

Opinion

Immunotoxins: A Review of Their Use in Cancer Treatment

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Abstract

Antibody therapies have become an important component in the management of malignant disease. Antibodies can block tumour growth factors or their receptors, activate immunological attack on the tumour, and are used to deliver payloads such as radioisotopes, cytotoxic drugs or toxins. Immunotoxins are a new class of antitumour agents consisting of tumour- selective ligands (generally monoclonal antibodies [MoAbs]) linked to highly toxic protein molecules and take the advantage of the exquisite specificity of antibodies to selectively target drug delivery and the potency of toxins to kill the target cells. Toxins are modified to remove their normal tissue-binding domains by genetic engineering. Analysis of the aminoacid sequence of the region specific for immunogenicity and the signal transduction mechanisms involved in the interaction of immunotoxins with tumour cells will give the clue for the development of most efficient immunotoxins.

Introduction

Definition of Immunotoxin: Immunotoxins are proteins that contain a toxin along with an antibody or growth factor that binds specifically to target cells. Immunotoxins are created by chemically conjugating an antibody to a whole protein toxin, devoid of its natural binding domain. Immunologic proteins that are smaller than monoclonal antibodies (MoAbs), like growth factors and cytokines, have also been chemically conjugated and genetically fused to protein toxins. Necessity For Alternative Therapy: Radiation therapy has been considered as more reliable one for cancer therapy. However, radiation therapy for cancer severely lowers the quality of life for the treated patients, resulting in nausea, hair loss, and a severe drop in energy. Drug therapy, or chemotherapy, most often is also accompanied by these side effects. The problem is that chemotherapeutics and radiation therapy are poorly selective in which cells they attack. By tethering therapeutic agents to antibodies, "magic bullets" could be produced that specifically bind and deliver the therapeutic agent to sick or kill cells, having little or no effect on healthy cells in the body. This works because antibodies are proteins that have exquisite binding selectivity, which can be produced to bind to only one targeted protein, while ignoring a multitude of other proteins they might come in contact with.

The Antibody Moiety

Antibodies used are generated using monoclonal antibody technology. The target protein (termed an antigen), usually a surface antigen of malformed cell is injected into a mouse, and when the mouse has developed a sufficient immune response to the antigen (including many protein-specific, antibody-producing B cells), its spleen cells (containing B cells) are harvested and fused to myeloma cells. Myeloma cells are an immortal cell line that will allow the fused cells to grow indefinitely at a fast rate. These myeloma-B cell hybrid cells are called hybridoma cells, and can be selected for and tested to verify

that they produce the desired antibody. The hybridoma clones that produce the antibody demonstrating the required specific binding activity can be grown in large quantities, and the monoclonal antibodies can be harvested for use in an immunotoxin.

Designing Antibody Fragments for unique Clinical Application:

Since antibodies have high molecular mass, they have a hard time penetrating solid cancer tumors where the blood supply is fairly restricted. As a solution, immunotoxins are created that utilize only the protein-binding part of the antibody, called the variable region. They do this by cleaving off this region with a protease (making a Fab fragment), or by cloning the variable region into bacteria and expressing as a single-chain antibody. Proteolysis however does not easily yield molecules smaller than a Fab fragment, and microbial expression of single chain Fv (scFv) is currently the favoured method of production. In scFv, the variable (V_H and V_L) domains are stably tethered together with flexible polypeptide linker. Smaller antibody fragments such as Fab or scFv exhibit better pharmacokinetics and also provide full binding specificity because antigen-binding surface is unaltered.

Toxins Used

Toxins used in immunotoxin constructs are derived from bacteria, fungi, and plants, and most function by inhibiting protein synthesis. Bacterial toxins commonly used in immunotoxins include Diphtheria toxin (DT) and the toxin from *Pseudomonas* exotoxin (PE). Plant toxins utilized in immunotoxins include the A chain of ricin (RTA), and the ribosome inactivating proteins (RIPs) gelonin, pokeweed antiviral protein, and dodecandron. Because it is an enzyme, one toxin molecule can work on many substrate molecules, having a devastating effect on the cell. Toxins such as diphtheria toxin (DT) and *Pseudomonas* exotoxin (PE) prevent protein synthesis by an effect on elongation factor 2 (EF-2). In order to be effective, however, immunotoxin must be internalized and route to the appropriate

intracellular compartment for translocation of their attached toxin into the cytosol. The targeting moiety and toxin are joined by a cross linker which is stable extracellularly but labile intracellularly so that the toxin can function in the cytosol.

Production of Immunotoxins

Immunotoxins are produced in *Escherichia coli* transformed with a plasmid encoding the recombinant toxin. A common method of producing material for clinical trials is harvesting recombinant protein from insoluble bacterial inclusion bodies. The insoluble protein can be washed extensively with detergent to remove endotoxin, solubilized, denatured, and reduced in guanidine-dithioerythritol solution. The recombinant protein is then renatured by rapid dilution into refolding redox buffer containing arginine and glutathione, and the dialyzed renatured protein purified by anion exchange and sizing chromatography. The other method is that harvesting the protein from cytoplasm or cell lysate and then using an affinity column to capture the dilute protein.

