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Chemoimmunoconjugates for the Treatment of Cancer

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I. Introduction: Concept of Targeted Chemotherapy

Targeted chemotherapy involves the specific carrier-mediated delivery of chemotherapeutic agents to tumors or other target tissues. This approach presumes the existence of some molecular, genetic or metabolic characteristic that differs between target and nontarget cells such as a structural membrane protein, a cell-surface receptor, an intracellular enzyme, or an altered sequence in the genome. Until recently, a problem existed in establishing a discrete and accessible difference between neoplastic and normal cells; however, the isolation of some oncogenes and their products and the production of monoclonal and polyclonal antibodies to tumor-associated antigens indicate that it is possible to biochemically distinguish normal and tumor cells (1, 2). In parallel with definition of differences between normal and neoplastic cells is the development of reagents with a high degree of selectivity for targets on the surface and within neoplastic cells. Over the past 20 years, considerable interest has been focused on targeting systems designed to permit selective delivery of drugs, radioisotopes, and toxins to tumors for both diagnosis and therapy and a great deal of this research has been performed utilizing antibodies as carriers (Table I). As vehicles for carrying cytotoxic agents to tumors, antibodies have the greatest potential; however, a number of other possible carriers have been investigated (Table II). The advantages of antibodies and other carriers include: (i) the selective delivery of the cytotoxic agent to the tumor cells; (ii) the slow release of the cytotoxic agent from the conjugate enabling prolonged exposure of the tumor cells to the cytotoxic agent; (iii) the preferential uptake of the cytotoxic agent–carrier conjugate by tumor cells; (iv) the use of extremely cytotoxic agents which cannot be used alone because of toxicity; (v) the binding of cytotoxic agents to carriers, which may protect the agent from enzymatic degradation and rapid excretion. Evidently then, the use of carriers to target cytotoxic agents is an attractive and provocative area of research; however, for the drug-targeting concept to succeed, both the cytotoxic agent and the carrier when conjugated must retain their

TABLE I
AGENTS CONJUGATED TO MONOCLONAL ANTIBODIES

Agent	Examples
Toxins (3)	Ricin, Pseudomonas exotoxin
Anticancer drugs	see Table III
Enzymes (5)	Cytosine deaminase, carboxypeptidase
Chemotactic factors (6)	fMLP
Cytokines (7)	IL-2
Isotopes (8)	^{90}Y, ^{131}I
Radiosensitizers (9)	Misonidazole
Photosensitizers (10)	Chlorin-e
Liposomes (11)	
Nuclear magnetic resonance contrast agents (12)	Gadolinium
Plasminogenactivators (13)	
Carborane cages (14)	
Iron oxide particles (15)	
Other (16)	Muramyl dipeptide

Note. References are in parentheses.

activity *in vivo*. For this and many other reasons outlined below, the development of the hybridoma technique to produce monoclonal antibodies (MAbs) has led to the production of more refined cytotoxic agent–carrier conjugates (33).

As indicated in Tables I and II, there are many carriers and many "bullets" which could be targeted. This review focuses on drug–antibody conjugates; the use of toxins, isotopes, and enzymes are extensively reviewed elsewhere (34)—some reference to them is included for comparative purposes.

II. Monoclonal Antibodies as Carriers

A. DEVELOPMENT

The use of antibodies as carriers for cytotoxic agents has been under consideration since the first recorded suggestions for targeting (35). The earliest studies made use of antisera raised by immunizing mice, rabbits, sheep, horses, and goats with tumor cells or their subcellular fractions (36–38). Antibodies reacting with normal tissue antigens were removed by absorption with normal tissue homogenates, thereby rendering the antisera relatively "tumor specific." These approaches

TABLE II
NONANTIBODY CARRIERS FOR CYTOTOXIC DRUGS, TOXINS AND RADIOISOTOPES

Macromolecules
DNA (17)
Bovine serum albumin (18)
Polyamino acid carriers (19)
Dextrans (20)
Lectins
Concanavalin A (21)
Hormones
Insulin (22)
Melanotropin (23)
Thyrotropin (24)
Microparticulate carriers
Liposomes (25)
Cells (26)
Microspheres (27)
Genetically engineered cytokines
IL2-PE (28)
IL6-PE (28)
IL4-PE (28)
TGFα-PE (28)
IGF-PE (28)
CD4-PE (29)
IL2-DAB_{486} (30)
Miscellaneous
Arachidonic acid (31)
Epidermal growth factor (32)

Note. References are in parentheses.

