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Perspectives and Commentaries

Limitations Associated with the Use of Labelled Antibodies Against CEA for Potential Tumour Localisation and Therapy

A Review

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INTRODUCTION

THE FIRST successful demonstrations that radiolabelled antibodies could be used to specifically localise xenografts of human tumours were reported by Goldenberg and Primus and their colleagues [1, 2] and Mach and his colleagues [3]. Extensive experimental studies followed which indicated that affinity purified anti-CEA polyclonal antibodies [4, 5] and monoclonal antibodies [6, 7] were likely to be more effective localising agents than the relatively crude antibodies used in the earlier studies. This is proving to be so at the clinical level where the procedure of radioimmunolocalisation (RIL) is beginning to complement other established methods of tumour detection [8].

The tumour to normal tissue discrimination achieved in both the xenograft model and in RIL using antibodies against various tumour markers, e.g. CEA, show that it is now reasonable to consider the use of radiolabelled or radionuclide-coupled antibodies for antibody-directed immunoradiotherapy. This idea, of course, is not new, but recent experimental studies in animals [9–11] and trials in patients [12] have produced encouraging results. In the next few years, the feasibility of immunoradiotherapy and its impact should become apparent. The problems are complex but, with the intensive investigations now being deployed in this area, it is likely that at least some progress will be made and some clinical benefit emerge.

The central problem undoubtedly, is to maximise the amount of antibody localising at the tumour site without excessive accumulation in normal organs. To achieve the necessary target to background ratio, that may be predicted on theoretical grounds, is a major challenge in cancer investigation and this has to be met before effective cancer immuno-radiotherapy can be achieved [13]. This review addresses some of the problems encountered and conveys some of the current thoughts on possible ways in which immuno-radiotherapy may be implemented.

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A USEFUL TEST MODEL

Antibodies against CEA have been used widely for RIL of colonic cancer and are likely to remain a potential targeting agent for therapy. Other promising markers, such as 17-1A, are emerging [14–16]. However, if immuno-radiotherapy is successful, it is likely that these will complement rather than replace anti-CEA antibodies to improve the diversity of the method. The CEA-anti-CEA system, therefore, provides a useful test model in which to investigate the various facets of antibody-directed tumour therapy.

Despite the limitations which I will discuss, the nude mouse tumour xenograft system has been extensively employed to identify localising antibodies. Moreover, this model also enables the pharmaco-kinetic factors such as absorption, distribution and clearance to be examined under different experimental conditions. Valuable information has been obtained from these animal tests, but they remain no more than a useful guide to manipulating antibody-directed targeting in man.

THE XENOGRAFT MODEL

The choice of the particular human tumour xenograft employed for *in vivo* studies is of necessity a compromise. First, the synthesis and secretion of CEA varies between different cell lines [17]. Different xenografts would therefore be expected to localise different amounts of specific antibody and this will affect the tumour to normal tissue discrimination reported by different laboratories. However, by choosing xenografts which secrete relatively large amounts of CEA, a comparative study on the effect of circulating CEA on tumour localisation could be made. Some human xenografts also secrete different amounts of CEA which is known to be related to the growth of the tumour [18]. This again must be taken into account when studying the dynamic aspects of antibody distribution since interaction with antigen, leading to immune complexes in the serum, could modify the mode of excretion of infused antibody and also its concentration at the tumour site. Variation between different CEA-producing xenograft systems, however, makes general comparison between different laboratories difficult.

Another problem encountered with xenografts derived from cell lines is their inability to express all the problems of heterogeneity of antigens as in naturally occuring human tumours [19, 20]. This means that some xenografts are more representative, in terms of epitope expression and secretion, than others in providing information pertinent to the human situation. It is likely that the use of mixtures of antibodies will reduce problems of heterogeneity, but selecting the best antibodies from experiments with single xenograft lines will be restricting. Preliminary experimental studies [21] and clinical studies by Chatal [16], however, have indicated favourably that combinations of antibodies may improve clinical tumour detection.

