The effect of circulating antigen and radiolabel stability on the biodistribution of an indium labelled antibody

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Summary This study has investigated two of the main problems with radiolabelled antibody imaging, the formation of circulating immune complexes (I.C.) and the non specific binding of radiolabel to the antibody molecule.

Patients undergoing immunoscintigraphy with 111In labelled monoclonal antibody ICR2 were divided into three groups who received either the radiolabelled antibody alone (control, n = 12), the radiolabelled antibody which was incubated with the chelating agent diethylene triamine pentaacetic acid (DTPA) prior to size exclusion chromatography (n = 6) or whose radiolabelled blood flow was measured by computer gamma camera with online computer and printout. In tumours and normal tissues by gamma well counting of biopsy. Circulating antigen and immune complex was measured by high pressure liquid chromatography (HPLC).

The sensitivity of tumour imaging and the tumour uptake of radiolabelled antibody was not significantly different between the groups. Patients with high circulating antigen levels developed high levels of circulating immune complex but also had high tumour uptakes of radiolabelled antibody. Administration of cold MAb increased the splenic, but did not effect the tumour uptake of radiolabelled antibody and only minimally reduced levels of circulating immune complex. Chelate administration reduced the urinary excretion of radioactivity but increased the liver uptake of radioactivity.

These results have shown that successful antibody imaging can be carried out despite high levels of circulating antigen, that large doses of unlabelled antibody are required to prevent immune complex formation and that removal of non specifically bound 111In does not reduce the liver uptake of radioactivity.

Many studies over the last 10 years have demonstrated that radiolabelled tumour associated antibodies may be successfully used for the imaging of a variety of human cancers including those of the colon (Chatal et al., 1984), ovary (Shepherd et al., 1987), breast (Rainbury et al., 1983), lung (Chan et al., 1986), skin (Carrasquillo et al., 1987) and bone (Armitage et al., 1986). These studies have reported the results of imaging but have not attempted to correlate the results with the biodistribution of the radiolabelled antibody. Of the few studies which have specifically analysed the biodistribution of radiolabelled antibodies in patients with cancer (Hnatowich et al., 1985; Rosenblum et al., 1985), none have explained the localisation of tumours using antibodies which recognise an antigen present in the circulation and which therefore forms a circulating immune complex. Increasing the dose of administered antibody has been shown in some studies to improve the quality of images produced but the mechanism by which this is achieved, and the relationship to circulating immune complexes, has not yet been elucidated (Lamki et al., 1985; Carrasquillo et al., 1988).

Possibly the greatest problem which is encountered in immunoscintigraphy is the background uptake of the radiolabelled antibody which results in poor quality images. This is partly attributable to clearance of the radiolabelled antibody by the reticuloendothelial system (RES) and partly due to instability of the radiopharmaceutical (Halpern et al., 1983; Goodwin et al., 1986). With antibodies labelled with charged metallic ions such as Indium-111 111In using a chelating agent (usually diethylene triamine pentaacetic acid (DTPA)), unstable non specific protein binding may be a major factor in the background uptake of radioactivity (Hnatowich & McGann, 1987). This problem is most evident with the liver which may accumulate 30–40% of the injected radioactivity (Rainsbury, 1984). The pretreatment of 111In labelled antibodies with metal chelating agents has been shown in tissue culture to reduce the uptake of radioactivity by hepatocytes and may, therefore, improve the results of immunoscintigraphy (Davidson et al., 1990). This has not been investigated in human studies.

The present study has analysed the importance of circulating immune complexes to the tumour uptake and biodistribution of novel 111In labelled monoclonal antibody ICR2. Immune complex formation and tumour uptake of radiolabelled monoclonal antibody was examined following administration of cold unlabelled antibody to avoid the administration of radiolabelled MAb being bound in circulating immune complex. Secondly the effect of 'scavenging' radioantigen non specifically associated with the MAb was evaluated. An initial in vitro study on immune complex formation was carried out and a subsequent clinical study in which the blood levels, urinary clearance, tumour and normal organ uptake of the 111In labelled antibody was measured along with levels of circulating immune complexes and the antigen expression of the resected tumours. This was then compared to the images and the biodistribution of the radiolabelled antibody in further groups of patients in whom the chelating agent DTPA was administered to the radiolabelled antibody preparation prior to gel permeation column chromatography, either with or without cold unlabelled antibody being administered to the patient prior to the labelled antibody.

