# Immunotoxins and Recombinant Toxins in the Treatment of Solid Carcinomas

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**Cancer remains the second most common cause of death in our society, and advanced disease is often refractory to surgical, chemotherapeutic, and radiologic interventions. One novel approach to cancer treatment involves targeting a cytotoxic agent to a cancer cell. Immunotoxins have been developed that contain a potent toxin (either *Pseudomonas* exotoxin, ricin toxin, or diphtheria toxin) coupled to a targeting moiety that directs the molecule to cells expressing a certain antigen. Chemically coupled immunotoxins have been developed over the past 12 years. These bind to and kill cells expressing many tumor-associated antigens. Initial clinical results were disappointing, but recent results have been more promising. Furthermore, newer immunotoxins have been developed that will soon be in clinical trials. Some of these are recombinant toxins that have been developed using techniques of genetic engineering. Transforming growth factor-α, acidic fibroblast growth factor, insulin-like growth factor-1, interleukin-2, interleukin-4, interleukin-6, the binding portions of monoclonal antibodies, and CD4 have been used to direct toxins to cancer cells or cells infected with the human immunodeficiency virus type 1. Efforts are under way to circumvent problems such as immunogenicity that may limit the clinical usefulness of immunotoxins.**

In our society, deaths from cancer are exceeded only by those resulting from cardiovascular disease, and one in five Americans will die of cancer. Localized tumors are highly curable, ranging from 50% to 80% in most sites, usually as a result of surgical excision. In addition, certain leukemias and lymphomas can be cured with conventional chemotherapy. In contrast, solid cancers with distant metastases have poor 5-year survival rates despite surgical, chemotherapeutic, or radiologic interventions.

In the war on cancer, attention has turned to novel treatment approaches using the techniques of molecular biology and genetic engineering. One therapy involves targeting a cytotoxic agent to a cancer cell. The targeting moiety is frequently an antibody produced by monoclonal antibody technology. In this technique, B lymphocytes from the spleen of mice immunized against specific cancer cells are fused with an immortal myeloma cell line. A single clone of the resulting hybridoma cells is selected that is immortal and produces a single antibody reactive with a tumor-specific antigen. Biologists have sought to use a monoclonal antibody directed against a single antigen as a means of detecting cancer cells among a large and varied population of noncancerous cells. A direct application of this idea is the concept of an immunotoxin, a specific monoclonal antibody chemically coupled to a toxin.

If a certain population of cancer cells have a characteristic antigen that is either poorly expressed or not expressed at all on normal cells, hybridoma technology may be able to produce a monoclonal antibody to this antigen (**Figure 1**). An immunotoxin containing this antibody would then be able to bind specifically to cancer cells expressing this tumor-specific antigen, and, when internalized by the cell, the toxin moiety would cause cell death. An immunotoxin that is tissue specific and binds solely to a nonessential organ, such as the prostate gland, would also be a useful cytotoxic agent, in this case for prostate cancer.

More recently, genetic engineering techniques have produced recombinant toxins by fusing modified toxin genes to DNA elements encoding growth factors. Certain cancers overexpress growth factor receptors; these cancer cells preferentially bind recombinant toxins containing the appropriate growth factor and are selectively killed. Molecular biology techniques have also been used to fuse modified toxin genes to DNA elements encoding solely the binding regions of monoclonal antibodies. These molecules are called recombinant immunotoxins and kill cells with which the antibody reacts.

This review briefly traces the development of immunotoxins and recombinant toxins. It is not intended as a complete review of the subject. Rather, it focuses on the prospects of using immunotoxins and recombinant toxins

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Manuscript submitted October 6, 1992, and accepted December 16, 1992.

to treat solid human cancers. Both the potential advantages as well as unresolved problems will be addressed to allow the reader a balanced perspective of these exciting new agents.

