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Review

Antibody-mediated targeting in the treatment and diagnosis of cancer: an overview*

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Summary. Enhancing the discrimination between tumour and host has been an underlying goal of efforts to improve the diagnosis and treatment of cancer. Over the past 15 years considerable interest has focussed upon targeting systems designed to permit selective delivery of a variety of agents, including drugs, radioisotopes and toxins, to tumours, for both diagnosis and therapy. A vast body of information has accumulated on this subject, and considerable emphasis has been placed on the use of antibodies as carriers, as at present they offer the greatest clinical potential. Many targeting systems have been evaluated in vitro and in pre-clinical models, but few, with the exception of antibody-radioisotope conjugates, have been evaluated in patients. However, systematic evaluation of the therapeutic potential of immunoconjugates in the clinic is planned or already under way. While reviews of some individual aspects of antibody targeting do exist, there are none that encompass this entire field. Our objective is to fill this gap with a concise overview of antibody-mediated targeting for diagnostic and therapeutic applications.

I. Introduction

In order to understand the re-emergence of interest in drug targeting, an insight into the current status of cancer chemotherapy is helpful. Progress and expectations for cancer chemotherapy have been reviewed recently [30] and indicate improved long-term disease-free survival (1973 compared with 1983 figures) in, for example, testicular cancer, diffuse histiocytic lymphoma and acute lymphocytic leukaemia. However, this optimism is tempered by the data from colon and lung cancer where, for example, chemotherapy has failed to improve the treatment results. This is true even in relatively chemosensitive small cell lung cancer; improvement in terms of long-term survival occurs only in 10%–15% of those patients with the most favourable prognostic factors and the optimal duration of chemotherapy, and its value for patients in relapse and the additional use of radiotherapy have yet to be defined [197]. With early breast cancer, analysis of 80 randomised trials of drug treatment indicate a reduction in mortality for women receiving tamoxifen or chemotherapy [174, 207]. Despite this, adjuvant chemotherapy for primary breast cancer is still considered to be in a transitional phase of clinical research, as optimal drug combinations, treatment intervals and duration of treatment have still to be elucidated [21].

One of the major limitations of current chemotherapy for cancer is lack of selectivity of the drugs for only the cancer cells. This often results in high toxicity for normal cells, necessitating a subsequent balance in treatment schedules between killing the tumour and excessive toxicitiy to the patient. As a result of this, new avenues that have been explored include the synthesis and testing of new analogues with either enhanced activity or less toxicity; optimisation of drug scheduling; and a better evaluation of the regional administration of these agents [30]. To indicate the magnitude of the problem, of 1000 cisplatin analogues screened only 2 were found to have less renal and ototoxicity without loss of antitumour activity [30]. For most of these compounds, the toxicity problem remains, and it is this that has provided the stimulus for exploring the targeting of drugs to cancer cells. The aim of investigators in this area is not to replace the current modalities used in the treatment of the cancer patient – surgery, chemotherapy and radiotherapy, but rather to enhance, if possible, the armamentarium available to the physician. With targeted therapy the aim is to deliver toxic agents to their site of action on or near the tumour cells, thereby reducing toxicity to normal cells and increasing the therapeutic index of the compounds.

II. Carriers

Although a wide variety of carrier systems for the delivery of therapeutic agents to target cell populations have been proposed, the theoretical prerequisites of each should be similar. That is, the carrier should guide the toxic agent to its target and allow it to exert its effect at that site; it should be able to be linked to toxic agents without excessive loss of specificity or reactivity and without loss of activity of the agents and it should remain as a complex until delivery to the target. Additionally, protection of the therapeutic agent from the host's natural defence mechanisms would be desirable, thereby preventing premature inactivation of the conjugate of carrier and toxic agent.

