Abstract

Conjugating cytotoxic agents to antibodies allows for site-specific delivery of the agent to tumor cells and should provide increased efficacy and reduced non-specific toxicity. These site-specific cytotoxic agents are known as immunotoxins or "magic bullets" and have demonstrated great promise as therapeutic agents for cancer and other diseases. The historical developments and future potential of this new approach to cancer therapy are reviewed.

Introduction

Cancer is responsible for approximately twenty-five percent of the deaths in industrialized countries and it is estimated that there are currently over seven million cancer patients in America. The American Cancer Society estimates that over one million Americans will be diagnosed with cancer in 1992 and that approximately 520,000 people will die of cancer, making it the second leading cause of death in America. Despite these alarming statistics, anticancer therapeutics account for only the eighth-largest pharmaceutical market in the United States.

Cancer causes far more morbidity and mortality than diseases that account for far larger drug markets and has shown much slower market growth. The slow market growth for the cancer therapeutic market is primarily because of the problems associated with low efficacy and the serious side effects of the majority of anticancer drugs. Even the most effective drugs, including some biotherapies, show as little as a fifty percent success rate. In addition, most cancer therapy is extremely toxic since chemotherapeutic agents kill normal cells as well as cancer cells.

Cancer therapies have historically consisted primarily of surgery, chemotherapy, and radiation. Because of the risks and invasive nature of surgery, and the adverse effects of radiation and chemotherapy, there is tremendous opportunity for new non-invasive therapies which offer improved efficacy while reducing associated side effects. Recent advances have provided opportunities for developing new alternative treatment strategies. One approach is to target a cytotoxic agent to the cancer cell through the development of immunotoxins. The term immunotoxin designates monoclonal antibodies (or antibody fragments containing their binding sites) linked to cytotoxic agents: drugs, toxins, radionuclides or cytotoxic cells of the immune system. To accomplish this, the cytotoxic agent is attached to an antibody or a growth factor that preferentially binds to cancer cells.

This exciting new technology has proven to be a feasible alternative to conventional chemotherapy and shows promise as an effective therapy for many cancers which have not responded well to conventional therapies. According to a 1991 Frost & Sullivan market report, the market for cancer therapy immunotoxins is projected to be approximately $720 million by 1996 and should continue to grow as technological improvements provide for higher clinical efficacy and general market acceptance [1].

This article will review the progress as well as several problems inherent to this approach to cancer therapy, and briefly highlight approaches taken at the NIH and elsewhere to advance this technology.
Historical Background and Current Developments

About 1913, Paul Ehrlich conceived the idea of therapeutics which function as "magic bullets" [2]. Ehrlich's "magic bullets" combined the targeting properties of antibodies with cytotoxic agents. Nearly eighty years later, this vision is beginning to reach fruition. The protracted period between Ehrlich's conception and the potential realization of immunoconjugate therapy for certain cancers underscores the numerous technological difficulties encountered in this field.

The first consideration in developing a "magic bullet" for cancer therapy is selection of an appropriate carrier or targeting agent to deliver toxic agents specifically to the tumor cells. In theory, the targeting agent could be any moiety capable of selective binding to a receptor on tumor cells. Indeed, anti-tumor reagents have been produced by attaching cytotoxic agents to numerous cell recognition proteins, including antibodies, alpha transforming growth factor, epidermal growth factor, interleukins, and transferrin [3,4]. Site-directed cytotoxicity is aimed primarily at cell-surface antigens or at receptors expressed in high numbers on cancer cells or other cells of interest. Toxic substances can be conjugated to antibodies or fusion proteins that recognize the cell-surface antigens characteristic of the specific cell type that is targeted for treatment. The toxin complex then specifically binds to the targeted cells resulting in a localized high dose of the toxin to them while sparing the normal cells. Early attempts to develop "magic bullets" using polyclonal antisera against tumor cells were frustrated by significant cross reactivity with surface antigens on normal cells. Even after rigorous absorption against normal tissues, polyclonal antisera preparations vary markedly in reactivity, specificity, and reducibility [5]. Coupling cytotoxic agents to such antibodies exacerbates the non-specificity, and results in therapeutic preparations with unacceptable toxic side effects.

