{131}I) activity in tumours and organs, divided by the same ratio in the blood; and

(b) absolute radioactivity, ie, disintegrations per minute per milligram of wet weight (dpm/mg).

We consider the localisation index to be an accurate expression of results, because it allows specific and non-specific uptake in tumours and organs to be compared, while simultaneously the relative blood concentrations are taken into account. This parameter also provides a standardised method of comparing values between various experiments.

Autoradiography was used to examine the pattern of accumulated monoclonal antibody within the tumour at a histological level. Tumours and other tissues were fixed in formol saline, conventionally processed, and embedded in paraffin wax, after which 5 $\text{m}\mu$ sections were cut. The tissue sections on slides were dipped in Ilford K5 emulsion at 55°C for 2 seconds and, after drying, were put in light-tight boxes and exposed for four to 35 days. They were subsequently developed (Kodak D19), fixed (AMFIX), and lightly counterstained with haematoxylin and eosin. Various pilot experiments were performed to establish the optimal exposure, subsequent processing, and staining. The slides were examined under both bright and dark field illumination. Calculations of radioactivity loss from the tumour during fixation and processing showed that only 9% to 15% of specific (^{125}I) activity was lost, while the loss of ^{131}I -mouse IgG was 40% to 60%. To prevent grains developing due to ^{131}I in each section, the slides were left for three to four weeks before being dipped in emulsion. Therefore, more than 95% of the grains seen by autoradiography on the sections are due to ^{125}I .¹¹

Results

The localisation index (see Material and methods) obtained in the tumour and in various organs at 24 and at 48 hours after injection of the radiolabelled antibodies is shown in the Table. Similar results were obtained at earlier time periods (not shown).

Localisation index at 24 and 48 hours

Tissue	Localisation index*		Specific radioactivity ratio†	
	24 hours	48 hours	24 hours	48 hours
Tumour	2.9	5.1	1.00	1.00
Liver	1.7	1.6	0.16	0.12
Salivary gland	1.4	1.5	0.16	0.12
Thyroid gland	1.6	1.6	0.13	0.11
Heart	1.6	1.5	0.12	0.08
Lung	1.4	1.5	0.13	0.08
Spleen	1.3	1.5	0.11	0.08
Stomach	1.2	1.6	0.09	0.07
Kidney	1.4	1.4	0.09	0.06
Intestine	1.4	1.4	0.09	0.06

*Tumour or tissue ^{125}I
 ^{131}I

Blood ^{125}I
 ^{131}I

†dpm/mg in tissue
 dpm/mg in tumour

Whereas the localisation indices in the organs ranged between 1.2 and 1.7 (the liver showed the highest uptake and the intestine the lowest), in the tumour the index is between 2.0 and 5.0 (Table) and in some tumours it reached 8.0. When the absolute amounts of radioactivity are considered, the tumour uptake is between six times higher than liver and 17 times higher than intestine.

From a further study of over 100 tumours in 69 animals (including unilateral and bilateral implantations), it has been consistently observed that the smaller the tumour the better the localisation (Fig. 3).

Autoradiography shows that the radiolabelled monoclonal antibody is seen only in viable parts of the tumour. Areas of necrosis and stromal tissue are generally devoid of grains. The antibody uptake follows two patterns. In the first, there is a high subcapsular concentration of radioactivity at the periphery of the tumour (Fig. 4). In the second pattern, scattered groups of highly concentrated grains are noted within the substance of the tumour, distant from the periphery. In most areas, the grains are seen around the periphery of the tumour cells (Fig. 3). Positive and negative chemography controls

Fig. 3 The localisation index is shown as a function of tumour weight in three separate experiments. Each point represents a tumour removed from an animal within 24 hours of receiving the antibody.

Fig. 4 Xenograft malignant teratoma. Autoradiograph to show the subcapsular specific radioactivity (a) and the location of the radioactivity in relation to the tumour cells (b). (a $\times 10$; b $\times 40$).

and sections of normal tissues did not show the above patterns. Normal tissue showed a generalised uptake of antibody distributed uniformly throughout the sections.

Discussion

The recent successful use of radiolabelled antibodies to detect *in vivo* both primary and metastatic tumours has reawakened interest in this sphere of laboratory-orientated diagnostic activity.³⁻⁵ In those studies, affinity-purified antibodies to CEA were employed. As CEA has a wide distribution in both normal and neoplastic tissues,¹² the use of antibodies with a higher degree of specificity would be advantageous. Monoclonal antibodies,⁷ which can be raised to antigens of choice, offer this possibility. Already, Ballou and his colleagues,¹³ using monoclonal antibodies against a murine teratoma, have successfully shown that radiolocalisation of tumours by γ -scanning is feasible.

Our results indicate that xenografts of human tumours can serve as ideal model systems to test the suitability of various monoclonal antibodies for radioimmunodetection. Using the present human teratoma xenograft model, we have found that the specific monoclonal antibody is taken up considerably more than non-specific IgG and that this specific uptake is considerably higher than in normal organs (Table). For tumour immunodetection by scanning to be possible, the tumour specific uptake should not only be greater than that of other organs but also be well above that of blood at the time of scanning. This has not been achieved by using conventionally raised antisera, and, therefore, reliance has had to be placed upon computerised blood-pool background subtraction.^{3,4} The results achieved to date demonstrate that in this system the levels of specific radioactivity localised in the tumour are such that blood-pool background subtraction and its limitations can be discarded, thereby facilitating camera localisation. Such scanning experiments are now in progress.

In our model system, the weight of the tumour is critical in obtaining optical localisation. Small tumours show the highest degree of specific uptake. This pattern is consistent in all our experiments. Furthermore, it is seen in any one animal which carries two tumours of different weights. One reason may be related to the appreciably greater amounts of necrosis in larger tumours. This observation, if true for other tumour systems and if it persists in the clinical situation, may be most valuable in assisting with micrometastatic detection.

The collection of radiolabelled monoclonal antibody in the periphery of the tumour and in scattered

groups within the tumour may be related to the vascular architecture. However, the importance of our autoradiographic studies lies in the demonstration that the specific radioactivity is located around viable tumour cells.

On the basis of our present results, we now plan to study the immunodiagnostic and therapeutic potential of this and other monoclonal antibodies in the *in vivo* clinical situation and at an immunocytochemical level in biopsies from sites where metastases from various tumours are known to occur. This latter approach has already been shown to be capable of detecting the presence of isolated small foci of metastases, even in the form of isolated single tumour cells.²

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