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The concept of using antibodies to target toxic agents is not new and can be credited to that "indifferent student" [65] Paul Ehrlich at the beginning of this century [46]. The use of antibodies to target "warheads" such as isotopes, drugs or toxins has great theoretical appeal because of the unique specificity of the antibody for the target antigen. However, the exploration of the use of antibodies for targeting drugs and toxins did not gain momentum until the early 1970s [43, 67, 70, 71, 103, 118, 179] and was hindered by lack of relevant preclinical in vivo models; apprehension regarding the consequences of administering large quantities of foreign protein to patients; development of an immune response to repeated antibody administrations; lack of specificity of the antibodies and poorly defined targets. Some of these obstacles have been reduced if not completely eliminated. The development of the human tumour xenograft model [186]; studies that showed xenogeneic antibody could be safely administered to patients [89, 149]; that it could localise in tumours in patients [76] and see below; that specific monoclonal antibodies could be produced [112]; and that these antibodies could be used to better define potential targets in human tumours (see section on targets); all this paved the way for a re-exploration of this approach. This has been strengthened by studies that show that not only can monoclonal antibodies be safely administered but that they can have a therapeutic effect on their own in xenograft models [95, 97, 108] and in patients with leukaemias and lymphomas [42, 134, 145, 176], and that they can also be demonstrated in melanomas after patients have received antibody infusions [189]. However, in pilot studies in gastrointestinal tumours [191] and melanoma [82] no objective responses have been noted. The use of monoclonal antibodies and problems in and approaches to enhancing their efficacy in cancer therapy have recently been reviewed [152].

Another carrier system which has been explored is the use of liposomes. These small spheres consisting of one or more concentric phospholipid bilayers separated by an aqueous phase enable a variety of substances, including drugs, enzymes and hormones, to be incorporated within either phase [86, 225]. Manipulation of membrane polarity may result in variable degrees of membrane "porosity", allowing drug leakage to proceed at a set rate, which may also be dependent upon the local milieu of a target tissue, organ or tumour [232]. Studies using cytosine arabinoside incorporated into liposomes or doxorubicin entrapped in cardiolipin liposomes have demonstrated prolonged survival of mice with L1210 [111] or P388 [167] leukaemias, respectively. Other anti-tumour agents, such as actinomycin D, 5-fluorouracil, methotrexate and bleomycin, have also been incorporated into liposomes [84, 85].

The problems with the use of liposomes have been recently documented [225] and include: uptake in liver and spleen; inability to escape the bloodstream; disruption by serum components; antigenicity and immunogenicity; toxicity and instability. In an attempt to improve the selective targeting of liposomes, antibodies have been incorporated into their outer layers. More recently, incorporation of monoclonal antibodies into liposomes containing the fluorescent dye carboxyfluorescein has been achieved with subsequent demonstration, by fluorescence microscopy, of the fluorochrome within mouse lymphoma cells [99] or within CEA-expressing tumour cells [88].

Cellular carriers have also been proposed for drug targeting, although it is difficult to envisage their use in the specific targeting of anti-cancer drugs to tumours at the present time. These systems include the use of neutrophils containing indium-111-oxine for selectively imaging inflammatory lesions in dogs [129] and indium-111 autologous leucocyte imaging in patients with acute pancreatitis [6], and the selective delivery of vinblastine to macrophages in vitro and in the treatment of patients with idiopathic thrombocytopenic purpura [2, 3].

The targeting potential of other proteins, enzymes or molecules has been investigated even less than the systems already mentioned, but examples of their use are: macromolecules [200]; DNA [209]; arachidonic acid [187]; insulin [161]; concanavalin A [230]; hormones, e.g., melanotropin, a tropic hormone which binds to surface receptors on mouse melanoma cells and, when conjugated to daunomycin, shows selective killing of melanoma cells [217]; and receptor-dependent photosensitization – low density-lipoprotein delivery of a photosensitising agent, pyrene, to cultured tumour and animal cells and subsequent cell death after the cells are exposed to ultraviolet light [142]. Also, a lipophilic high-molecular-weight anti-cancer agent, poly(styrene-maleic acid) conjugated neocarzinostatin, when in lipid contrast medium has been shown to achieve both drug delivery and image enhancement in vivo in human solid tumours [113].

However, of all the carrier systems postulated, antibodies have shown the greatest potential and have been the most intensively investigated to date. The remainder of this review will be restricted to their use for targeting.

