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Monoclonal Antibody Targeting of Anti-cancer Agents: Mühlbock Memorial Lecture*

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THE Mühlbock Memorial Lecture to the European Association for Cancer Research is dedicated to Professor Otto Mühlbock, who was closely involved in the founding of the Association and was its first President. I had the privilege of spending many hours with Otto Mühlbock on affairs of the EACR during my term of office as President from 1973 to 1979. During this time I came to respect this man of science who devoted his career in pursuit of the 'cancer problem'. He was a distinguished investigator and it is indeed fortunate that his thinking is still alive today through the researches of his students and colleagues at the Netherlands Cancer Institute. He constantly strived to forge links between the laboratory and the clinic and so while perhaps not a 'devotee' of tumour immunology he was prepared to concede that it 'might be useful'. He would, therefore, have been well satisfied to find that monoclonal antibodies are finding increasing usage in the detection and therapy of cancer including breast cancer.

The notion that tumour-localizing antibodies might be used for targeting therapeutic agents has become more appealing following the development of monoclonal antibodies which recognize antigens associated with many types of human cancer [1]. This may increase the therapeutic effectiveness of agents either by improving their localization in tumours, especially metastatic deposits, or by minimizing toxic reactions which represent a major limitation in cancer therapy with cytotoxic drugs. In principle, it is desirable to target highly toxic agents such as plant toxins or their A chain moieties (immunotoxins) which can kill target cells following internalization of only a few molecules [2]. In this case the antibody vector ideally should be highly specific for the tumour target cell and, following antibody–toxin conjugate binding to tumour cells, the toxin moiety should be efficiently internalized. An alternative approach is to target cytotoxic drugs which are already in clinical use and where toxic side-effects are considered acceptable although limiting in relation to therapy [3]. Here there are several pathways exploitable for antibody-directed delivery of drugs. As with immunotoxins, drugs may be targeted to tumour cells as antibody conjugates and exert their cytotoxic interactions after internalization. Additionally, where drug conjugates are constructed using biodegradable linkages, drug moieties can be released extracellularly following antibody conjugate localization and thereafter function as free drug. In this case antibodies do not have to bind to antigens associated with tumour cell surface membranes, although this is desirable. This increases the potential of antibody conjugates since agents can be targeted to extracellular tumour products such as carcinoembryonic antigen (CEA) in colorectal cancer and α-foetoprotein in hepatocellular carcinoma.

TUMOUR LOCALIZING MONOCLONAL ANTIBODIES

Antibody targeting of therapeutic agents requires that the antibody localizes in a tumour and also ideally uniformly penetrates regions of the tumour which contribute to its progressive growth. Also, the antibody should not react with normal cells or tissues or at least this reactivity should be sufficiently low as to ensure preferential uptake into tumour tissue.

The repertoire of murine monoclonal antibodies reacting with antigens associated with human tumours is now considerable and preparations are available against most of the major types of human cancer [1]. It must be recognised, however, that few, if any, have shown the desired absolute tumour specificity, since they recognise antigens (epitopes) shared with normal cells, albeit at reduced levels compared with tumour cells. Therefore the search continues for antitumour monoclon-

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al antibodies produced by murine, and more recently human, hybridomas with better tumour specificity. Even so, a limited number of antibodies have been found to localize *in vivo* in human cancers, this evidence being generated in studies where radioisotope-labelled preparations have been used in gamma camera imaging of patients [1]. These include anti-CEA antibodies in colorectal cancer [4] as well as monoclonal antibodies 791T/36, 19–9 and 17–1A [5, 6]. Ovarian-cancer-localizing monoclonal antibodies include 791T/36 and anti-human milk fat globule membrane antibody HMFG2 [7–9], whilst breast cancer localization has been demonstrated with anti-HMFG antibodies and 791T/36 [10, 11]. There are also several monoclonal antibodies which localize in malignant melanoma [12] and 791T/36 has been used to localize bone and soft tissue sarcomas [13].

Analysis of the biodistribution of radiolabelled antibodies in patients provides an assessment of their tumour to normal tissue localization, this being important with respect to the development of antibody targeting of drugs. This is illustrated by studies on the localization of ^{131}I- and ^{111}In-labelled 791T/36 monoclonal antibody in colon cancers when compared with adjacent 'normal colonic mucosae', the tumour to normal tissue ratio of radioactivity ranging from 2:1 to 8:1 [5, 14, 15].