## BACTERIAL AND PLANT TOXINS

To be an effective cytotoxic agent, a growth factor or antibody must be either chemically coupled or fused to a potent toxin. Most immunotoxins contain modified forms of bacterial toxins secreted by *Pseudomonas aeruginosa* or *Corynebacterium diphtheriae* or contain the plant toxin ricin, a product of castor beans. Diphtheria toxin and ricin each consist of two chains (**Figure 2**). The B chain mediates binding to cells and is joined through a disulfide bond to the A chain, which contains enzymatic activity. *Pseudomonas* exotoxin A has been more extensively studied. This toxin is a 66,000-dalton protein that is organized into three structural domains that act in concert to cause cytotoxicity (Figure 2). Domain I is responsible for cell binding. Following receptor-mediated endocytosis of *Pseudomonas* exotoxin A by cells, domain II allows translocation of domain III—which contains enzymatic activity—into the cell cytosol. In the cell cytosol, each of these three toxins irreversibly inactives protein synthesis and causes cell death. Since these toxins are catalysts with a high turnover, few molecules need to reach the cytosol to kill the target cell.

## CHEMICALLY COUPLED IMMUNOTOXINS

The construction of chemically coupled immunotoxins dates to the early 1980s. Researchers at several institutions coupled mouse monoclonal antibodies directed against tumor-associated antigens to the ricin A chain. The *in vitro* activity of immunotoxins was measured by the inhibition of incorporation of radioactive amino acids into protein by cultured cells. Immunotoxins that acted specifically caused nearly 100% inhibition of protein synthesis in cultured cancer cells expressing the tumor-associated antigen but had no effect on cultured cancer cells not expressing the tumor-specific antigen. The activity of specific immunotoxins could also be completely inhibited by adding excess monoclonal antibody to compete for binding to the tumor-specific antigen.

Immunotoxins have been constructed using *Pseudomonas* exotoxin A, diphtheria toxin, and ricin to target many tumor-associated cancer antigens (**Figure 3**). The production of immunotoxins by chemical coupling, however, is expensive because it requires large amounts of monoclonal antibody and toxin. Furthermore, the chemical conjugation methods produce heterogenous products. The ideal immunotoxin contains a single toxin molecule covalently bound to a monoclonal antibody at a site removed from the antigen-binding site. These are often mixed with immunotoxin molecules containing more than one toxin molecule per monoclonal antibody or with immunotoxin molecules in which the toxin is coupled near the antibody-binding site, preventing the monoclonal antibody from efficiently recognizing its target antigen. It has become possible to overcome these difficulties by creating cytotoxic agents using genetic engineering techniques. Both *Pseudomonas* exotoxin A and diphtheria toxin have been used to make recombinant toxins.

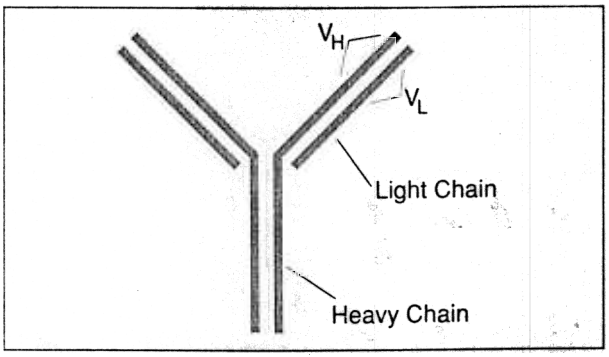


**Figure 1.** Schematic diagram of a typical monoclonal antibody. Each antibody consists of two heavy and two light chains. The variable regions of the heavy chain (VH) and light chain (VL) are indicated and define the antigen specificity of the bivalent antibody.

## RECOMBINANT TOXINS

In most recombinant toxins, DNA encoding the binding domain of *Pseudomonas* exotoxin A has been deleted and replaced with DNA encoding either a growth factor or the variable region domains of a monoclonal antibody that contains the antigen-binding site. These chimeric genes are then expressed into protein in *Escherichia coli*, from which the recombinant proteins are purified to homogeneity. Recombinant toxins have been produced using the leukocyte antigen CD4, acidic fibroblast growth factor, insulin-like growth factor-1, interleukin-2, interleukin-4, interleukin-6, and transforming growth factor-$\alpha$ (TGF$\alpha$) (Figure 2). In *Pseudomonas* exotoxin A-containing recombinant toxins, the binding domain of the native toxin has been deleted, resulting in mutant forms of *Pseudomonas* exotoxin A called PE40 and PE38. These modified toxins retain full enzymatic activity but depend on the appropriate growth factor or monoclonal antibody variable region domains for binding to target cells.