Development of antibodies specific to tumor cells was revitalized with the introduction of hybridoma technology in 1975 which permits production of monoclonal antibodies against a selected antigen [6]. Despite this significant advance over polyclonal antisera, many monoclonal antibodies to tumor cells retain some degree of cross-reactivity with normal cells. Anti-tumor immunotoxic conjugates displaying cross-reactivity to normal tissue must be employed judiciously to minimize toxic side effects. The search for new monoclonals with greater specificity against tumor cells is an ongoing endeavor. For example, NIH scientists have patented or have pending patent applications claiming an expanding portfolio of selective monoclonal antibodies useful for treating a wide variety of cancers, including medulloblastoma, glioblastoma, adenocarcinomas, squamous cell carcinomas, breast, colon, prostatic, ovarian, cervical, and esophageal cancer.

Most currently available monoclonals against tumor cells are derived from murine hybridomas. Multiple administration of such monoclonals stimulates immunological responses by the human host against the foreign mouse immunoglobulins [7]. Neutralizing human anti-mouse antibodies compromise the efficacy of immunoconjugate therapy. The ultimate solution to this problem, of course, would be to utilize human monoclonal antibodies rather than murine species. Progress continues in the developing field of human hybridoma technology. A group from the National Cancer Institute and Bionetics Research (a division of Organon-Teknika) has reported testing the immunogenicity of conjugates incorporating two human immunoglobulins directed against colorectal cancer. To date, the study has confirmed the general expectation that immunogenicity of human antibodies will be low [1]. However, nagging technical problems remain with establishing human hybridomas which prevent them from being a reliable source of human monoclonals in the near term.

In lieu of human hybridomas, much work has been directed toward "humanizing" murine monoclonal antibodies. A simple technique which markedly reduces anti-mouse immunoglobulin effects is to use only those portions of the immunoglobulin molecule responsible for binding affinity and specificity. Consequently, immunoconjugates have been constructed using Fab, Fab' or F(ab')2 fragments rather than intact immunoglobulin [8]. This eliminates the immunogenic epitopes of the Fc region of the mouse antibody. Two additional benefits may accrue from removal of the Fc portion of the antibody. Firstly, large immunoglobulin conjugates have difficulty permeating solid tumors. This problem is reduced substantially when smaller antibody fragments are employed. Secondly, such fragments eliminate non-specific binding to
non-target cells mediated via the Fc region; e.g., binding to cells of the reticuloendothelial system. These potential benefits must be weighed against negative consequences of using antibody fragments. For example, antibody fragments are known to be cleared from circulation more quickly than intact immunoglobulins [9]. Additionally, Fab fragments would not be indicated in situations where the Fc portion of the antibody is critical to the biological function of the antibody. For example, Michael Kaliner of the National Institute of Allergy and Infectious Diseases has developed a model for selective destruction of cells expressing high affinity IgE Fc receptors (e.g., mast cells in either malignant systemic or benign systemic mastocytosis) employing IgE immunotoxin conjugates [10].

Another approach to "humanize" murine monoclonals for human therapy relies upon application of recombinant DNA technology. Chimeric (or mouse/human) antibodies have been created whereby the constant regions of human immunoglobulins are fused to the variable regions of mouse monoclonal antibodies [11]. These chimeric antibodies retain the antigen binding specificity of the mouse monoclonal, elicit reduced human anti-mouse antibody responses in patients, and are not subject to the enhanced clearance rates of Fab fragments.

An exquisite extension of the recombinant approach involves constructing mouse/human chimeric antibodies which incorporate only the complementarity-determining regions (CDRs) from the mouse. CDRs are the portions of the antibody molecule which guide the antibody to its binding ligand. The remainder of the chimeric antibody structure is human, including the framework residues (FR) which support the CDRs and determine the disposition of the CDRs relative to one another [12]. A further variation on this technique, called 'veneering', was developed as a joint invention by Merck & Co., Inc. and Eduardo Padlan of the National Institute of Diabetes and Digestive and Kidney Diseases. Veneering judiciously replaces mouse exterior amino acid residues in the variable region of the antibody with those of the human. The premise of "veneering" is that the key residues in CDRs (i.e., those involved in preserving ligand binding) are "interior" and interdomain contact residues. Consequently, surface amino acid residues of mouse origin, which can be "seen" by the immune system in its immune surveillance, may be changed to their human counterpart without affecting ligand binding properties.