Materials and methods

In vitro study on cold antibody administration

The possible effect on immune complex formation of administering unlabelled antibody to patients prior to injection of the radiolabelled antibody was investigated using serum samples from normal controls and colorectal cancer patients.

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These were mixed with unlabelled antibody to give a 1:1, 3:1, 10:1 and 100:1 ratio of unlabelled antibody to the subsequently added 125I-ICR2. Following the addition of the radio-labelled antibody the samples were incubated for 1 h then analysed by size exclusion HPLC (Zorbax GF250, Du Pont).

**Clinical study**

**Patients**

Twenty-two patients undergoing immunoscintigraphy with Indium-111 labelled monoclonal antibody ICR2 for known or suspected colorectal cancer were investigated in the present study. Clinical details of the patients are given in Table 1.

The patients were divided into three groups. The first group was administered radio labelled antibody alone (control group A), the second group had their injectate treated with chelating agent during preparation (chelate group B) and the third group, group C, had the injectate pre-treated with chelate and in addition the intravenous infusion of 5 mg of cold unlabelled antibody over the 30 min preceding radio labelled antibody administration.

**Radio labelled antibody**

The rat IgG2a MAB ICR2 (Imrie et al., 1990) was used in the present study. This antibody was raised in Chester Beatty Hooded rats using defatted human milk fat globule membrane as immunogen. Reactivity of ICR2 with colorectal cancers and an immunohistochecmical comparison of its binding to epithelial membrane antigen (EMA) with an antibody to carcinoembryonic antigen has been described previously (Davidson et al., 1988; 1989a).

The MAB ICR2 was conjugated to DTPA using the bicycl anhydride (cdDPTA) method of Krejcarek and Tucker (1977). Conjugation of cdDPTA with ICR2 at a 2:1 molar ratio gave a molar substitution ratio of 0.7:1 and an optimal immunoreactivity (> 85%) as determined with a competitive binding radioimmunoassay using the epithelial membrane antigen expressing cell line MCF7 as target.

One milligram of the DTPA-ICR2 conjugate was labelled with 100–200 MBq of 111In by incubating the conjugate with 111In acetate pH 6 for 20 min. Removal of the unbound 111In was effected by gel filtration chromatography using a PD10 column (Pharmacia, Uppsala, Sweden). The pooled protein fractions were then sterilised by micropore filtration through a 0.25 μm filter (Millipore, France) and subjected to thin layer chromatography just before use to determine stability of the 111In label. In order to facilitate the removal of any 111In which may have been non specifically bound to the monoclonal antibody (not chelated to the DTPA group) some samples of the freshly radio labelled antibody were incubated with free DTPA (500 μmol for 15 min) prior to gel filtration chromatography.

The radio labelled antibody was administered to patients by an intravenous bolus having excluded their hypersensitivity by skin testing.

**Imaging and organ uptake**

Images were obtained of the anterior and posterior abdomen and pelvic outlet at 10 min and 24, 48 and 72 h after administration of 111In-ICR2 using a gamma camera (Siemens) fitted with a medium energy collimator and connected to a computer for data analysis and information storage (Nodecrest). A positive tumour localisation was assessed as a focal uptake seen in the images at 24, 48 and 72 h which was not present on the blood pool image and which corresponded to a known tumour site on subsequent laparotomy.

The gamma camera was also used to assess the uptake of radio labelled antibody in the liver, spleen, kidneys and bone marrow using an anthropomorphic phantom and region of interest (ROI) analysis for the liver and ROI analysis alone for the spleen, kidneys and bone marrow (Davidson, 1989b).

**Determination of EMA in serum**

Normal sera contains EMA or EMA like substances that bind to ICR2. To determine the amount in the patients sera a competitive radioimmunoassay was used that is based on the binding of 125I-ICR2 to plates coated with purified EMA (Ormerod et al., 1983). Serial dilutions of sera in phosphate buffered saline containing 0.5% bovine serum albumin (PBS/ BSA) were made in triplicate and 30 μl aliquots of each were mixed with an equal volume of 125I-ICR2 (105 c.p.m. MBq−1). Aliquots of 50 μl of the mixture were then transferred to 96 well plates (Nunc) that had been pretreated with EMA by covering each well with 50 μl of PBS/BSA containing 5 μg ml−1 of EMA and then blocking for 1 h at 37°C with 200 μl well of PBS/BSA. Unbound radioactivity was removed by repeated washing with PBS/BSA and then the bound 125I-ICR2 detected by counting each well in an Innol on Hydragamma 16 well counter (John Cant, Evesham, UK). Standard curves were prepared by mixing known concentrations of EMA with 125I-ICR2 and incubating with the EMA coated multiwell plates.