III. Target

To increase the selective delivery of toxic agents to cancer cells it is desirable to have clearly identified targets which, ideally, are expressed on the membrane of the cancer cells and not on normal cells. With the possible exception of the surface immunoglobulin idiotype expressed by certain B cell lymphomas [201], there is no conclusive evidence for human tumour-specific antigens. Many potential antigens have been identified, but all have been found to be tumour-associated rather than tumour-specific, three of the best known examples being alphafetoprotein (AFP) [1]; carcinoembryonic antigen (CEA) [74] and the common acute lymphoblastic leukaemia antigen (CALLA) [83]. The development of monoclonal antibodies [112] of reproducible specificity raised hopes that unique cell surface antigens in embryogenesis, differentiation and oncogenesis would be identified. While some of the antigens identified are mainly carbohydrate structures of glycoproteins and glycolipids present in many cell types [54], others have been shown to recognise non-carbohydrate determinants on glycoproteins. For example, monoclonal antibodies 9.2.27 and 155.8 have been shown to recognise different determinants of the core protein of a high molecular weight proteoglycan found on melanoma cells [91]. Although the early claims for tumour specificity of the antigens identified by some of these antibodies [9, 94] have not been realised [229], production and characterisation of monoclonal antibodies has resulted in the identification of tumour-associated membrane markers of potential value in diagnosis and therapy in a variety of cancers – melanoma [23, 91, 138] lung cancers [37]; breast cancers [188]; leukaemias and lymphomas [11, 120, 144, 175], to name just a

few. They have also been of value in clarifying the antigen expression of tumours, e.g. CEA expression by neuroblastomas, which have been shown to lack the antigen when tested with monoclonal anti-CEA antibodies [60].

For targeting with antibodies absolute specificity may not be necessary, as a higher density of expression by tumour versus normal may be sufficient to produce a relative improvement in the therapeutic index of a cytotoxic agent. Conversely, if the target is not expressed by key normal cells, such as those of the haematopoietic system, these might be spared the toxicity normally produced by the targeted agent. A potentially more serious limitation is that of antigenic heterogeneity both within and between tumours [38, 45], as well as between primary and autologous metastatic lesions [98, 147]. Attempts to circumvent this problem have been made in histopathology with the use of panels of monoclonal antibodies for the diagnosis of human malignancy [66] and the definition of both prognosis and high-risk patients in acute lymphoblastic leukaemia by immunological phenotyping [31, 110, 234].

The improved definition of the 'older' tumour-associated antigens, CEA and AFP, with monoclonal antibodies recognising different epitopes [35, 93, 133, 165, 222] and the immunochemical and biochemical characterisation of the newer candidates [8, 22, 24] have provided the necessary impetus for exploring the use of antibodies to these antigens as targeting agents.

IV. Antibody targeting

a) Diagnosis

An important consideration for the use of antibodies as carriers has been the demonstration that they can reach tumour cells and bind to them – in vitro, in vivo with animal tumours and human tumours growing as xenografts in immunodeprived mice, and in patients with cancer. Publications that demonstrate that antibodies can localise in vitro on human tumour cell lines or tissue sections using indirect immunofluorescence, immunoperoxidase or radiolabelled antibodies are legion and beyond the scope of this review. It was Pressman in the early 1950s who pioneered the use of anti-tumour antibodies as carriers of radioisotopes to cancer cells in animal models [114, 163]. Twenty years later, localisation of radiolabelled polyclonal anti-CEA antibodies was convincingly demonstrated with human CEA-producing colonic carcinomas growing either in the hamster cheek pouch [75] or as xenografts in nude mice [125]. These observations have been confirmed and extended for polyclonal anti-CEA antibodies using colon [62, 121] and breast [139, 141] xenografts and with antibodies directed at human chorionic gonadotrophin (hCG) which localised in human choriocarcinoma xenografts [190].