TISSUE LOCALIZATION OF MONOCLONAL ANTIBODIES

Ideally, for effective delivery of agents linked to monoclonal antibodies, the conjugates should uniformly penetrate regions of tumour contributing to its progressive growth and then bind directly to all, or almost all, individual tumour cells. In fact, autoradiography of tumour tissue sections obtained following injection of radioisotope-labelled antibodies indicate a non-uniform deposition. For example, localization of monoclonal antibody 791T/36 in human osteogenic sarcoma xenografts was predominantly at the periphery of the tumour with only low levels of penetration [16]. Also, even though anti-CEA antibodies as well as $F(ab')_2$ were found to localize in colon carcinoma xenografts, their distribution was not uniform [17]. This was also the experience in a clinical trial on the distribution of ^{131}I-labelled monoclonal antibody 791T/36 in colon carcinoma, where antibody was predominantly localized in tumour pseudoacini and stroma [5] even though tumour cells derived from primary and metastatic colon carcinomas bind this antibody [18].

Vascularization of tumour, antibody transport across capillary endothelium and tumour diffusion are important factors in antibody localization. However, heterogeneity of antigen expression in tumour cell populations may be a more fundamental problem. Immunohistological staining with many anti-tumour monoclonal antibodies shows that tissue staining is generally quite variable with regions of intense antibody localization through to areas showing no reactivity. This is further illustrated by flow cytometry tests in which monoclonal antibodies have been reacted with tumour cells derived from primary and metastatic colon carcinomas. Whilst more than 90% of the cell preparations reacted with a monoclonal antibody recognizing tumour-membrane-associated carbohydrate antigen (Y hapten), the intensity of antibody binding was quite variable. When quantitated in terms of fluorescence signal/cell, 10% of tumour cells showed strong staining, 60% showed medium staining but 20–30% reacted weakly or not at all [18]. There was a similar distribution of colon carcinoma cells reacting with anti-CEA monoclonal antibodies, with approximately 10% of tumour cells only reacting weakly. But in this respect there is little understanding of the biological properties of tumour cells in relation to their reactivities with monoclonal antibodies. For example, in testing the reactivity of five monoclonal antibodies with colon carcinoma cells it was found that they bound most strongly to the aneuploid populations [18]. Related studies also showed that clonogenic cells isolated from primary colon adenocarcinomas demonstrated enhanced reactivity with several monoclonal antibodies. In this study with 50 colorectal carcinomas only one tumour failed to react with at least one of a panel of five monoclonal antibodies, and this was from a patient who had received radiotherapy prior to resection of the tumour. This emphasizes the view that by using a 'cocktail' of monoclonal antibodies it is possible to obtain positive reactions with all or almost all colon carcinomas.

IMMUNOTOXINS

Conjugates of plant toxins such as ricin, or more especially their A chain components, with tumour-localizing monoclonal antibodies represent potentially highly specific reagents for destroying malignant cells [2]. It has been clearly shown that ricin A chain-containing immunotoxins exhibit specific *in vitro* cytotoxicity for target tumour cells which react with the conjugated monoclonal antibody. But they have not proved uniformly effective *in vivo* when tested against murine tumours or human tumour xenografts. One factor here is the reduced cytotoxicity of A chain–antibody conjugates when compared with that of whole ricin. This is illustrated by studies on ricin A chain conjugates with monoclonal antibodies reacting with human T leukaemia cells (anti-T65 immunotoxin) and hu-

man melanoma cells (anti-p97 immunotoxin). Kinetic studies established that the time to reduce protein synthesis by 90% following incubation with target cells was 1.4–1.6 hr for ricin but 60–65 hr with the immunotoxins [19]. The rate-limiting step here probably is the transmembrane passage of the A chain following antibody binding to tumour cells, which evidently is less effective than that produced by the B chain component of the whole ricin [2]. Following on from the finding that ammonium chloride increased the *in vitro* cytotoxicity of immunotoxins, other lysosomotropic amines and ionophores including amanadine and monensin have been used as potentiators. Whether such agents can be used to potentiate *in vivo* responses is unclear, but another approach involves dual targeting of ricin A chain and ricin B chain conjugated to antibody [20].

The *in vivo* stability of immunotoxins is another factor which will considerably influence their therapeutic efficacy. This is emphasized by biodistribution trials with a number of ricin A chain conjugates showing that there was rapid degradation and release of the A chain moiety. This is especially important in designing treatment regimes. Even with these limitations ricin A chain immunotoxins have proven therapeutic activity when tested against xenografts of several human tumours, including osteogenic sarcoma, colorectal carcinoma and malignant melanoma. Based upon these investigations, clinical trials have been initiated in malignant melanoma and the outcome is awaited of these investigations with respect to therapeutic response and overall toxicity.