Selection of appropriate and optimal cytotoxic components for immunoconjugates also has been an area of active research. Early anti-tumor immunoconjugates combined antibodies with known low molecular weight chemotherapeutics such as radionuclides, DNA alkylating agents, and anti-metabolites. Recently, Otto Gansow of the National Cancer Institute has reported encouraging results using yttrium-90 conjugated to anti-interleukin-2 receptor antibody for treating T-cell leukemia patients. The greatest interest, however, has been in the use of bacterial and plant toxins as the cytotoxic element of therapeutic immunoconjugates. The best studied of these toxins are diphtheria toxin (DT) from Corynebacterium diphtheria, the lectin ricin from the seeds of Ricinus communis, and pseudomonas exotoxin A (PE) from Pseudomonas aeruginosa.

Both DT and ricin are heterodimeric molecules consisting of A- and B-chains. In both toxins, the B-chain is responsible for cellular binding and entry into the target cell, and the A-chain is a potent inhibitor of protein synthesis. DT bound to cell surfaces by the B-chain enters the cell via receptor-mediated endocytosis. Within the resulting endosomes, the B-chain of diphtheria toxin undergoes a conformational change which permits the A-chain to translocate into the cytoplasm. Once in the cytoplasm, diphtheria toxin A-chain irreversibly inhibits the protein translation machinery. Specifically, A-chain inactivates elongation factor 2 (EF-2) via an ADP-ribosylation reaction. Ricin binds to cells via affinity of its B-chain for terminal galactose residues of cell surface glycoproteins and glycolipids. Analogous to diphtheria toxin, surface-bound ricin becomes internalized within vesicle structures, and the B-chain facilitates the translocation of the ricin A-chain ("ricin A") out of vesicles into the cytoplasm. Ricin A inhibits protein synthesis via selective N-glycosidase activity which cleaves a specific adenine residue in the 28S ribosomal subunit. Both toxins are extremely potent; a single molecule is sufficient to inhibit protein synthesis within a cell.

Both DT and ricin have been conjugated to antibodies producing immunotoxins with potent cytotoxic activities [13]. Such immunotoxins, however, exhibit serious non-selective binding due to the binding
properties of their respective B-chains. Attempts to remedy this problem by conjugating only the A-chain of the toxin to the antibody produces immunotoxins with good selectivity, but variable cytotoxic potency [14]. Genes for both diphtheria toxin and ricin have been cloned, and recombinant constructs containing mutant B-chains are being tested for reduced cell binding. Richard Youle and colleagues at the National Institute of Neurological Disorders and Stroke have developed a recombinant DT with reduced cell binding properties [15]. When conjugated to anti-human transferrin receptor or anti-CD3 antibodies, this recombinant DT demonstrated up to 1,000 fold reduction in cell binding; yet was equal to wild-type immunotoxin in cytotoxic potency. David Neville's research group at the National Institute of Mental Health has developed similar immunotoxins utilizing recombinant DT.

Another immunotoxin system demonstrating exceptional promise has been developed in Ira Pastan's laboratory at the National Cancer Institute. This system utilizes the bacterial toxin pseudomonas exotoxin A (PE). Analogous to the B-and A-chains of DT and ricin, PE contains domain I and domain III which confer cell binding and cytotoxicity, respectively [16]. Cytotoxicity is accomplished by the same mechanism as in diphtheria toxin; i.e., ADP-ribosylation of elongation factor 2. Unlike ricin and DT, pseudomonas exotoxin A additionally has a domain II which mediates translocation of the toxic domain III across cell membranes. Consequently, it has been possible to abolish cell binding (i.e. domain I function) without disturbing membrane translocation functions [17]. Recombinant pseudomonas exotoxins, with truncations in domain I (PE40 and PE38), have been prepared. These modified forms of pseudomonas exotoxin are as potent as native PE, but are 100 fold less toxic to nontarget cells [3,18].

PE40 and PE38 have been used also to construct completely recombinant immunotoxins by fusing them to DNA fragments encoding growth factors, antibodies, and antibody-fragments. In this way, PE40 and PE38 have been joined to the carboxyl end of the fragment variable (Fv) portion of antibodies to produce, so called, "recombinant single chain immunotoxins". The Fv region is the smallest antibody fragment capable of binding antigen. They consist of two chains, each about 110 amino acids in size, held together by a linking peptide about 15 amino acids in length. Recombinant single chain immunotoxins have been constructed to selectively bind the human transferrin receptor, human IL-2 receptor, and an antigen, recognized by monoclonal antibody B3, found on many human carcinomas (e.g., prostate, colon, stomach, breast, ovary, lung, and bladder). These recombinant immunotoxins are particularly attractive in that they can be produced in large amounts in E. coli, have reduced animal toxicity, and appear to be well suited to penetrate solid tumors by virtue of their small size [19-21].