Apart from immunological reactivity other factors, relating to tumour vascularity and lymphatic drainage, may play a big part in determining antibody accumulation and release at the tumour site. These factors are likely to vary from one xenograft to another and between the mouse and the patient.

In our CEA xenograft studies we have used the human colon tumour 'MAWI'. This xenograft was derived from a mucoid adenocarcinoma of the colon which expresses moderate amounts of CEA. Notwithstanding the restrictions noted above, antibodies accumulating in this tumour have shown a favourable 'localising potential' in a large number of patients with CEA-producing tumours.

Antibodies vary considerably in the absolute concentration localising in the MAWI xenograft [22] and this has been found not to correlate with *in vitro* binding affinity (Table 1). It can be argued that antibodies which show relatively poor accumulation are unlikely to localise tumours in patients. However, this is not always the case. An example is the monoclonal antibody H58 which was raised to CEA expressed on HT29 colon tumour cells [23]. After labelling with 125-Iodine, with minimal loss of immunological activity, the amount of this antibody localising in the xenograft was found to be 0.56% of the injected dose at 24 hr falling to 0.08% by 7 days [22]. This compares with between 3 and 5% localising in tumour for relatively good antibodies [22, 24]. The use of H58 for RIL, however, showed that it was capable of locating tumours in the patient (Begent, unpublished observation). The reason for this may be related to the immunological specificity of H58 which is directed at a high mol. wt form of CEA [23].

The absolute amount of antibody localising in tumours of patients is generally much less than

Table 1. *The concentrations of five monoclonal anti-CEA antibodies in 'MAWI' tumour* 48 hr *after injection together with binding data relating to radiolabelled CEA* in vitro. *Data for a polyclonal anti-CEA* PK1G *is included for reference*

Antibody	Affinity (l/mol) in PBS	Affinity (l/mol) in 0.02M Tris	Isotype	Uptake in tumour†
1B1	8.8×10^7	9.1×10^8	IgG 1	0.05 (± .02)
H58	4.6×10^7	3.2×10^7	IgG 1	0.30 (± .07)
1C12	6.2×10^7	6.0×10^7	IgG 2a	3.10 (± .40)
MA/200	1.3×10^{10}	8.3×10^9	IgG 1	2.00 (± .10)
1H12	approx. 10^8	–	IgG 1	5.00 (± .70)
PK1G*	5.6×10^9	–	–	2.90 (–)

*PK1G is a goat polyclonal anti-CEA used routinely for R.I.L. in our laboratory. This antibody gave a straight line Scatchard plot.

†The uptake is measured as % injected dose of antibody/g tumour at 48 hr after injection.

that found in experimental systems. Apart from likely differences in tumour vascularity and excretory mechanisms, the larger blood volume in the patient leads to a lower initial concentration and a slower turnover of localising antibody in the blood pool. This in turn would reduce the net uptake in the tumour. An attempt to increase this uptake by use of combinations of antibodies is more likely to work if the epitopes recognised become saturated. Experimental studies (Rogers, unpublished data) suggest that extremely large doses would be necessary to achieve this unless the epitopes are sparingly expressed.

LOCALISATION OF ANTI-TUMOUR ANTIBODIES AND RESIDENCE AT THE TUMOUR SITE

Recent studies by Harwood [24] have shown that, as expected, the rate of accumulation of antibody in tumour xenografts is proportional to its concentration in the blood pool. Moreover the amount localising is similar to that encountered by normal organs such as colon and stomach. Tumours differ from these organs in the mode of clearance of antibody. Whereas the antibody concentration in normal tissues starts to decline after about 4 hr, it is maintained at the tumour site presumably by an impaired lymphatic drainage. The mechanism for this is unclear but a possible explanation is that immune complexes, in the extra-vascular spaces at the tumour site, form a lattice network which slows down the excretion. Antibodies which reside in the tumour for relatively short periods may form more soluble or smaller complexes than those which exhibit a prolonged localisation like the monoclonal antibody to CEA (1H12) [24] and the unrelated monoclonal antibody B-72/3 reported by Colcher [25]. If this interpretation is correct then the residence time of an antibody at the tumour site, and its absolute concentration, may depend on its valency of binding which determines the extent of lattic formation. In practice one should be able to increase the valency, and improve lattice formation, by constructing bi-specific antibodies which recognise different unrelated epitopes on CEA.