A major step forward with the practical implications of these results was the demonstration by Goldenberg and his group that ^{131}I-labelled goat anti-CEA antibodies could be used to detect a variety of carcinomas in vivo in patients using computer-assisted gamma camera subtraction scanning [76]. The use of polyclonal anti-CEA antibodies for radioimmunolocalisation has been subsequently demonstrated for breast [40] colorectal and gastrointestinal [44, 126], ovarian [216], and a variety of other cancers [166]. Antibodies to renal cell carcinoma [15]; alphafetoprotein (AFP) [78]; human chorionic gonadotrophin (hCG) [77] and ferritin [119] have also been shown to localise in vivo.

With the advent of hybridoma technology and the development of monoclonal antibodies, radioimmunolocalisation studies have been considerably extended. With xenografts, localisation has been reported with monoclonal antibodies to CEA [122, 159]; osteogenic sarcoma [158]; epithelial proliferating antigen [48]; melanoma [72]; germ cell tumours [140]; HLA [223]; breast tumours [33]; placental alkaline phosphatase [106]; colorectal and gastrointestinal tumours [34, 96] and lung tumours [233]. While these studies have amply demonstrated antibody localisation, the nature and specificity of the localisation is still under investigation, as it has been shown that polyclonal anti-CEA antibodies interacted predominantly with CEA in the extracellular tumour space, rather than on the cell membrane or cytoplasm of breast xenografts [141], and that while antibody specificity could be demonstrated after localisation of monoclonal anti-melanoma antibodies in xenografts, imaging of tumour deposits in this system could involve a non-specific element which was largely dependent on tumour weight [127]. However, use of an anti-melanoma monoclonal antibody (9.2.27) labelled with ^{125}I convincingly revealed preferential localisation of antibody for antigenexpressing melanomas but not for low-antigen-expressing tumour xenografts, and non-specific immunoglobulin of the same IgG2a subclass could not localise in the melanoma [102]. While the total amount of antibody accumulated in the tumour was directly correlated with size, the specific radiation in smaller tumours was, interestingly, found to be higher than in larger tumours. Another factor shown to be of potential importance in the immunoscintigraphy of xenografts with monoclonal anti-CEA antibodies is ionic strength [92].

Use of monoclonals for radioimmunolocalisation has been extended to clinical practice, and localisation of antibodies in primary and metastatic breast carcinomas [169, 202, 228] colorectal tumours [53, 164]; melanoma [117]; and a variety of other tumours [49, 195] using monoclonals recognising a variety of antigens including CEA and AFP [18, 104] have been reported. Although the feasibility of localising antibodies in patients has been convincingly demonstrated, the present technology does not generally reveal lesions of less than 1.5 cm, although detection of occult lesions has been reported in colorectal [80] and ovarian cancers [50]. Also, not all tumour sites in a patient and not all patients show localisation [44, 80], which may be related to size as well as to the antigenic heterogeneity of the lesions.

A variety of approaches have been tried to circumvent these limitations: The use of antibody fragments [Fab und $F(ab')_2$] to reduce accumulation in the reticuloendothelial system and to ensure a more rapid clearance from the circulation [25]; the use of different radioisotopes, for example, indium labelled antibodies [52, 143, 168]; the use of two antibodies which would complement each other e.g. anti-CEA and anti-colon specific antigen-p (CSAp) [62, 148]; and liposomally entrapped second antibody (LESA) [14]. While these approaches have met with some success, for example, with the detection of microfoci of colon adenocarcinoma cells in a lymph node less than 1 cm in size using ^{131}I-$F(ab')_2$ fragments [136] significant improvements over the results originally obtained by Goldenberg et al. [76] have yet to be made. The limitations tend to be techno-

