Antineoplastic Immunoconjugates and Immunotoxins

Victoria F. Roche
School of Pharmacy and Allied Health Professions, Creighton University, 2500 California Plaza, Omaha NE 68178

PROLOGUE
This paper discusses the scientific rationale, synthesis, therapeutic utility and clinical limitations of cancer chemotherapy with antineoplastic drugs or toxins covalently linked to monoclonal antibodies directed against tumor cell antigens. Chemical issues that must be considered when designing an effective antineoplastic immunoconjugate or immunotoxin (including purity, functional group suitability for conjugation, antigenic specificity, stability, solubility, potency and immunogenicity) are discussed. Synthetic strategies used in the generation of immunoconjugates and immunotoxins are presented, and the use of flexible spacer molecules as adjuncts to conjugation is described. The use of targeting antibodies conjugated to enzymes that selectively activate antineoplastic prodrugs on or near cancer cells (known as antibody-directed enzyme prodrug therapy, or ADEPT) is also addressed. The paper ends with a brief discussion on the development of chimeric and humanized antibodies, which are designed to decrease the incidence of the human anti-mouse antibody (HAMA) response that is so often responsible for treatment failure with these targeted anticancer agents.

INTRODUCTION
Cancer is among the most prevalent and devastating diseases that plague the human family. Surgical removal of solid tumors is done when possible, but there are times when surgical intervention is simply not feasible (e.g., in cancers involving blood cells or when tumors project into or around vital organs). Even when surgery is an option, follow-up treatment with poisons (which we call anticancer drugs) and/or radiation is often recommended to ensure that all neoplastic cells have been destroyed. At times, chemotherapy and radiation are all that are recommended to combat this potentially fatal disease.

The cell toxins used to treat cancer can be grouped into seven major classes based on structure or primary mechanism of antineoplastic action. These include:

- Alkylating agents, (e.g., nitrogen mustards, N-nitrosoureas) electrophilic species that target nucleophilic nucleotides in DNA. These compounds induce DNA crosslinking and inappropriate base pairing, and leave the DNA unable to replicate.

- Organometallic compounds (e.g., cisplatin, carboplatin) which, like alkylating agents, crosslink DNA after binding irreversibly with selected nucleotides.

- Antimetabolites, (e.g., methotrexate, 5-fluorouracil, mercaptopurine, 6-thioguanine, cytarabine) false substrates that inhibit enzymes required for vital cell functions and inhibit the de novo synthesis of DNA and RNA nucleotides.

- Hormones and their antagonists (e.g., tamoxifen, diethylstilbesterol, leuprolide, testolactone) which are often used in the treatment of cancers where tumor growth or cellular proliferation is dependent upon the action of endogenous sex or adrenocorticotropic homones.

- Antibiotics (e.g., anthracyclines, mitoxantrone, dactinomycin, bleomycin) which inhibit DNA-dependent RNA synthesis by interfering with normal cellular processes and functions, often by inhibiting key enzymes like topoisomerase II and via the production of cytotoxic free radicals.

- Mitosis inhibitors, (e.g., vinca alkaloids, podophyllotoxins, paclitaxel) which commonly interact with tubulin to inhibit the formation of active and functional microtubules that form the mitotic spindle essential to proper cell division.

- Biological response modifiers (e.g., interleukins, interferons) which attempt to augment the body’s own chemical mechanisms of cell growth control.

An ideal antineoplastic would be tissue specific (for the organ or tissue with the cancer), cell specific (for malignant cells) and non-toxic but, unfortunately, the ideal is not anywhere near the reality. Despite the best efforts of chemists to design and synthesize antineoplastic agents that selectively target tissues and cells exhibiting uncontrolled growth, all antineoplastics marketed today are highly toxic molecules, especially for cells with short half lives (e.g., GI tract, hair follicles, bone marrow). In addition to retarding the proliferation of the cancer, these agents induce a myriad of unpleasant, debilitating and sometimes life-threatening side effects including nausea and vomiting, hair loss, bone marrow suppression, hemorrhagic cystitis, myocardial toxicity and severe fatigue(1).