The influence of the Fc moieties of antibodies on their residence at the tumour site has been investigated by comparison with antibody fragments [7, 26–29]. Time studies by Harwood [29] have shown that the amount of F(ab')2 fragment localising in tumour at 24 hr after injection was the same as that of the corresponding intact antibody. The F(ab')2 fragment, however, partly escaped from the tumour site usually by 48 hr, whereas the intact antibody generally resided for much longer periods. Time studies beyond 24 hr showed a lower concentration of the F(ab')2 fragment, compared to whole antibody, at the tumour site. Experiments with a high affinity monoclonal IgM antibody to Stage Specific Embryonic Antigen-1 and its divalent fragment F(ab')2 have supported this conclusion [30]. These findings suggest that the Fc moeity of the antibody, presumably because of its ability to complex, is important for prolonged retention at the tumour site.

It may be possible to trap previously localised F(ab')2 antibody fragments at the tumour site with use of a second antibody Fab fragment directed at F(ab')2 and thereby increase lattice formation. This idea is appealing since it would afford an opportunity of increasing the amount of radiolabel at the tumour site. Bi-specific chimaeric antibody F(ab')2 fragments [31, 32] may achieve a similar role if they are directed at both the tumour target antigen and have specificity for a synthetic peptide which can be radiolabelled. These ideas, although theoretically possible and certainly worth considering, may have limitations imposed by pharmaco-kinetic factors.

DOSE ESCALATION STUDIES

An obvious way of increasing the amount of antibody at the tumour site is to increase the administered dose. With anti-CEA antibodies like 1H12, prolonged localisation at the tumour site should allow normal organ clearance to take place without incurring appreciable loss of antibody from the tumour even with large doses [24]. Therefore the tumour to normal tissue discrimination would be essentially maintained and the effective dose of 1H12 should increase. The problem of normal tissue toxicity, however, would still be dose limiting depending on the relatively slow circulatory clearance of the intact antibody.

Two approaches are being considered to accelerate circulatory clearance. First, the use of a second 'clearing' antibody which is discussed in the next section and secondly, the possibility of therapy using the smaller F(ab')2 fragments with their improved clearance over intact IgG.

Dose escalation experiments in the nude mouse model with F(ab')2 fragments against CEA have recently been reported in preliminary form [33, 34]. The main conclusions of these studies are that when the fragment was administered as a bolus, increasing doses up to 100-fold (380 μg) caused an increased demand on the mouse antibody clearance mechanisms resulting in a decrease in the tumour to normal tissue ratios at 24 hr after injection. By 48 hr, however, the tumour to normal ratios were no longer dose-dependent. The magnitude of the dose of fragment may therefore only be a limiting factor at time points up to 48 hr and may only be restricting when using therapeutic doses of antibody-isotope conjugates with

relatively short physical half-lives. However, it was noteworthy in these studies that at all doses up to 100-fold, circulatory clearance was incomplete at 24 hr but essentially complete by 48 hr after injection. This indicated that normal tissue toxicity would not be as severely affected by a dose increase of fragment compared with intact IgG.