**Circulating levels of immune complex**

The levels of immune complex in the serum were measured at intervals after the administration of radio labelled antibody by high performance liquid chromatography (HPLC) on a Zorbax GF250 (DuPont, Massachusetts). The serum taken from patients before administration of the radio labelled antibody was also tested by HPLC for immune complex formation with 125I-ICR2. 125I-ICR2 was added to the serum to give a concentration of 0.3 μg ml−1 which is equivalent to the concentration found by administering 1 mg of MAB to a patient with a plasma volume of 3 litres (blood volume of 5 litres and haematocrit of 40%).

**Blood clearance, urinary excretion and tumour uptake**

Following administration the clearance of radio labelled antibody from the blood was determined by gamma well counting of paired 2 ml serum samples taken at 10 min, 30 min, 24, 48 and 72 h.

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**Table 1** Details of patients undergoing immunoscintigraphy

| No. | Initi. | Age | Diagnosis | Site | Treatment |
|-----|--------|-----|-----------|------|-----------|
| 1   | MD     | 60  | Ca        | R/S  | Lt Hemi   |
| 2   | MB     | 83  | Ca        | R/S  | Lt Hemi   |
| 3   | RS     | 53  | ?recur    | -    | Laparotomy|
| 4   | RN     | 66  | Ca        | Hep flex | Rt Hemi |
| 5   | DN     | 51  | Ca        | Hep flex | Rt Hemi |
| 6   | JL     | 71  | Ca        | Rectum | Ant resect|
| 7   | JH     | 79  | Ca        | Trans | Lt Hemi |
| 8   | BT     | 58  | Ca        | Rectum | Ant resect|
| 9   | JP     | 59  | Ca        | Sigmd | Lt Hemi |
| 10  | KH     | 65  | ?recur    |       | -         |
| 11  | MJ     | 67  | Ca        | Sigmd | Lt Hemi |
| 12  | BB     | 54  | Ca        | Rectum | Ant resect|
| 13  | VD     | 74  | Ca        | Sigmd | Lt Hemi |
| 14  | FH     | 76  | Ca        | Sigmd | Lt Hemi |
| 15  | BS     | 60  | Ca        | Sigmd | Lt Hemi |
| 16  | RD     | 64  | Ca        | Sigmd | -         |
| 17  | JP     | 76  | Ca        | Spl flex | Lt Hemi |
| 18  | EB     | 73  | Ca        | Adenoma | Caeum | Rt Hemi |
| 19  | DN     | 73  | Ca        | Sigmd | Lt Hemi |
| 20  | DF     | 79  | Ca        | Adenoma | Rectum | Laser |
| 21  | GX     | 67  | Ca        | R/S  | Ant resect|
| 22  | JB     | 56  | Ca        | Sigmd | Ant resect|

No.: number in series; Initi.: patients initials; Ca: carcinoma; ?recur: suspected recurrent tumour; R/S: rectosigmoid; Hep flex: hepatic flexure; Tran: transverse colon; Spl flex: splenic flexure; Lt Hemi: left hemicolectomy; Ant resect: anterior resection.
The urinary excretion and uptake of radioactivity by the tumour was calculated as a percentage of the injected dose of radioactivity (% I.D.) by preparing a known dilution of the injectate and determining its radioactivity at the same time as that of the sample of tumour or urine being measured, again using a gamma well counter.

Results

In vitro analysis

When $^{125}$I or $^{111}$In-labelled ICR2 which has been prepared for in vitro or clinical studies was analysed by HPLC it was found to elute as a single peak corresponding to a molecular weight of 150–160,000. When serum samples from either healthy volunteers or patients with cancer were mixed with either of the radiolabelled antibodies two peaks eluted from the column. The first, comprising approximately 30% of the labelled activity, eluted within the void volume of the column and had a molecular weight $>600,000$. The second peak contained the remainder and corresponded to the molecular weight expected for IgG (150–160,000).