CD4-PE40 has been the focus of recent attention as a new therapy for the acquired immunodeficiency syndrome (AIDS). The human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, binds to lymphocytes containing CD4 through the viral coat protein gp120. Infected cells subsequently produce HIV-1 proteins, including gp120, that are expressed on the cell surface. CD4-PE40 binds specifically to and kills cells expressing gp120. When given in combination with the antiviral agent zidovudine, CD4-PE40 completely sterilizes cultured cells infected with HIV-1. This recombinant toxin is currently being evaluated in a clinical trial of patients with AIDS.

A second recombinant toxin that has entered clinical trials is TGF$\alpha$-PE40. This recombinant toxin was constructed by replacing the binding domain of *Pseudomonas* exotoxin A with TGF$\alpha$. TGF$\alpha$-PE40 selectively binds to and kills cells expressing the epidermal growth

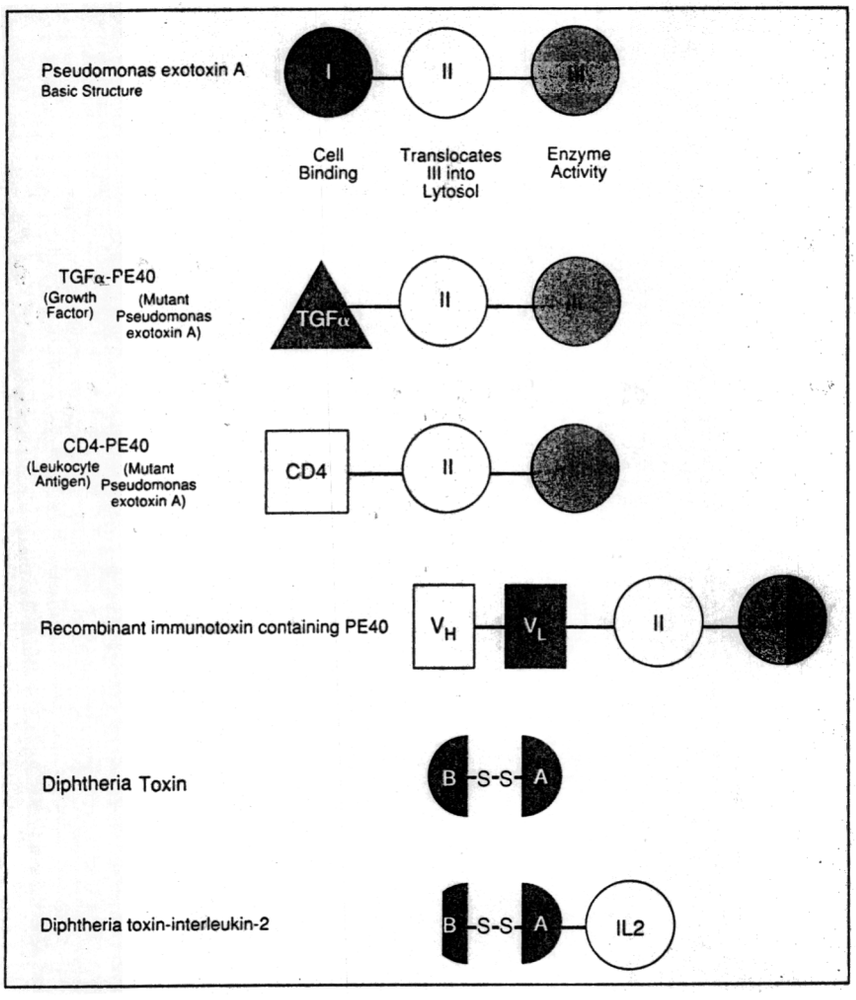


**Figure 2.** Representative examples of bacterial toxins and recombinant toxins containing either *Pseudomonas* exotoxin A or diphtheria toxin. Domain I of *Pseudomonas* exotoxin A has been removed and replaced with various ligands or ligands have been fused to diphtheria toxin with a deletion of part of the binding domain. PE40 is a mutant form of exotoxin A that needs a growth factor or a monoclonal antibody to bind to target cells.