TUMOUR TO NORMAL TISSUE DISCRIMINATION

Studies with F(ab')2 antibody fragments have indicated that their faster clearance, compared to intact antibodies, is the most important factor for superior imaging. Elimination of the Fc-part of the antibody eliminates receptor binding to cells in the liver, lung and spleen and greatly improves tumour to normal organ localisation ratios both experimentally and clinically [5, 27, 28]. Non-specific localisation of intact anti-tumour antibodies, largely due to Fc receptor binding, is supported by the failure of specific F(ab')2 fragments to accumulate in unrelated non-antigenic tumours and by the failure of non-specific fragments to localise in specific tumours [14, 26, 29].

The low overall tumour concentration of antibody fragments, discussed above, however, is one of the main arguments against using them for therapy. Unless the residence of fragments in tumour can be extended or compensated for by increased or repeated doses, whole immunoglobulin may be favoured for experimental and clinical investigations aimed at therapy despite the slower clearance from normal organs. One way round the latter problem has been to use a second antibody which, if administered after the localising antibody has accumulated in the tumour, will accelerate circulatory clearance of radiolabel with improved tumour: normal tissue ratios [35, 36]. In this way it may be possible to increase dose levels and the amount of radiation received by tumour cells, with minimum toxicity to normal organs.

Use of a liposome-entrapped second antibody to accelerate circulatory clearance of a goat anti-CEA has been demonstrated in the nude mouse model bearing a human colon tumour [37] and in patients. However, whilst this manoeuvre greatly improves tumour : blood ratios, recent studies have shown that entrapment into liposomes is unnecessary at least for imaging purposes. Unfortunately, studies in the xenograft model have shown that the use of second antibody without the liposomes causes the amount of localising antibody in tumour to be reduced by about 3–4-fold, while the amount in the spleen is increased (Rogers and Keep; R.B. Pedley, unpublished data). Moreover, prolonged splenic uptake is also evident in patients undergoing attempted therapy with localising and clearing antibodies (Begent, unpublished observation). The above findings imply that the second antibody is able to complex with localised primary antibody at the tumour site and then facilitate excretion possibly by an Fc receptor mediated mechanism. This premature loss of primary antibody from the tumour site would appear to be a serious limitation in therapy unless much larger compensatory doses of localising antibody can be administered. A more elegant way of solving this problem might be to resort to liposome entrapment of second antibody where, if the liposomes are stable, their effect will be restricted to intra-vascular clearance only.

The effectiveness of second antibody mediated clearance has been shown by Keeling and his colleagues [36] to involve Fc-receptor uptake which is species dependent. Unfortunately these studies indicated that mouse antibodies had a high affinity for human Fc-receptors which could explain non-specific uptake encountered in patients with some potentially good monoclonal anti-tumour antibodies. The severity of this restriction will undoubtedly depend on the affinity of receptor binding or on whether F(ab')2 or Fab fragments can be used.

EFFECT OF CIRCULATING ANTIGEN

The amount of circulating CEA in patients with gastrointestinal cancer varies widely. It has been shown that circulating CEA is capable of binding to infused antibody but imaging of tumours has been successfully accomplished under these conditions as reported by Primus *et al.* [38]. To account for this it was considered that the dose of antibody administered might exceed the blocking capacity of free antigen or that the binding affinity might be sufficiently low to minimise blocking. In addition *in vitro* studies have shown that a radiolabelled immune complex retains its ability to react with a CEA-immunoadsorbent. Thus, unless antigen exchange occurs, circulatory immune complexes may retain a free antigen combining site capable of reacting at the tumour site. However, because of the variation between patients it is difficult to assess the likely effects of circulating CEA on the amount of antibody localising and image quality.

Of greater potential importance are the possible effects of complexes on toxicity in normal excretory organs by therapeutic doses of radio-labelled antibody and also the diminution of radiolabel at the tumour site [18]. Toxicity may be particularly severe with antibody linked metal chelates since the presence of circulating antigen would cause rapid transport to the liver where breakdown would lead to accumulation of free metal ions. One way round these problems might be to inject small amounts of unlabelled antibody, possibly incor-

porated into liposomes, to reduce free circulatory CEA, to be followed, by the radiolabelled antibody shortly afterwards. This method may increase the effective antibody concentration at the tumour site and reduce toxicity if the doses and timing are optimised.