Like all cells, cancer cells express specific proteins on their surface that can act as antigens in the production of antibodies(2). When administered parenterally, antibodies generated against these surface antigens would selectively seek them out and bind tightly to them. If the antibody was also covalently bound to an antineoplastic drug or toxin, it could serve as a “homing device” to carry the toxic structure directly to the neoplastic cell in a type of “seek and destroy” mission. Once bound, the antibody-drug macromolecule (called an antineoplastic immunoconjugate) would be internalized by the cancer cell, and the internal machinery of that cell would liberate the toxin from its carrier antibody to do its cell-killing work right where it’s needed.

Although not devoid of therapeutic problems, this scenario suggests a chance for the relatively safe and selective delivery of cell toxins to individuals suffering from cancer.

1Professor of Medicinal/Pharmaceutical Chemistry.

Am. J. Pharm. Educ., 64, 197-204(2000); received 1/17/00, accepted 3/17/00.
Better drug targeting can often result in the use of lower drug doses, which reduces the chance of drug-induced side effects. Distinct improvement in the drug-induced toxicity profile of immunoconjugates when compared to the administration of unconjugated drug have been observed in some preclinical studies(3-5).

MONOCLONAL ANTIBODIES
Hybridoma technology has made it possible to generate the large quantities of homogeneous antibodies (called monoclonal antibodies or MoAb) needed to make therapeutic use of these proteins. Antibodies utilized clinically are most commonly murine-based IgG immunoglobulins, which are “Y” shaped proteins with four subunits (two heavy and two light). The chains are connected through several disulfide bonds. The two arms of the “Y” bind antigen, and are therefore known as the Fab domains. The stem of the “Y” is called the Fc domain since it crystallizes readily, is relatively constant in structure, and binds a cell-destroying mediator called complement. This antibody domain is responsible for anchoring the antigen-antibody complex to cells of destruction (cytotoxic lymphocytes and macrophage, in addition to complement proteins). Murine IgM antibodies have also been used therapeutically because they fix human complement better than IgG immunoglobulins. Their disadvantages include a sticky quality and a larger size, which makes them more difficult to handle in vitro(6).

MoAb generated against tumor cell proteins can specifically recognize both bound and circulating tumor products (e.g. a-fetoprotein and HCG in testicular cancer, and carcino-embryonic antigen in colorectal cancer), permitting both therapeutic and diagnostic utility(7). Antigen expression can be augmented by concomitant treatment with lymphokines (e.g., interferons) if necessary, which facilitates antibody recognition of the target cancer cell(6). Native (unconjugated) antibodies can be used therapeutically when cell destruction through phagocytosis is sufficient for clinical response(6), and some success has been observed in the treatment of breast cancer(8), colorectal carcinoma(9), malignant melanoma(10-12), ovarian cancer(13), lymphoma(12,14-15) and leukemia(12,15-16) with these proteins. Unfortunately, the beneficial effects were sometimes transient.

Antibodies against neoplastic stem cells have also been used to cull out cancerous cells in leukemia patients undergoing autologous bone marrow transplants(17). Side effects from this form of therapy result when the surface antigens of healthy cells demonstrate cross-reactivity with the tumor cell-generated antibody.

ANTINEOPLASTIC IMMUNOCONJUGATES
As previously noted, an antineoplastic immunoconjugate is made by covalently joining a MoAb generated against a tumor cell antigenic protein to an anticancer drug or toxin that would be appropriate to use in the treatment of that cancer. Nitrogen mustards, anthracyclines, vinca alkaloids, antifolates, 5-fluorouridine and its derivatives, maytansinoids and mitomycin-C have all been successfully conjugated to a targeting antibody(3-4,18-26). Some tumors that were refractory to other therapies have responded to immunoconjugate therapy, specifically breast cancer(27), malignant melanoma(28), lung cancer(23,29), lymphoma(30) and leukemia(27,31). Unfortunately, life-threatening toxicities have also been noted by many investigators, which has limited their widespread use in cancer therapy. Still, they may be beneficial in patients who have relapsed or who have failed to respond to more conventional agents.