logical and biological. Problems arise with both subtraction scanning and attempts not to substract; the fact that the use of monoclonal antibodies on their own has so far not improved discrimination, or antigenic heterogeneity of in vivo tumour deposits. A potential improvement, which has been suggested when the objective is to detect or to treat tumour in regional lymph nodes, is SC injection of antibody with subsequent lymphatic distribution. Using this approach, 2 mg guinea pig hepatocarcinoma line 10 could be identified in regional lymph nodes after injection of ^{125}I-labelled antibody and gamma camera imaging [226]. Such an approach has also been used clinically by Goldenberg's group, who administered polyclonal ^{131}I-labelled anti-CEA antibodies SC in the finger webs of patients with breast carcinoma. Subsequent scintigraphy showed localisation of radioactivity in the ipsilateral axillary metastases of all patients and in the contralateral axillae in three [40]. Similar results were also obtained with patients with gastrointestinal, genitourinary, lung and vulval tumours, although radioactivity was also noted in the nodes of patients without metastases, possibly due to the presence of CEA in the nodes draining the tumour site [41]. Radioimmunolocalisation is still in its relative infancy, and the role of radioimmunodetection in the management of cancer is still being actively evaluated, for example, in the staging of gastrointestinal tumours as a guide to second-look laparotomy [5].

b) Therapy

Most attempts to use antibodies as carriers for therapeutic purposes have involved their linkage to radioisotopes, chemotherapeutic drugs and toxins. The demonstration that anti-L1210 leukaemia antibodies conjugated to methotrexate led to improved survival of treated mice [128] stimulated renewed interest in this approach. Although radiation therapy is well established in cancer treatment, the systemic administration of radioisotopes has found limited application. One exception is in the treatment of thyroid cancer, where the selective uptake of radioactive iodide by thyroid tissue, both malignant and normal, is exploited. A question that is still unanswered in the choice of radionuclides for antibody delivery is the optimal particle emitter [20]. Isotopes emitting alpha particles have been used infrequently, owing to their general instability, although monoclonal antibody has been labelled with astatine-211 (^{211}At) with apparent preservation of antibody specificity in vitro and in vivo [218, 219]. Attention has focussed mainly upon beta emitters, such as ^{131}I or ^{32}P [20, 193] although others such as scandium-47, have been suggested as having potential [7]. Since α and β particles can exert a toxic effect without being in close proximity to the target cell's nucleus, they have a clear advantage over γ-emitters, and the ability of beta radiation to penetrate several cell layers has potential application in the treatment of tumours with poor vascularity and generally inaccessible target cells. Antigenic heterogeneity is theoretically less of a problem with this approach, as it should require relatively few antigen-expressing cells to target the radioisotope to the tumour where a toxic effect would be exerted regardless of antigen expression. The major disadvantage of this approach is that of non-specific irradiation of normal tissues during the distribution of the radionuclide throughout the body, an intrinsic property of the warhead independent of the specificity of the carrier.

Interest in the use of toxins arises from their intrinsic potency which, theoretically, offers the greatest chance of killing tumour cell populations that are inaccessible to conventional chemotherapy or that lack sufficient cell surface antigen density required of immunochemotherapy [171, 193, 205]. The basic structure of toxins is essentially similar, comprising two polypeptide chains (A and B) linked by a disulphide bond. A prerequisite for internalisation of the A chain, which interacts with ribosomes and inhibits protein synthesis, is binding by the B chain to surface cellular receptors. It has been estimated that the entry of just one toxin molecule into a cell is sufficient to kill it [231]. The use of intact toxins is, however, hampered by non-specific toxicity, which occurs as a result of interaction between B chains and normal cells, and this has been mainly responsible for preventing the administration of toxin-carrier conjugates to patients at the present time. However, efforts to overcome this by coupling A chains directly to carriers, e.g. antibodies or their fragments, have often resulted in loss of conjugate potency although selectivity of action has been demonstrated [87, 105]. Blocking of intact B chains using monoclonal antibodies, or through competition with excess galactose or lactose (essential components of cellular receptors that interact with abrin or ricin B chains), has been successful in vitro in preventing non-specific toxicity [204]. While the clinical application of this approach with non-covalently bound sugars is probably limited to bone marrow purging, conjugation of sugars to antibody, or incorporation of sugar into B-chain-binding sites in vitro may enhance in vivo applicability of intact toxins in the future.