Cross-reactions of monoclonal antibodies with an antigen on granulocytes and red cells can impose severe limitations on RIL and therapy in humans. The study by Dillman [39] showed that toxicity was mainly related to destruction of these cells and an uptake of antibody in the spleen. These reactions, which may be related to Fc-receptor binding, were apparently unrelated to the known reactivities of anti-CEA due to normal cross-reacting antigen (NCA) on granulocytes or anti-H blood group activity on red cells. Fortunately these cross-reactions can easily be recognised by immunohistochemical techniques. Some monoclonal antibodies against CEA which show only feeble and sporadic reactivity with NCA containing macrophages, but not red cells, have been satisfactorily employed for RIL of human tumours.

METHODOLOGIC CONSIDERATIONS

It is not the aim of this review to consider the technical aspects of antibody production and labelling methods but there are a number of important points which are relevant. A major problem concerns the *in vivo* stability of the radiolabelled antibody. Particularly with iodinated antibodies, overlabelling can lead to diminished immunological activity or loss of affinity. This effects its biodistribution resulting in increased uptake in the liver and spleen [40] and failure to locate tumour. A more intractable problem with iodinated antibodies, however, is their tendency to rapidly dehalogenate *in vivo*, leading to urinary excretion and a tendancy of free iodide to localise in the thyroid and gastric mucosa [8].

In therapy extensive dehalogenation is an obvious limitation reducing the effective dose of iodinated antibody at the tumour site. This has been compared with that of indium-labelled antibodies against CEA where much higher amounts of label are acquired by the tumour [41]. The use of antibody–chelate conjugates offers the versatility of readily selecting different metal radionuclides with regard to their physical half-life and radiation energy. However breakdown of metal chelates takes place in the liver and, unless more stable chelates can be synthesised, subsequent excretion of the metal from this organ is very slow leading to high background and toxicity. The immunological tolerance of an antibody to radiolabelling, the type of isotope or radionuclide used and its *in vivo* stability are therefore of paramount importance when optimising the dosimetry of therapeutic antibodies.

FUTURE PROSPECTS

The aim of current work in the field of antibody-directed therapy is to establish ways of delivering the maximum dose of radioisotope to a tumour whilst minimising the toxicity to normal tissues. Some of the major obstacles concerned principally with physiological factors have been considered in this review. Non-specific accretion of antibodies due to Fc-receptor binding, increasing the residence time of antibodies at the tumour site and improving the tumour to normal tissue discrimination are major considerations. Antibodies against CEA and against other secreted antigens, are likely to play an important part in establishing the best approaches for immuno-radiotherapy. Currently a number of monoclonal antibodies to cell-surface antigens are being evaluated as possible vehicles for immunolocalisation and therapy. It should be informative to compare these with antibodies against markers which are secreted. Among the cell surface markers is 17–1A which has been shown to locate colonic tumours experimentally [14], and by RIL in patients [15, 16], and is showing promise as a potential targeting agent. The intensive studies on cell-surface glycolipids, especially gangliosides [42] and carbohydrate antigens [43], may provide antigen targets with greater cancer discrimination.

Bi-specific antibodies [31] which are directed at both the tumour target and either a synthetically constructed radioactive source, or a T-cell receptor as suggested by Staerz [44], may have therapeutic implications. In particular, recent studies by Reardon [45] have demonstrated the production of monoclonal antibodies against metal chelates which may facilitate the delivery of metals like indium to the tumour with minimised liver toxicity.

In conclusion, this review has focussed on some of the experimental aspects of antibody distribution which could have a bearing on attempts at experimental and clinical tumour therapy. Despite the problems, there is ground for optimism that antibody-directed tumour therapy may play a part, at least in conjunction, with other methods of treatment.

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