Chemical Considerations
There are several important chemical concepts to consider when linking a drug to an antibody protein for clinical use. An immunoconjugate that is to be administered to a patient must be free of both unconjugated antibody and free drug. Prior to conjugation, MoAbs are extensively purified via protein precipitation and fractionation, ion-exchange, high pressure and/or affinity chromatography to isolate the desired protein and remove DNA and endotoxin contaminants. After conjugation these same techniques are employed to remove unconjugated or improperly conjugated protein which could precipitate an immune response in the patient and/or direct a toxic antineoplastic agent to healthy tissues through cross-reactive binding to structurally similar cell surface proteins. Purification of bound and unbound therapeutic proteins must be carried out in accordance with regulations and Good Manufacturing Practices (GMPs) established by the FDA for the production of MoAbs destined for clinical use. Unbound antineoplastic drug of low molecular weight should also be removed to avoid toxicities that would result from the non-selective distribution of free drug to healthy cells. Gel filtration and dialysis are purification methods commonly employed for this purpose(32).

While many drugs are released from the antibody protein prior to exerting their cytotoxic effects, some can act at target cell receptors while bound to antibody. Due to the natural variation in the extent and rate of release of free drug from the antineoplastic immunoconjugate in individual cancer patients, it is generally wise to avoid utilizing drug functional groups that are essential to receptor affinity for conjugation to antibody amino acid residues. For example, methotrexate’s N1 and the amino groups at C2 and C4 are important for binding the antitumor agent to its target enzyme, dihydrofolate reductase (Figure 1). These potential conjugation sites should be avoided in favor of the carboxylate groups of the glutamate tail, which promote cell penetration but do not bind with high affinity to the reductase(33-34). Likewise the mustard nitrogen of chlorambucil should not be covalently joined to antibody, since it must be free to generate the toxic aziridinium ion responsible for DNA alkylation (Figure 1).

The bond between the antineoplastic drug and targeting antibody must be stable enough to make the trip from site of administration to site of action (the tumor cell). This, of course, means stability in the blood and other body fluids. But, as it is common to plan for the eventual cleavage of active drug from the antibody, the chemical link between the two entities must have some vulnerability to metabolizing enzymes. Amide and ester linkages appear ideal candidates to meet this metabolic need, and both have been successfully employed in the synthesis of antineoplastic immunoconjugates.

Unless they are sterically or electronically hindered, esters are much more vulnerable than amides to hydrolysis in the blood. If the immunoconjugate is hydrolyzed prematurely, free drug will distribute wherever its physico-chemical properties

![Fig. 1. Structures of the antineoplastic agents Methotrexate (antifolate) and Chlorambucil (nitrogen mustard).](image-url)
permit both cancerous and healthy cells will be destroyed as free drug travels throughout the body, and the selective targeting purpose of the immunoconjugate will be defeated. Side effects (from both free drug and free murine antibody protein) will be the invariable result.

Amide linkages result in highly stable immunoconjugates. Amidase activity is very low in the serum, but high in lysosomes. An antineoplastic agent coupled to a targeting antibody by an amide bond should stay bound until the conjugate is delivered to the tumor cell. After the antibody anchors the immunoconjugate to the surface antigen, the complex is internalized and transported to the lysosome for digestion by amidase enzymes and/or the acidic environment in this organelle (pH approximately 5). The liberated drug can then diffuse into the tumor cell cytoplasm to inhibit cell growth by its structurally dictated mechanism.

Another critical chemical consideration that must be addressed when generating immunoconjugates is antigenic specificity. An antibody raised against a target antigen will only continue to recognize that antigen if its own structure remains relatively undisturbed. However, antibody proteins can undergo significant conformational change as a result of forming new covalent bonds with drug molecules. If these changes result in a loss of affinity for the target antigen, the immunoconjugate will lose its therapeutic utility. The massive antibody protein has a great number of reactive amino acid residues, each of which could conceivably be joined to drug through ester or amide bonds, but the risk of affinity-destroying conformational change increases with each drug unit added. This is particularly true if drug molecules are conjugated to residues that are close to the region of the protein that binds antigen. Some antibodies can tolerate loads of up to 30 drug molecules per antibody(36-38) while others lose reactivity if more than four drug molecules are attached(39). Lys residues (which exist predominantly in cationic form at pH 7.4) are particularly important sites for conjugation, but if too many of them are covalently bound to drug, water solubility is lost and inactivating precipitation results. For all of these reasons, it is very rare to see more than ten molecules of drug linked to a therapeutic antibody protein(36).