Relatively large numbers of chemotherapeutic drugs have been investigated as warheads since Mathé's report [128]. This reflects the outlook that the use of anti-cancer drugs may represent the most clinically relevant model presently available for targeting. Anti-tumour antibodies linked to chlorambucil, in which antibody specificity and drug activity were retained, were found to be effective in a variety of animal models – EL4 murine lymphoma [70]; Ehrlich ascites tumour [57, 69] and rat Novikoff hepatoma [196]. The documentation, however, that antibody and chlorambucil could react separately but synergistically with tumour cells [39, 185] raised questions as to the mechanism of action of these conjugates in vivo. A range of methods have been used to produce antibody-drug conjugates, including direct covalent conjugation and the use of intermediate carriers such as dextrans or polyglutamic acid, in order to increase the number of drug molecules associated with the carrier [179]. Although there is indirect evidence that antibodies can deliver drugs to target cells, the exact mechanism of action of these complexes at the cellular level is not known. While many drugs exert intracellular effects that would require either internalisation of the conjugate or its dissociation at the site of action with the liberation of free drug, others, such as adriamycin, are able to exert a cytotoxic affect without entry into the cell [208].

In vitro efficacy has been demonstrated for antibody-drug conjugates in animal polyoma-transformed BHK21/C13 cells [185]; YAC lymphoma [100]; AFP-producing mouse hepatoma [16], and human systems – with, for example, anti-CEA antibodies [17, 107, 181]. As with the localisation studies, monoclonal antibodies offered the potential of better defined targets on the tumour cells and

very specific and pure antibodies. Demonstration of in vitro efficacy for monoclonal antibodies against human tumour-associated targets when conjugated to drugs or toxins has been extensive. For example, antibodies to a variety of human tumour-associated products when linked to chemotherapeutic drugs have been shown to be effective in the following systems: CEA [61, 181, 182]; osteogenic sarcoma [47, 63, 64]; melanoma [182, 213]; neuroblastoma [101]; and lymphoblastoid [212]. In addition, with monoclonals linked to the chemotherapeutic drug vindesine (VDS) in vitro efficacy of conjugate has been demonstrated to correlate with antigen density with VDS-96.5, a mouse IGg2a specific for p97, a melanoma-associated antigen [184]; VDS-791T/36, a mouse IgG2b raised against osteogenic sarcoma [47]; and VDS-11-285-14, a monoclonal antibody to CEA [61]. In vitro efficacy has also been demonstrated for toxins linked to polyclonal antibodies with animal [137, 150, 206, 215] and human [87, 105, 172, 203] cells; and with monoclonal antibody-toxin conjugates with cells from human colon cancer [73]; leukaemia [124, 173, 210, 227]; melanoma [28]; retinoblastoma [130]; ovarian [160]; breast [19] and cervical [211] cancers; and a variety of other human cancers [4]. Gelonin, a polypeptide plant extract with A chain activity, has also shown selective activity when used as an immunotoxin conjugate, but as with other A chain conjugates, this has been at the expense of potency [199]. An interesting approach has been the independent delivery of A and B chain conjugates in vitro, which has proved to be more effective than the use of A chain immunotoxins alone, possibly as a result of synergism between the two conjugates [220]. A novel, practical laboratory application of immunotoxins has been the recent report of the selective killing of contaminating human fibroblasts in epithelial cultures derived from colorectal tumours, using an anti Thy-1-antibody-ricin conjugate [156].

A limited number of additional cytoxic agents have also been successfully targeted to tumour cells in vitro with subsequent demonstration of selective killing, although none has yet been used in vivo. A glycoprotein component of cobra venom, when conjugated to a monoclonal antibody raised against a human melanoma-associated antigen, was shown to be selectively toxic for cultured melanoma cells [221]. A number of enzymes, such as glucose oxidase and phospholipase C, have also been targeted and shown to be selectively toxic [58, 157]. Another avenue which is being explored is the use of antibodies to direct photochemicals to tumour cells in an attempt to reduce their toxicity to normal tissues. Monoclonal anti-mouse rhabdomyosarcoma antibodies linked to haematoporphyrin have been shown to specifically kill target cells in vitro and to inhibit tumour growth in vivo after light activation [131]. This same group have also demonstrated selective in vitro killing of human tumour cell lines bearing a common leukaemia-associated antigen with monoclonal haematoporphyrin conjugates which were activated with a laser light source [132]. As pointed out by the authors, however, the possible use of this approach in vivo remains to be established, particularly in view of the non-specific light sensitivity that circulating haematoporphyrin can produce.