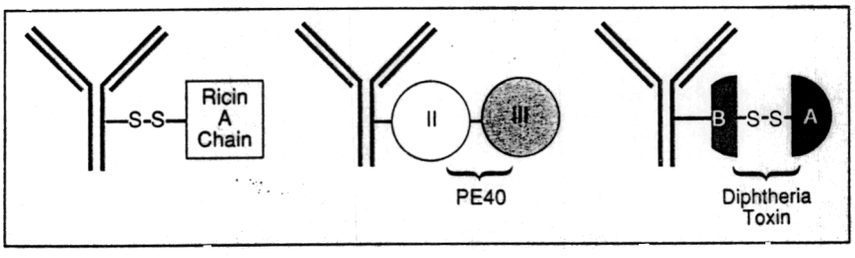


**Figure 3.** Schematic diagram of typical immunotoxins. Antibody is bound through a disulfide bond to ricin A chain, is covalently linked to a modified form of *Pseudomonas* exotoxin A lacking the native binding domain (domain I), or is covalently coupled to diphtheria toxin with a deletion of part of the binding domain.

factor receptor. Low doses of TGFα-PE40 have been shown to cause partial regression of subcutaneous human cancer xenografts growing in nude mice. Because epidermal growth factor receptors are abundantly expressed on normal hepatocytes, higher doses of TGFα-PE40 cannot be given systemically without causing death from chemical hepatitis. Therefore, attention has focused on using this recombinant toxin for the local therapy of cancer.

Nonmetastatic bladder cancer is suited for local therapy via intravesical therapy. Bacillus Calmette-Guerin (BCG), an attenuated strain of *Mycobacterium bovis*, has become the intravesical treatment of choice for transitional-cell carcinoma *in situ*. Systemic complications are unusual because BCG is not absorbed well from the bladder. The effects of BCG may be immunologic but may also include a component of direct cytotoxicity.

Epidermal growth factor receptors are found on human bladder cancers as well as normal bladder mucosa. Many bladder carcinomas overexpress the epidermal growth factor receptors on the superficial layers of tumor cells that contact the urine. In contrast, the epidermal growth factor receptors of normal bladder cells are located on only the basal layers of urothelium, away from the bladder cavity. The intravesical therapy of bladder cancer with TGFα-PE40 takes advantage of the unique distribution of epidermal growth factor receptors on bladder cancer cells and has now been initiated in a clinical trial. The pleural cavity and subarachnoid space are two other

sites where recombinant toxins have been contemplated for local cancer therapy.

## CLINICAL ANTICANCER STUDIES WITH IMMUNOTOXINS

Most clinical trials using chemically coupled immunotoxins were phase I studies using escalating doses of intravenously administered immunotoxin containing ricin A chain. Unequivocal clinical responses were not observed in patients with solid carcinomas. A pattern of mixed regression—which is defined as a 50% or greater reduction in the area of one or more metastases combined with an increase in the size of one or more concurrent lesions or the appearance of a new lesion after the initiation of therapy—occurred in a minority of patients with metastatic malignant melanoma and colorectal cancer treated with immunotoxins specific for each of these cancers.

Clinical responses in patients with hematologic malignancies have been more encouraging. In patients with B-cell lymphoma, immunotoxins containing monoclonal antibodies directed to B-cell antigens coupled to either ricin A chain or a blocked form of ricin itself produced partial regression—which is defined as a 50% or more reduction of the overall tumor burden—in about 40% of the patients.