Some drugs (e.g., vendesine) have shown a lower antineoplastic potency when coupled to antibody than they do in the free state(40). Others (e.g., phenylenediamine mustard, doxorubicin and daunorubicin) have shown superior activity in conjugated form(18, 26, 41). The reasons for this disparity are not always clear, but may be related to differing antibody drug loads. Some investigators have also suggested that selective targeting of bound drug to the cancer cell, as well as differences in metabolic stability between bound and unbound drug, can sometimes explain the potency differential.

Even if a drug loses some of its antineoplastic potency when bound to antibody, the enhanced selectivity gained from immunoconjugation maybe a worthwhile trade-off if immunogenicity to the murine-based antibody can be controlled. Immune responses to the foreign mouse protein (known as HAMA or human anti-mouse antibody response) vary in intensity from patient to patient, and can range from a mild allergic reaction all the way to anaphylactic shock(42). As repeated exposure to the immunonoconjugate is often required for therapeutic benefit, a strong HAMA response can render this targeted approach to cancer treatment ineffective. Drug design chemists and molecular biologists are attempting to overcome this therapeutic drawback. Some approaches taken to date include the coupling of polyethylene glycol or low molecular weight dextran to the antibody, the use of the less antigenic Fab antibody fragment (rather than the complete antibody protein), and the generation of chimeric (partially humanized) and totally humanized antibodies(42).

**Imunoconjugate Spacers**

As we have already noted, antibodies are very bulky structures, as are many of the antineoplastic drug products used in immunoconjugate formation. Getting the coupling functional groups on drug and MoAb close enough to one another to react in high yield can be a very tall synthetic order. Even if the chemist can induce the covalent bond between the two players to form, the steric hindrance that results around the new covalent bond may be too great to allow for the metabolic release of drug by tumor cell enzymes. Rather than trying to conjugate the two large structures directly, an inert spacer molecule can be used as a bridging unit(36). In order to do its chemical connecting job, the spacer must be slender in structure, flexible, and reactive on both ends (bifunctional) so as to access “hard to reach” functional groups and form covalent bonds with both drug and antibody. The use of these chemical bungee cords in immunoconjugate synthesis has been shown to promote improved conjugation yields, enhanced drug loads on a single antibody, and faster rates of release at target cells by minimizing steric hindrance to coupling and hydrolytic reactions.

Amide or ester linkages between both drug and antibody and the spacer are routinely employed. Therefore, aliphatic, amino and carboxylic acid functional groups are commonly found in spacer structures used in immunoconjugate synthesis. Peptides like polyglutamate or chains containing Gly, Ala and other non-bulky residues lend themselves well to use as spacer structures (Figure 2). Succinic acid and cis-aconitine have also found favor in immunoconjugate chemistry.

A representation of a desacetylvinblastine immunoconjugate that utilized a succinate spacer is provided in Figure 3. In this conjugate, the free aliphatic hydroxy group on the vinca alkaloid-based antineoplastic drug was generated by hydrolysis of the original acetate ester. This alcohol was then conjugated to one of the COOH groups of succinic acid, forming an ester linkage. The second spacer COOH was conjugated with
the e-amino group of a MoAb Lys residue, resulting in an amide link between spacer and protein. As we will soon see, these chemical reactions must be coaxed along with compounds that activate the carboxylic acid-containing reactant.

Chemical Reactions Used in Immunoconjugate Synthesis

To make an ester bond between drug and MoAb or spacer requires the condensation of an activated carboxylic acid with an alcohol. An amide is formed by reacting a carboxylic acid and an amine. Therefore the antibody amino acid residues which contain these reactive functional groups (Asp, Glu, Lys, Ser and Thr) will be prime choices as sites for immunoconjugate formation(42). The same can be said for COOH, NH2 and OH groups on drug structures (the ones that are not essential for receptor interaction) and spacer, if used. The Tyr residue contains a phenolic OH group, but this reactive amino acid is most commonly used to introduce a radioactive iodine atom into the antibody protein. Even though OH groups on drugs could conceivably be reacted with a MoAb or spacer COOH to make an ester, they are most often modified to form reactive carbonyl-containing groups which can then be coupled with Lys residues on an antibody protein to generate the more biologically stable amide linkage. The following reactions have all been employed in the synthesis of immunoconjugates(36,42-43).