While demonstration of in vitro efficacy is a necessary part of the evaluation of conjugates, in vivo efficacy is the key aim. The exploration of antibody linked to radioisotopes as therapeutic agents was pioneered by Ghose in Nova Scotia, and he and his colleagues demonstrated that ^{131}I-labelled anti-EL4 lymphoma antibodies were capable of curing mice as long as 3 days after the injection of EL4 cells [68]. More recently it has also been shown that radioimmunotherapy with anti-CEA antibodies labelled with ^{131}I could inhibit the growth of colon cancer xenografts in hamsters [79] and that boron-labelled polyclonal anti-CEA antibodies could be prepared for the evaluation of neutron capture therapy [135] and be successfully used to target boron-10 to CEA human xenografts in hamsters [81]. Studies using nude mice bearing human tumour xenografts are less well advanced, but initial reports have indicated that antibodies linked to drugs can be effective in suppressing growth of human tumours in vivo. One of the most extensively studied systems has been with monoclonal antibodies linked to the drug vindesine (VDS). In vivo efficacy with VDS-monoclonal conjugates has been shown with osteogenic sarcoma [10, 179]; melanoma [180] and with VDS-anti-CEA [180, 183, 184]. In the CEA model, studies with VDS-11-285-14, an anti-CEA monoclonal, have demonstrated that in vivo efficacy with lung and colorectal xenografts is related to target antigen expression, indicating the selectivity of the effect [29]. Similarly, a monoclonal antibody 9.2.27 directed against a human melanoma associated antigen has been shown to suppress in vivo tumour growth when linked to diptheria toxin A chain [27] and intratumoral injections of antibodies linked to ricin have resulted in specific damage to T-cell leukaemia xenografts [224]. In addition, increased efficacy of ricin A chain immunotoxins has been demonstrated in an L1210 leukaemia model when animals were treated simultaneously with conjugate and conventional anti-cancer drugs [194].

It is now 14 years since the first reports of the use of antibody-drug conjugates to treat human cancer, pioneered by the Halifax group [70, 71]. While regression was noted in two patients, not all nodules responded and evidence of antigenic modulation was seen. Subsequently, Oon et al. [154] reported patients (1 with melanoma, 3 with neuroblastoma) treated with conjugates of human antibody and chlorambucil, noting minor therapeutic effects. However, further clinical evaluation of conjugates awaited the events set out earlier in this section, and cautious evaluation of conjugates in the clinic has started again with, for example, the demonstration that polyclonal VDS-anti-CEA conjugate, when labelled with ^{131}I, localised in five of eight patients with advanced ovarian and colorectal tumours [59]. Also, polyclonal anti-ferritin, anti-CEA and anti-AFP antibodies linked to radioisotopes have been used clinically in attempts to achieve therapeutic benefit [51, 123, 155]. Toxicity was reported not to be a major problem in these studies, and in an attempt to prevent potential systemic toxicity ^{131}I labelled monoclonal antibody directed against a tumour associated antigen has been administered via intracavity routes in three patients [90]. Studies such as these are, however, still in the very early days of their evaluation.

An important promising area in which antibodies on their own, in conjunction with complement, or linked to drugs, toxins or isotopes are undergoing clinical evaluation is in the elimination of leukaemic cells from human bone marrow prior to autologous marrow rescue for patients undergoing high-dose chemotherapy. In animal models depletion of tumour cells in bone marrow by treatment with polyclonal [116] or monoclonal [206] antibody-

ricin conjugates and subsequent transplantation of marrow back into the animals has been shown to be effective in preventing tumour recurrence. In vitro elimination of cancer cells from human bone marrow with monoclonal antibody and complement [12, 13, 26, 151] or a pokeweed anti-viral protein immunotoxin [214] have also been reported. A novel approach has been attempted in neuroblastoma by first inoculating the bone marrow cells with a panel of monoclonals to neuroblastoma and then with goat anti-mouse Ig-coated microspheres containing magnetite. When placed in a magnetic field, 99% of neuroblastoma cells could be removed [109].