With all serum samples the amount of high molecular weight complex formed with radiolabelled antibody was reduced by first incubating the serum with unlabelled antibody. The percentage of $^{125}$I or $^{111}$In labelled ICR2 present in the complex was reduced with progressive increase in the concentration of the cold antibody. In the example shown in Figure 1(a–d) immune complex was reduced from 37.6% of the total activity at a ratio of unlabelled to labelled of 1:1 (1a) to 5.7% at a 100:1 ratio (1d).

Clinical studies

Imaging results

Of the 22 patients to whom radiolabelled MAb was administered two were not imaged, three had benign tumours and two were shown not to have recurrent colorectal cancers. Of the 15 patients with primary cancers the number of tumour sites localised were not significantly different in control group (Group A) (five or six + ve), the group given antibody pretreated with chelate (Group B) (four of six + ve) or the group pretreated with unlabelled antibody (Group C) (three of three + ve). Similarly the level of background radioactivity in the images produced was not found to be different in an objective assessment by a nuclear medicine clinician.

Blood clearance

The clearance of radioactivity from the blood is shown as a percentage of the level at 10 min in Figure 2. Here, the mean effective half life of the circulating activity was 39 h for controls (Group A), 33 h for the Group B given chelate treated antibody and 27 h for Group C pretreated with cold antibody. Furthermore the level of radioactivity in the blood was significantly less in Group B at 48 h ($P = 0.005$) and in Group C at both 24 h ($P = 0.034$) and 48 h ($P = 0.000$) compared with controls. We conclude that the rate of clearance of antibody from the blood was enhanced by pre-treatment of radiolabelled antibody with DTPA or by pretreatment of the patient with unlabelled ICR2.

Figure 1(a–d) The effect of cold antibody administration on immune complex formation. HPLC analysis of human serum following reaction with $^{111}$In-ICR2 demonstrated a high molecular weight peak formed by immune complex and a low molecular weight peak formed by monomeric IgG. The effect of adding cold unlabelled antibody to the serum prior to radiolabelled MAb administration at concentrations of 1:1 a, 3:1 b, 10:1 c, and 100:1 d are shown. The area under the curve has been integrated to give the percentage of the total circulating activity forming the high molecular weight complex.

Figure 2 Blood clearance of radioactivity. The level of radioactivity in the circulation with time is shown compared with samples taken at 10 min after radiolabelled MAb administration. The results are mean ± standard deviation for the controls, chelate and cold antibody groups.
Urinary excretion

The urinary excretion of radioactivity was significantly reduced over the first 12 h period when DTPA treated 111In-ICR2 was administered (16.2 ± 6.94 ± 2.9 ± 1.8% of injected dose, \( P = 0.007 \)) but excretion was not affected by the administration of unlabelled ICR2.

Tumour uptake

The absolute uptake of activity in the tumours as determined by gamma well counting of specimens obtained at surgery ranged from 0.0016% I.D. g⁻¹ to 0.016% I.D. g⁻¹ with a tumour to normal colon ratio of 2.1 ± 0.915 (mean ± s.d.). There was no significant difference in the value between the three groups of patients.

Organ uptake of radiolabelled antibody

By 48 h after administration about 20% of the injected dose had accumulated in the liver of patients in the control group A (21.9 ± 4.6, Table IIa). An increased percentage of the injected dose was found to accumulate in the liver of patients in group B given antibody pretreated with DTPA at 45 min (22.2 ± 2.6 vs 18.1 ± 3.2, \( P = 0.049 \)), 24 (26.0 ± 4.9 vs 20.7 ± 3.5, \( P = 0.029 \)) and 48 h (31.6 ± 3.7 vs 21.9 ± 4.6, \( P = 0.002 \)). The preadministration of cold MAB to patients in Group C led to a reduced liver uptake at 48 h (26% vs 32%, \( P < 0.05 \)) but no significant differences were observed at the earlier times.

By 48 h after administration 2–3% of the injected dose had accumulated in the spleen in the control group (2.44 ± 1.4). Analysis of the counts accumulating in the spleen per MBq of injected activity showed this to be significantly increased in the other groups (Group B, \( P = 0.05 \)) (Group C, \( P < 0.05 \)) (Table IIb). No significant differences were found between the groups regarding the renal uptake or that of the bone marrow (data not shown).