The mixed anhydride method of generating an amide bond is valued for its simplicity (Figure 4). Let’s assume we are attempting to join a carboxylic acid-containing drug with a MoAb Lys e-amino group. The carboxylic acid group of the drug is first reacted with an activated carbonyl group like that found on isobutyl chloroformate. The HCl generated in the reaction is consumed by a scavenger base (e.g., tributylamine), which drives the reaction forward. The product formed is termed a mixed anhydride because the two groups on either side of the (OC-O-CO) linkage are different. Water must be strictly avoided in this reaction, as it can destroy both the chloroformate reactant and the anhydride product. The carbonyl carbon of the drug, now activated by the electron-attracting anhydride structure, is attacked by the nucleophilic Lys e-amino group of the MoAb. The “non-drug” portion of the structure is displaced as a carbonate, and the amide-linked immunoconjugate is generated.

In the activated ester method of amide synthesis, the carboxylic acid on a drug is activated for attack by antibody Lys through the formation of a succinimido ester (Figure 5). The activated hydroxy group of N-hydroxysuccinimide attacks the carbonyl carbon of the drug to form the activated ester. The reaction is driven by the dehydrating agent dicyclohexylcarbodiimide (DCC), which reacts with water generated in the reaction to form dicyclohexyleurea (DCU), which precipitates out. As in the mixed anhydride synthesis, the carbonyl carbon attached to the drug is now highly electrophilic and will be readily attacked by the nucleophilic amino group of an antibody Lys to generate the amide conjugate. The N-hydroxysuccinimide catalyst is regenerated in the process.

When an antineoplastic drug contains an amino group suitable for immunoconjugate formation, it is popular to join it to an antibody NH2 group via a heterobifunctional reagent. Like the name implies, heterobifunctional reagents have two chemically distinct and reactive ends. Among the most versatile of these is the pyridyl disulfide reagent N-succinimidyl-3-
Fig. 6. The use of the heterobifunctional reagent SPDP in immunoconjugate synthesis.

(2-pyridyldithio)propionate (SPDP). This compound has a reactive succinimidyl unit on one end, and a reducible disulfide bond on the other. As in the activated ester method of immunoconjugate formation, the carbonyl carbon of the succinimidyl moiety will be readily attacked by the nucleophilic ε-amino group of the drug, forming an amide link and displacing N-hydroxysuccinimide (Figure 6). The disulfide bond of this intermediate can be reduced with dithiothreitol (DTT) to provide a highly reactive sulfhydryl moiety. Pyridine-2-thione is released in the process. Another molecule of SPDP is allowed to react with a MoAb Lys-NH₂ to generate a disulfide reagent that is susceptible to attack by the free sulfhydryl group of the activated drug in a fashion identical to that exhibited by DTT. The result is a drug-antibody conjugate in which both are linked by amide bonds to a disulfide spacer(43-44).

Other effective reagents used to join the amino groups on drugs and antibodies through a disulfide-containing spacer include 2-iminothiolane (2-IT) and N-succinimidyl-acetyl-S-thioacetate (SATA). These synthetic tools permit the chemist to control the number of antibody residues conjugated and minimize affinity-destroying conformational change in the protein. The structures of these and other conjugating and heterobifunctional reagents, are provided in Figure 7. The maleimido and haloacetyl moieties react selectively with sulfhydryl groups while N-hydroxysuccinimide and imidoesters are amine-selective reagents(43-44).

Homobifunctional reagents (which have the same reactive functional group on each end of the molecule) are available for immunoconjugate synthesis but they are, by nature, either amine specific or thiol specific in their reactivity. The structures of some popular reagents are provided in Figure 8. The use of homobifunctional cross-linking reagents is sometimes associated with inactivating protein aggregation(42).