These studies have been extended into the clinic with the treatment of human bone marrow with monoclonal antibody alone [55, 162] or immunotoxins [56] to prevent graft-versus-host disease, or monoclonal antibody and complement for removal of tumour cells followed by reinfusion into patients with leukaemia and lymphomas [146, 177]. While these studies are preliminary, the results obtained have been encouraging and indicate one of the areas, initially at least, where antibody targeted chemotherapy may have a valuable role.

V. Conclusions and future

Within the next 5 years a number of well-conducted clinical studies evaluating the toxicity and efficacy of immunoconjugates in patients with cancer will be reported. The improved specificity of the carrier antibodies; the better defined targets; considerable evidence in human tumour xenografts and patients of the ability of antibodies to localise in tumours and to exert an effect on their own; and the demonstration of immunoconjugate/immunotoxin efficacy both in vitro and in xenograft models have all paved the way for such studies. Problems still remain, including antigenic heterogeneity, development of an immune response by the patients to the immunoconjugates, which could limit the number of treatments, and definitive proof at present that the carriers do deliver the toxic agents to the cancer cells. Progress is being made in the resolution of some of these problems. For example, the use of 'cocktails' of immunoconjugates recognising different antigens on target cells could overcome the problem of heterogeneity. Also, production of human monoclonal antibodies, while still in its infancy, has had a measure of success [36, 192], and improvements in the technique, e.g. the production of better fusion partners by producing hybrids between human myeloma cells and lymphoblastoid cell lines [115], should increase the availability of such reagents in the future. At least in theory, human monoclonal antibodies would not elicit the same intensity of immune response as xenogeneic antibodies, and this could considerably enhance the therapeutic potential of antibodies, on their own or as carriers of toxic agents. However, the antiglobulin response in patients receiving murine monoclonals is very variable and depends on a number of factors, such as antibody preparation, dosage and route of administration. It has yet to be demonstrated in the clinic that use of human antibodies will result in a reduced antiglobulin response and their potential in experimental cancer research has been recently summarised [153]. While the development of monovalent or hybrid antibodies [32, 198] has great potential in reducing the limitations imposed by antigenic modulation and low target antigen density, such antibodies have a major limitation in that having only one combining site for antigen can greatly reduce their affinity, with a resultant decrease in delivery capability. The recent production of monoclonal bispecific antibodies which recognise both CEA and vindesine (Corvalan, Smith and Brandon, personal communication) may hold interesting prospects for the future. Also, data are becoming available on the immunological and biological stability of immunoconjugates in vivo. Studies in rabbits with monoclonal antibodies linked to the toxin pokeweed anti-viral protein (PAP) have indicated that the conjugates have a half-life of up to 24 h and that they retain immunological and biological activity [170]. In addition, studies of the pharmacokinetics of ^{111}In-labelled anti-p97 monoclonal antibody in patients with metastatic malignant melanoma have indicated that increasing the total antibody dose can in fact decrease the distribution of the antibody to the liver [178], which may have important implications for reducing the nonspecific hepatotoxicity in imaging and immunoconjugate studies. With the current reappraisal of surgery, radiotherapy and chemotherapy in the management of the cancer patient there is an urgent need for the evaluation of other approaches, and targeted chemotherapy is one of those being explored. Clinical studies which are planned or under way will determine whether or not this potential is realised.

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Note added in proof: Since preparing this review the reports of the clinical experience with ^{131}I-antiferritin therapy in advanced Hodgkin's disease (Lenhard RE et al. 1985, J Clin Oncol 3: 1296–1300) and hepatoma patients (Order SE et al. 1985, J Clin Oncol 3: 1573–1582), as well as the first ever report of a Phase I trial of a monoclonal antimelanoma antibody-ricin A chain immunotoxin (Spitler LE et al. Xoma Corporation, presented at the International Conference on Monoclonal Antibody Immunoconjugates for Cancer, San Diego, March 6–8, 1986) emphasise that systematic evaluation of antibody mediated targeting in the clinic has started.