The application of recombinant toxins has markedly reduced toxic side effects associated with the native molecules. These still are foreign proteins, however, and repeated administration leads to host immunological responses against the toxin. Richard Youle's laboratory has developed an approach to further "humanize" immunotoxins. They constructed a recombinant immunotoxin, where the traditional bacterial or plant toxin is replaced by a human enzyme, angiogenin [22]. Angiogenin is a protein found in normal blood plasma, and has homology to pancreatic Rnase. While not cytotoxic toward intact cells, angiogenin is a potent inhibitor of protein synthesis once it gains access to the protein synthesis machinery within the cytoplasm. Attachment of angiogenin to antibodies directed against cell surface antigens results in endocytotic incorporation, followed by inhibition of protein synthesis. Using recombinant techniques, angiogenin was fused to a mouse/human chimeric antibody heavy chain gene. This antibody-angiogenin fusion protein was introduced into a transfectedoma which secreted the chimeric light chain of the same antibody [23]. The resultant F(ab')₂-like antibody-angiogenin fusion protein has the "magic bullet" properties of antibodies linked to plant/bacterial toxins, but elicits a reduced immune response in the host.

Also, recent studies are beginning to demonstrate synergistic antitumor effects when immunonconjugates are used in conjunction with other treatment modalities. Because of the advantages of site directed specificity and the potential for synergistic effect, immunonconjugates are expected to replace or supplement, in increasing measure, the use of unconjugated chemotherapeutics and radionuclides in therapy [1].
Market Outlook

The primary criteria for an immunotoxin are specificity and high potency, i.e., the toxin must be delivered to a specific cell type and must be able to get into the cells for maximum cytotoxic effect. The immunotoxin market is currently dominated by the use of one of three toxins, i.e., ricin toxin, pseudomonas exotoxin, and diphtheria toxin.

Although immunoconjugates show much promise for cancer therapy, they will certainly not replace surgery as the primary therapy whenever surgery is feasible. Even when it is known that not all malignant sites can be resected, it is important to decrease the tumor burden as much as possible, so that the non-invasive treatments, and the patient's own defenses, have a reduced task. Large tumor masses, which are most amenable to surgical resection, are also least accessible to conjugates and other pharmacological agents, because of their poor internal circulation [1]. Immunoconjugate therapy will most likely be the primary therapy of choice for inoperable cancers and for cancer micro-metastases, i.e., when cancerous cells move to various locations throughout the body.

Currently, the major obstacle to overall market acceptance of immunotoxins for therapeutic applications is non-specific toxicity. Price is also an obstacle: initially the cost will be $2,000 - $5,000/course of treatment for therapeutic immunoconjugates [1]. This increased cost for treatment may however, be justified by the decrease in required hospital care, i.e., the immunoconjugate therapies may prove to be more effective and efficient for treating numerous diseases with fewer side effects, and consequent faster discharge from the hospital.

What role immunoconjugates will play in future cancer therapies is not yet clear, but in solid tumors therapeutic immunoconjugates will probably be most useful for eliminating residual or occult sites of malignancy after surgery, and to reduce the tumor burden, prolong life and improve the quality of life for patients with advanced disease [1].

Immunoconjugate therapy is most obviously applicable to cancer therapy, but may have significant market penetration in numerous other therapeutic applications including: rheumatoid arthritis, diabetes, infectious diseases, AIDS, and graft vs. host disease.

Scientists at the NIH have played a major role in the development of this promising technology and continue to be at the forefront of new discoveries in the fight against cancer. These new developments are brought forward to the market place through the patenting and licensing efforts of the Office of Technology Transfer at NIH. Technology transfer is the process by which the discoveries of laboratories are brought forth into practical knowledge and useful products. The NIH Office of Technology Transfer's primary mission is to facilitate the transfer of technology from Federal laboratories into the private sector for further development and commercialization for the benefit of world health.

It is critical to the medical community and the public welfare that these new technologies find their way to the market place as quickly and safely as possible. The continued efforts of NIH scientists have resulted in major advances. Through the technology transfer process, the promise of "magic bullets" appears closer to realization.
References