If a desired reactive functional group is not normally present on the drug desired for immunoconjugate synthesis, it can often be placed there through creative chemistry. For example, a primary alcohol can be condensed with succinic anhydride to form a hemisuccinate intermediate. The free COOH remaining on the succinate, after activation by reaction with N-hydroxysuccinimide, can then be condensed with Lys from the MoAb as described for the synthesis of activated esters (Figure 5). Hydroxy groups on carbohydrates can be converted to aldehydes through periodate oxidation. An antibody Lys NH₂ can then attach through reductive amination to provide a stable saturated amine link between drug and antibody. Those interested
in these, and other, chemical conversions are encouraged to review the pertinent reactions in several readily-available references(36,42-45).

IMMUNOTOXINS

While not generally considered drug molecules per se, ribosomal inhibitory proteins such as gelonin(46-48) and saporin(49), plant toxins such as ricin(50-52), bacterial toxins like diphtheria toxin(32) and cobra venom(53-54) have all been conjugated with antibodies raised against tumor antigens. These immunotoxins have been used with some success in bone marrow transplants and in the treatment of leukemia, lymphoma, melanoma, ovarian cancer, small-cell lung cancer and other solid tumors.

Ricin and diphtheria toxin contain two chains, A and B, which are connected through disulfide bonds. The A chain is responsible for cell destruction through the inhibition of protein elongation. The B chain serves to ferry the A chain into the cell by binding to membrane glycoproteins (specifically, to galactose residues on the cell membrane), which stimulates endocytosis. The A chain alone is relatively non-toxic, since it cannot gain access to the intracellular protein synthesis machinery without the assistance of the B chain. Immunonojugates of these toxins are often made with only the cytotoxic A chain(55). The targeting antibody serves the same purpose as the B chain (i.e., allowing the toxin to bind to the outside of the cell and be internalized) but, unlike the natural B chain, does so selectively. The antibody will only deliver toxin to cells that present the antigen against which it has been developed (e.g., the tumor cells). If the antibody is cleaved from the A chain protein prior to docking on the target cell, the toxin will be unable to enter cells on its own, and should not cause use-limiting nonspecific toxicity. The highly basic ribosomal inhibitory proteins gelonin and saporin are not heterodimeric, and are non toxic until conjugated with a cell-targeting antibody(55).

Disulfide linkages, rather than amide bonds, are much preferred when toxins are the therapeutic agents being conjugated to antitumor antibodies(55). The disulfide bond mimics the natural form of the dimeric (two chained) structures, and provides conjugates that are often active intact. The A chains of both ricin and diphtheria toxin have free sulfhydryl groups which can be linked to MoAb Lys residues that have been treated with SPDP to provide a disulfide bridge suitable for attack by the toxin (refer to Figure 6). Ribosomal inhibitory proteins can also be modified by reaction of an amino group with SPDP or 2-IT to provide a reactive sulfhydryl group. This new protein SH moiety can be coupled to another SH generating on the MoAb through modification of a Lys residue with the SPDP reagent(55,56).

In addition to disulfide bonds, thioether linkages between toxin and antibody have been successfully employed(55-58). These stable bonds are most commonly generated through reaction of the antibody with maleimidobenzoyl N-hydroxy succinimide ester (MBS, Figure 7) followed by selective coupling of the maleimido moiety with a toxin SH group that is either present in the native toxin or incorporated through the use of SPDP or 2-IT. Unlike the disulfide bond, the thioether is nonreducible. The thioether conjugates of the A chain of both ricin and diphtheria toxin are less active than the disulfide, which implies that free toxin must be released before it can be internalized and effect cell destruction(55). If the B chain is present, the thioether linked conjugates retain full cytotoxic potency, indicating that reduction of the disulfide bond between the two toxin chains is sufficient for access to the cellular cytoplasm.

Like many antineoplastic drugs (especially the antibiotics and mitosis inhibitors) plant, bacterial and reptilian toxins are very large structures, and steric hindrance to reaction with equally bulky antibody proteins can be a major obstacle to efficient immunotoxin formation. Fortunately, flexible spacer molecules such as those shown in Figure 2 have been used successfully in the synthesis of immunotoxins that would have otherwise been very difficult, if not impossible, to produce(55).