MONOCLONAL ANTIBODY–DRUG CONJUGATES

Conjugation of drugs to monoclonal antibodies aims to introduce the maximum number of residues under conditions which ensure optimal retention of both drug and antibody reactivities. Direct linkage of agents to antibody can be effected through a number of interactions, depending upon the availability of reactive groups in the drug. But only a limited number of drug residues can be introduced by direct linkage to antibody without producing protein denaturation and loss of antibody reactivity. In general, substitution ratios of greater than 10:1 with respect to IgG antibodies produce marked loss of antibody reactivity and in some cases substitution of as few as 3–4 drug residues/antibody molecule is sufficient to produce antibody damage. For example, a conjugation ratio of only 3:1 could be achieved when desacetylvinblastine azide was linked to an anti-CEA antibody, whereas ratios up to 10:1 with anti-melanoma antibody 96.5 yielded conjugates with adequate antibody reactivity [21]. Since most drugs are generally less cytotoxic than plant toxins, drug carrier systems have been introduced to increase the drug:antibody ratio. This involves first linking the drug to a carrier molecule which expresses multiple combining sites and then the drug-carrier complex is linked to monoclonal antibody. A variety of carriers have been used for drug conjugation, including human serum albumin (HSA) and dextran. For example, methotrexate conjugates using HSA carrier have been produced with monoclonal antibody 791T/36 to produce products containing 30–40 mol of drug per mol of antibody [22]. The design of drug–carrier–antibody conjugates is still undergoing development, but even so, products have been produced which retain adequate levels of antibody reactivity and drug cytotoxicity as assessed by *in vitro* testing [23–25]. Furthermore, evidence is accruing that conjugates suppress growth of human tumour xenografts. Whilst many of these trials have not been fully developed and evaluated, findings indicate that in some cases the therapeutic effectiveness of drug–antibody conjugates is superior to that of unconjugated drug. This is illustrated by trials with methotrexate–HSA conjugated to monoclonal antibody 791T/36 which effectively suppressed growth of xenografts of osteogenic sarcoma 791T cells [23]. When expressed as a ratio of tumour weights in treated compared with control mice (T/C ratio), free methotrexate reduced tumour growth by 50% (T/C:0.5) at a dose of 24 mg/kg body wt. Methotrexate–791T/36 monoclonal antibody conjugates produced a more pronounced therapeutic response, with a T/C ratio of 0.5 being obtained at a dose of methotrexate of 14 mg/kg body wt. Also a dose of 18 mg/kg of methotrexate in antibody-conjugated form almost completely suppressed sarcoma xenografts whereas the maximum dose of free drug tested (60 mg/kg) only reduced tumour growth (T/C:0.30). The therapeutic potential of methotrexate–antibody conjugates is further evidenced by trials with colon carcinoma xenografts, which are not susceptible to free drug but are suppressed by conjugates [26].

Similar trials are in progress with a range of drug–antibody conjugates, and whilst few are as advanced as the tests with methotrexate–monoclonal antibody 791T/36 conjugates, they do indicate that antibody conjugates have therapeutic potential. These include trials with vindesine linked to several monoclonal antibodies including anti-melanoma antibody (96.5), anti-CEA (11.285.14) and 791T/36 tested against xenografts of melanoma, colon carcinoma and osteogenic sarcoma [27].

In addition to improving therapeutic responses, drug conjugation to monoclonal antibodies often results in a significant reduction in drug toxicity. For example, with daunomycin, the LD_{50} for mice

given twice weekly treatment is 14 mg/kg body wt while drug conjugated to monoclonal antibody 791T/36 showed no toxicity at doses up to 30 mg/kg body wt. Similarly, acute toxicity tests with vindesine indicated an LD_{50} of 6.7 mg/kg whereas no significant toxicity was observed at doses up to 90 mg/kg in terms of drug when linked to antibody [27].

THERAPY WITH RADIOISOTOPE-LABELLED MONOCLONAL ANTIBODIES

The use of radioisotope-labelled monoclonal antibodies for radiotherapy is being actively explored following on from the gamma camera imaging trials. In one trial ^{131}I-labelled monoclonal antibody to human milk fat globule membrane (HMFG), which recognises a differentiation antigen on carcinomas, has been given to patients directly into sites of malignant effusions [28]. Dosimetry studies indicated that the dose delivered to malignant sites was 5000–7000 cGy, to normal organs 20–200 cGy and 10–25 cGy to whole body. No toxic side-effects was observed in patients and significant clinical responses have led to further testing of this approach.

CONCLUSIONS

The brief review of monoclonal antibody targeting of cytotoxic/cytostatic agents, although by no means exhaustive, illustrates the potential of these approaches to cancer treatment. It should also be noted that significant therapeutic effects have been observed by following treatment with antibody alone [29–31]. These responses may involve complement-mediated effects, although modulation of effector cells, including macrophages and possibly natural killer cells, is considered particularly important [32]. Accordingly, antibody conjugates with immunomodulating agents, including interferon [33, 34] and muramyl dipeptide [35], are being examined for enhancing cellular reactions to tumours. These approaches further illustrate the diverse options available for the development of antibody-targeted agents with potential for limiting the growth of tumours.

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