were limited, principally because the reagents still lacked specificity for tumor antigens; however, many preparations were of value in formulating procedures for coupling antibodies to cytotoxic drugs (38–40). The desire for monospecific antibody reagents and some of the earlier difficulties with cell-mediated immunity to detect human tumor antigens provided some of the impetus for developing MAbs (41) and the advent of the hybridoma technology and MAbs represented a real advance in the field of tumor immunology (33). As a result of this technology, the production of many MAbs and the subsequent identification of tumor-associated antigens have considerably extended the possibilities of targeting cytotoxic agents to tumors. MAbs, by virtue of their unique specificity, the ability to select for the desired affinity, and ease of production, have surpassed polyclonal preparations as carriers for targeted delivery of cytotoxic agents to tumors. Indeed,

the prospect of using antibodies as vehicles for isotopes, drugs, and toxins only became a reality with the development of MAbs with some degree of specificity for tumors. Reexploration of this approach using MAbs has been strengthened by studies which demonstrated that xenogeneic MAbs could not only be safely administered to patients and localize in tumors (42) but could also have a therapeutic effect of their own in xenograft models (43, 44) and in patients with leukemia and lymphoma (45, 46). Although therapeutic effects against tumors have been obtained using MAbs alone, and these responses have involved complement-mediated effects or modulation of effector macrophages and natural killer cells (48), clinical responses to serotherapy have been variable (49, 50), and animal studies indicate there are limitations to this approach (51). The variable antitumor effects of MAbs, however, may well be improved by conjugation to cytotoxic agents, given that the cytotoxic potential and mechanism of action of many drugs and toxins are well understood as many have already been used in the clinic.

B. Antibodies Alone

Why not antibodies alone? They clearly function *in vivo* after active or passive immunisation, particularly for infectious disease. In practice, the use of passively administered antibodies, in cancer, has rarely been successful. With regard to antibodies only OKT3 (52) and Campath 1 (53) appear to be active in transplantation (both) and in lymphoma–leukemia (Campath-1). The reasons have been discussed elsewhere, but essentially there are three major problems: (a) amount of antibody bound; (b) poor mobilization of effector mechanisms by mouse antibodies; and (c) the development of immune responses to the foreign immunoglobulin—refered to as human antimouse antibodies (HAMA). Recombinant monoclonal antibodies consisting of murine variable sequences and human constant domains are now available and some have been tested in Phase I clinical trials (54). These recombinant antibodies where the variable domains of the mouse antibodies are engineered onto human constant domains, binds complement and have antibody-dependent cellular cytotoxicity (ADCC) activity and therefore may activate effector function in man. Alternatively, antibody constant domains have been modified (e.g., altered hinged region) to improve various functional activities (55) for improved therapy in humans. How these modifications effect the HAMA response is discussed below. More recently, additional approaches to increase the antitumor activity of monoclonal antibodies *in vivo* have been studied by administering biological response modifiers such as interferons (56), interleukins (57), and colony-stimulating factors (58).

C. Specificity and Localization

1. Targets

To increase the selective targeting of cytotoxic agents to neoplastic cells, it is desirable to have clearly defined targets which ideally are expressed on the cell surface of tumor cells but not on normal cells. Despite the repertoire of murine MAbs reacting with antigens associated with human tumors (59), there is no conclusive evidence for the existence of human tumor-specific antigens detected by murine MAbs—with the possible exception of the idiotype of surface immunoglobulin on B cell lymphomas (60). While the search continues for specific antitumor MAbs produced by murine and more by human hybridomas, the targeting of cytotoxic agents with MAbs of absolute specificity may not be necessary. For example, an antigen which has a higher expression on tumor than normal cells or is absent on vital normal cells (e.g., hemopoietic stem cells) may be a suitable target for the delivery of cytotoxic agents. Many potential antigens have been found to be highly tumor-associated, three of the best known examples being α-fetoprotein (AFP) (61), carcinoembryonic antigen (CEA) (62), and common acute lymphoblastic leukemia antigen (CALLA) (63).

A better definition of the known tumor-associated antigens, such as CEA and AFP, has been possible using MAbs recognizing different epitopes (64–66). CEA is representative of many tumor-associated antigens and is one of the most widely studied tumor markers. It is immunologically a complex macromolecule, expressing both protein and carbohydrate determinants on colon carcinoma cells (67) and has been reported to be cross-reactive with NCA-1 (68), NCA-2 (69), normal biliary glycoprotein (70), and some circulating cells (71). These types of cross-reactivities with normal tissues, displayed by many MAbs-binding tumor-associated antigens, make it necessary to clearly define the properties of the MAbs both biochemically and by immunohistochemical techniques before they are used as carriers for cytotoxic agents. Epitope analysis and immunohistology has allowed a number of CEA-specific and cross-reactive antibodies to be identified, providing the opportunity of using different mixtures of antibodies to overcome heterogeneity of CEA epitope expression found within individual tumors and between different patients (72). The isolation and characterization of cDNA clones encoding CEA reveal a highly conserved repeating structure (73). Antibodies to various parts of the CEA molecule have been made as an effort to obtain more specific antibodies (74). MAbs against CEA have proved to be of value for the radioimmunoassay of human circulating CEA (75), for the radioimmunolocalization of tumors (76), and as carriers of cytotoxic drugs such as

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