Analysis of immune complexes in vivo

Serum from 16 of the 22 patients undergoing immunoscintigraphy were analysed by HPLC. When these sera were reacted in vitro with 125I-ICR2, a mean of 31.5 ± 3.6% of the radioactivity was found in the immune complex of >600 kD. When serum samples taken from the patients were analysed by HPLC the amount of the high molecular weight circulating immune complex was found to decrease with time after administration of the radiolabelled ICR2. When 5 mg of cold MAB was injected before the administration of the radiolabelled antibody only a small reduction in the quantity of immune complex was observed from 34.2% to 30.2% of the circulating radioactivity.

Circulating antigen levels

The apparent levels of EMA in the patients serum varied from 60–1150 ng ml⁻¹ with a mean level in the total patient group of 344 ± 340 ng ml⁻¹. No significant differences were noted between the rate of clearance of 111In-ICR2 from the blood of patients with low (n = 9, <500 ng ml⁻¹) or high (n = 6, > 500 ng ml⁻¹) levels of circulating antigen. A strong correlation was found, however, between the level of EMA in the patients serum prior to 111In-ICR2 administration and the amount of immune complex formed following administration, patients with higher circulating antigen levels forming greater amounts of circulating immune complex (\( n = 11, r = 0.61, P < 0.05 \)) (Figure 3). Circulating antigen levels also had a direct correlation with tumour uptake of radiolabelled antibody (\( n = 9, r = 0.65, P = 0.05 \)) (Figure 4).

Discussion

This study has shown that when the anti-EMA antibody ICR2 was injected intravenously into patients with colon cancer a high molecular weight immune complex was formed in the blood with antigen or antigen-like material.

Studies in vitro show that the same high molecular weight complex was formed when the anti-EMA antibody ICR2, labelled with either 111In or 125I, was mixed with serum from patients with colon cancer or normal controls. The reduction in amount of radioactivity present in the high molecular weight complex by the addition of cold antibody would suggest that it is a true immune complex. It would seem likely, although it is rarely reported, that radiolabelled monoclonal antibodies raised against other secreted tumour associated antigens (NIH consensus statement 1981; Magnani et al., 1983; Canney et al., 1984; Nouwen et al., 1985; Chia et al., 1983; Hilkens et al., 1986; Klug et al., 1986; Ashorn et al., 1988; Ben-Mahrez et al., 1988) would also form circulating immune complexes when administered for immunoscintigraphy (Mach et al., 1980a; Primus et al., 1980; Dillman et al., 1984; Zalutsky et al., 1988). Although it has been suggested by some authors that the presence of antigen in the blood does not significantly affect the results of imaging (Goldenberg et al., 1978; Mach et al., 1980b) other studies have shown that when an increased dose of antibody has been administered the number

| Time after | Patient group | Control | Chelate | Cold antibody |
| administration | (A) | (B) | (C) |
|---|---|---|---|
| 45 min | 18.7 ± 6.0 | 22.2 ± 2.6* | 22.8 ± 2.4* |
| 24 h | 20.7 ± 3.5 | 26.0 ± 4.9* | 25.8 ± 4.0* |
| 48 h | 21.9 ± 4.6 | 31.6 ± 3.7* | 25.8 ± 4.0* |

Organ uptake as a percentage of the injected dose (mean ± standard deviation). Statistical comparison with controls \( * P = 0.049; \* P = 0.029; \* P = 0.002; \* P < 0.05. \)

(b) Spleen

| Time after | Patient group | Control | Chelate | Cold antibody |
| administration | (A) | (B) | (C) |
|---|---|---|---|
| 45 min | 433 ± 154 | 603 ± 124* | 815 ± 157* |
| 24 h | 470 ± 159 | 615 ± 141* | 859 ± 110* |
| 48 h | 450 ± 149 | 526 ± 76* | 800 ± 143* |

Uptake as counts/5 min MBq of injected activity (mean ± standard deviation). Statistical comparison with controls. \( * P = 0.05; \* P < 0.05. \)

![Figure 3](image-url)
of tumour sites detected on immunoscintigraphy is increased (Halpern et al., 1985; Murray et al., 1985; Carrasquillo et al., 1986). This conflict of opinion on the significant of circulating immune complexes may be explained by differences in the antigen associated with the tumour and those present in the circulation and also in their affinity of binding to administered radiolabelled antibody. Such differences explain variations in the reported value of administering unlabelled MAb during immunoscintigraphy.