ANTIBODY-DIRECTED ENZYME PRODRUG THERAPY (ADEPT)

ADEPT(59,60) represents a new twist on conventional immunoconjugate therapy. In this approach an unconjugated chemotherapeutic prodrug (which is, by definition, inactive) is administered after treatment with a MoAb that has been conjugated with the drug’s activating enzyme. Although the prodrug is not targeted specifically to the cancer cell, higher concentrations of active drug accumulate there because of the high density of activating enzyme. As the prodrug is metabolically converted to its cytotoxic form, it penetrates the cell bearing the bound enzyme as well as “naked” cells that are nearby. The catalytic enzyme is regenerated after each activation and able to react anew with additional prodrug molecules. Thus, since less conjugate can be given due to the catalytic nature of the enzyme and ability of activated drug to diffuse to cells not bound to the MoAb-enzyme conjugate, there is a decreased risk of use-limiting HAMA responses. Toxicity can be further decreased by administration of a highly galactosylated “clearing antibody” (which reacts with unbound conjugate and promotes hepatic elimination) prior to administration of the prodrug(61). The ADEPT approach of selective delivery of antineoplastic drugs to tumor cells has been utilized with prodrg versions of nitrogen mustards(62-64), doxorubicin and daunorubicin (65-67), methotrexate and other inhibitors of thymidylate synthase(68-70) and amygdalin(71).

LIMITATIONS OF IMMUNOCONJUGATE THERAPY

As scientifically promising as immunoconjugate therapy appeared to be when first envisioned, significant problems have surfaced with clinical use(32,35,42). An obvious problem is the possibility of side effects caused by drug or toxin that is allowed access to non-target cells. The specificity of the targeting antibody for the antigen against which it was raised is not absolute, and there will invariably be cross-over reactions getting antibody for the antigen against which it was raised(55). Like many antineoplastic drugs (especially the antibiotics and mitosis inhibitors) plant, bacterial and reptilian toxins are very large structures, and steric hindrance to reaction with equally bulky antibody proteins can be a major obstacle to efficient immunotoxin formation. Fortunately, flexible spacer molecules such as those shown in Figure 2 have been used successfully in the synthesis of immunotoxins that would have otherwise been very difficult, if not impossible, to produce(55).

Another clinical issue is the propensity of the Fc domain of the antibody to direct some of the immunoconjugate to macrophage-like cells of the reticuloendothelial branch of the immune system. These important cells may be destroyed by the toxic conjugate molecules in the process. Poor penetration of the toxic macromolecular conjugates into solid tumors may also preclude therapeutic utility in some cases. As previously noted, the HAMA response can be life threatening, and has been responsible for treatment failure in several clinical trials. This reaction to foreign protein has been proposed to occur in between 50 and 100 percent of people...
who receive murine-based immunoconjugates (72-73). Better tolerated chimeric antibodies (with murine Fab domains and a human Fc domain) and humanized antibodies (where only the antigen binding loops are murine) can be obtained through genetic engineering, fragment grafting, or antibody veneering, and mice bred to produce antibodies with human Fc regions (transgenic mice are now available (42). Clinical trials utilizing humanized antibodies in the diagnosis and treatment of cancer are ongoing (42) but documentation of efficacy and toxicity in comparison with murine-based antibody therapy is hard to find (74-75). Studies involving non-human primates are promising, however (76). It is possible that, with the extended availability of "primatized" and humanized antibodies anticipated in the future, the HAMA response will cease to plague patients, and they will be able to receive the repetitive doses of conjugated drug necessary to effect remission or cure.

In summary, intense interest in making immunoconjugates and immunotoxins safe and effective in the treatment of cancer has been demonstrated by both basic scientists and clinicians. The persistent and concerted effort of chemists, molecular biologists, geneticists, pharmacologists, biopharmaceuticalists and health care providers should eventually win out, and permit these targetted delivery systems to realize their full therapeutic potential in the treatment of neoplastic diseases.

References