A single phase I trial using immunotoxin containing *Pseudomonas* exotoxin A has been conducted. A monoclonal antibody reactive with a human ovarian tumor-associated antigen chemically coupled to *Pseudomonas* exotoxin A (called OVB3-PE) was administered via the peritoneal cavity to patients with refractory ovarian cancer. Unequivocal clinical responses were not observed, although one patient who was treated with conventional chemotherapy as well as OVB3-PE remains disease free more than 5 years after treatment. Many patients experienced abdominal pain and a mild chemical hepatitis. This was expected since chemical hepatitis is the dose-limiting toxicity of *Pseudomonas* exotoxin A-containing immunotoxins in mice. At higher doses of OVB3-PE, however, two patients experienced a toxic encephalopathy that necessitated termination of the study. This unexpected toxicity was the result of the systemic absorption of the immunotoxin from the peritoneal cavity and binding of OVB3-PE with a normal central nervous system antigen. Cross-reactivity to this antigen was not recognized in preclinical screening.

## BARRIERS TO TUMOR PENETRATION BY IMMUNOTOXINS

The lack of efficacy of immunotoxins in clinical studies can be attributed to a number of factors beyond the obvious problem of treating refractory cancer patients with large tumor burdens in phase I escalating-dose studies. In the human body, an immunotoxin is subjected to an environment much more complex than that of cell culture. Many factors affect how well a specific cytotoxic agent will work *in vivo*. Analysis of these factors follows most easily from an analysis of the physiologic and molecular hurdles that prevent an immunotoxin from reaching cancer cells expressing a target antigen.

An immunotoxin depends on the vascular system to reach the tumor bed. Many tumors produce only a modest angiogenic response that produces leaky blood vessels at the tumor-host interface, with very few tumor-penetrating vessels. These tumors tend to outgrow their blood supply and undergo central necrosis. Treatment of these tumors would appear to be most effective during the early stages of tumor growth. Well-vascularized tumors are more suited for immunotoxin therapy. Even in well-vascularized tumors, however, centrally located tumor cells may be remote from blood vessels and not readily accessible to circulating immunotoxins.

Once an immunotoxin reaches a tumor-penetrating vessel, it must diffuse into the interstitial fluid that bathes the tumor. Blood vessel permeability is affected by a molecule's charge and size. A smaller molecule, such as a recombinant immunotoxin (molecular weight of 65,000 daltons), diffuses better than a larger chemically coupled immunotoxin (molecular weight of 200,000 daltons). The fact that human cancer xenografts growing in nude mice are more sensitive to recombinant immunotoxins than to chemically coupled immunotoxins may be indicative of this fact.

Once an immunotoxin is within the interstitial space, it must bind to the target antigen. The affinity of the immunotoxin for its antigen is important to ensure maximal binding at low doses of immunotoxin. However, at higher immunotoxin doses such as those that would be used in the clinical setting, the most important factor that maximizes immunotoxin uptake by a tumor appears to be the number of antibody-binding sites present in the tumor. In sum, a clinical trial using an immunotoxin would appear to be most successful in patients with a low tumor burden and a highly vascular tumor that abundantly expresses the target antigen.

The monoclonal antibody B3 chemically coupled to PE38 is an example of an immunotoxin that has circumvented many of the hurdles mentioned above and produces dramatic effects *in vivo*. B3 recognizes a complex polysaccharide antigen that is abundantly expressed on many human cancers including those derived from breast, colon, stomach, lung, and squamous cells. The human breast cancer cell line MCF7, for example, contains more than 100,000,000 antigens per cell. A single intravenous dose of B3-PE38 causes complete and sustained regression of 5 × 5-mm subcutaneous human cancer xenografts growing in nude mice without adverse sequelae. In contrast, many of the immunotoxins that were used in clinical trials heretofore caused only partial regression of tumors of similar size.

Preclinical studies of B3-PE38 are currently under way. Attention has focused on avoiding the problem of unsuspected toxicity from cross-reactivity of the monoclonal antibody to normal human tissues. Immunohistochemical staining of a variety of human tissues has showed reactivity with gastric mucosa and tracheal and bladder epithelium, but not to any life-sustaining tissues incapable of regeneration. A similar reactivity pattern was observed in monkey tissue specimens, indicating that the monkey should be a useful preclinical model for human toxicity from B3-PE38. If B3-PE38 can be tolerated by monkeys without unexpected dose-limiting toxicity,