Based on the in vitro data a group of patients in the present study were given a small dose (5 mg) of unlabelled ICR2 prior to injection of the 111In-labelled antibody. This treatment did not, however, result in a significant improvement in the number of tumour deposits localised on imaging, nor did it enhance the tumour to background ratio of radioactivity or lead to a greater uptake of radioactivity by the tumours. This finding contrasts with those of previous studies where it was concluded that administration of cold antibody was beneficial. The earlier studies, however, claimed an improvement only in the number of tumour deposits localised in patients with multiple tumours and have not demonstrated increased tumour uptake of radioactivity (Halpern et al., 1985; Murray et al., 1985; Carrasquillo et al., 1986).

The biodistribution analysis suggests a reason why the tumour uptake of radiolabelled MAb was not significantly increased by the preadministration of unlabelled antibody in that it was found that the percentage of radioactivity forming immune complexes in the blood was reduced by only 4% following administration of 5 mg of unlabelled antibody. A large dose of cold antibody would therefore be required to prevent or significantly reduce the formation of immune complexes. A better understanding of the importance of circulating antigen in immunoscintigraphy would be achieved if accurate analysis of the biodistribution of radiolabelled antibody was performed routinely.

Clearly the problems associated with circulating antigen need to be overcome. One approach would be to use antibodies to tumour associated antigens which are not secreted or alternatively antibodies which recognise epitopes not present on the secreted antigen molecule.

A correlation was found between the level of circulating antigen and the uptake of radiolabelled ICR2 into the immune complexes, those patients with highest levels of circulating antigen having the greatest amount of circulating immune complex in the blood. However, the strong correlation between the amount of circulating immune complex present in the blood and the uptake of radioactivity by the tumour would not have been expected and has not been reported previously. This finding would suggest that patients with large amounts of circulating immune complexes and small amounts of free radiolabelled antibody have the best uptake of radiolabelled antibody into their tumours. The relationship between the expression of antigen at the tumour cell surface ad the amount shed into the circulation is controversial and is likely to vary from one tumour associated antigen to another (Wagener et al., 1981; Martin & Halpern, 1984; Philben et al., 1986). It is possible that satisfactory antibody imaging may be achieved despite high levels of circulating antigen if the tumour associated antigen is strongly expressed on the cell surface or the affinity of the antibody was greater for the tumour bound antigen than to that in the circulation.

The pretreatment of the radiolabelled antibody with chelate has not been found to be of any value in this clinical study. The uptake of radiolabelled antibody in the liver and spleen was greater in the group receiving chelate treated antibody than in the untreated controls, suggesting that this uptake of activity is largely due to stable radiolabelled antibody rather than dissociated 111In. This result contrasts with the situation in vitro where a significant reduction in the uptake of 111In-MAb by hepatocytes was achieved using chelating agents without reducing the tumour uptake (Davidson et al., 1990). The difference between the results found in vitro and in the clinical investigation may, however, be due to differences in the concentration of chelate in the environment of the hepatocytes, a high local concentration of chelate being required to prevent the uptake of 111In by hepatocytes. The concentration required to prevent hepatocyte uptake in vitro would not be safely obtainable in vivo. Indeed, studies on the use of chelating agents to inhibit the liver uptake of radiolabelled antibodies have been carried out in animals but the results have failed to establish a useful role for this procedure (Ward et al., 1986).

Before immunoscintigraphy can become routinely employed there are a number of problems which must be addressed. Firstly there is a need for antibodies with a greater tumour specificity so that the uptake by normal tissues is reduced. Second, it is essential to lower the non specific uptake and retention of radionuclide by organs such as the liver. Here the use of Iodine-124 or 99Tc would be expected to lead to substantial improvements (Goldenberg et al., 1990; Lamki et al., 1990). The use of bispecific antibodies which recognise both a tumour antigen and a low molecular weight probe that may be radiolabelled also offers a number of advantages (Goodwin et al., 1988), not least that administration of the radiolabelled probe can be done after antibodies that are non specifically bound to normal tissue are cleared from the cell surface. Other techniques have been employed to improve immunoscintigraphy. Paik and colleagues (1989) describe the use of a metabolisable linker joining the MAb with the radiolabel and showing increased tumour to background ratios in an animal xenograft model. The use of the biological linkage of avidin and biotin has also been investigated although the results of these studies remain preliminary (Kalofonos et al., 1990). Other methods for optimising immunoscintigraphy are required along with careful evaluation of the results.
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