Immunotoxin Mechanism

The mechanism by which immunotoxins work to kill diseased cells in the body is quite simple. Using AIDS therapy as an example, let's say we have developed an immunotoxin to kill HIV-infected cells by raising antibodies that bind to GP120, a viral protein found on the outside of only HIV-infected cells. Once an AIDS patient has been treated, the immunotoxin floats around in the bloodstream until it binds to a GP120 molecule on the outside of an infected cell. Once bound, the GP120-immunotoxin complex gets pulled inside the cell by endocytosis, where it is either localized to an acidified endosome (if DT is the toxin), or the endoplasmic reticulum (ER) and trans-golgi apparatus in the cell. Inside these organelles, the linker holding the toxin to the antibody is cleaved. Usually the linker is made with an internal disulfide bond, so that it is stable in the oxidizing atmosphere outside the cell and cleaved by reduction in

the reducing environment inside the cell. Once freed from the antibody, the toxin now catalytically inactivates the protein synthesis machinery of the cell. The bacterial toxins perform this by inactivating the ribosome accessory protein elongation factor-2 (EF-2). The plant RIPs accomplish their task by cleaving a single adenine base from the ribosomal RNA so that it can no longer bind EF-2. Either way, the inactivation of protein synthesis leads to the death of the cell.

Targets of Immunotoxins

Immunotoxins in the Treatment of Hairy Cell Leukemia:

Hairy Cell Leukemia, (HCL) is a rarely seen cancer of cells known as B-lymphocytes. The new experimental therapy involves the injection of an immunotoxin designed to destroy the cancerous cells. This immunotoxin, known as LMB-2, is made by using recombinant DNA technology to attach part of an antibody molecule (designed to recognize a substance called CD25) to the toxin produced by bacteria called *Pseudomonas*.

Immunotoxins in the Treatment of Acute Myelogenous Leukemia:

An immunotoxin is developed as a conjugate of a monoclonal antibody that binds CD33, a cell-surface molecule expressed by the cancerous cells in acute myelogenous leukemia (AML) but not found on the normal stem cells needed to repopulate the bone marrow and calicheamicin, a complex oligosaccharide that makes double-stranded breaks in DNA. Commercially this immunotoxin is named as Mylotarg®. Mylotarg® is the first immunotoxin to show promise in the fight against cancer.

Immunotoxins in the Treatment of Lymphomas:

A conjugate of monoclonal antibody against the CD22, a molecule found on the surface of some leukemias and lymphomas with *pseudomonas* exotoxin, a bacterial product that blocks protein synthesis in cells is developed. Commercially this immunotoxin has been named as BL22.

Immunotoxins in the Treatment of Solid Tumour:

Targeting solid tumors with immunotoxins is much more difficult than targeting hematologic tumors. Not only are the cellular junctions tighter and the tumor cells more tightly packed, but the patients are less immunosuppressed and more likely to make neutralizing antibodies to the toxin. Clinical trials are being carried out for the treatment of breast cancer and renal cancer. The monoclonal antibody 8H9 was produced by immunizing human neuroblastoma cells to mice. 8H9 was highly reactive against human brain tumours, childhood sarcomas, and neuroblastomas. The characterization of 8H9 and its antigen presented on the surface of cancer cells suggests that 8H9 may be useful for targeted cancer therapy. Two types of 8H9 based immunotoxins, 8H9(scFv)-PE38 and 8H9(dsFv)-PE38 were constructed. The scFv immunotoxin is a single peptide protein. The toxin PE38, a 38-KDa truncated mutant form of *Pseudomonas* exotoxin A, is fused to the C-terminal of Fv portion of 8H9. The recombinant immunotoxin is expressed in *E. coli*. The dsFv immunotoxin is a two subunits protein. The small subunit is the V_L domain of 8H9. The large subunit is the V_H domain fused with the PE38. The two subunits are separately expressed in *E. Coli*. The dsFv is prepared by combining inclusion body protein of the two subunits. (Table 1)

Problems and Scope of Immunotoxin Development

There are challenges associated with the development of many immunotoxins for cancer therapy. Several of these problems, including immunogenicity, unwanted toxicity, difficulty in production, limited half-life, and resistance, will be considered below, along with potential opportunities for improved development of immunotoxins.

Immunogenicity:

One of the problems with monoclonal antibodies is their mouse, or murine, origin. A human patient's own immune system will

recognize the murine antibody as foreign, and will clear the antibody from the bloodstream quickly, greatly reducing the immunotoxin's effectiveness. To combat this, laboratories have engineered "humanized" antibodies where the part of the antibody that the human immune system identifies as of mouse origin, called the constant region of the antibody, is swapped out for a human constant region. The method most useful for other biologic agents, such as interferon and L-asparaginase, is PEGylation, which not only blocks immunogenicity but also prolongs half-life.

Unwanted Toxicity:

A variety of toxicities have been observed with immunotoxins that have limited the dose and hence the efficacy. The most common toxicity is Vascular leak syndrome (VLS). Studies have shown that RTA binds directly to endothelial cells, while truncated PE requires a ligand that cross-reacts with the endothelium. Other studies have suggested that specific residues on RTA and also truncated PE and IL-2 can bind to endothelial cells and can elicit VLS by a mechanism independent of the normal toxin-induced cell death. Such studies led to a mutant form of RTA that shows less VLS in an animal model. Hepatotoxicity, a typical side effect of recombinant immunotoxins, is due to the binding of basic residues on the Fv to negatively charged hepatic cells. Hepatotoxicity appears to be related to cytokine production, possibly by the Kupffer cells of the liver. Although recombinant immunotoxins that specifically bind to antigens expressed on the liver are not well tolerated systemically, recombinant immunotoxins like LMB-2 and BL22 that cause transaminase elevations are not associated with decreased hepatic function. Renal toxicity due to immunotoxins could be nonspecific because the kidneys are the dominant route of excretion of recombinant immunotoxin.

Conclusions

In the past 3 to 4 decades, a wide variety of immunotoxins have been tested against a wide variety of malignancies in cell culture, in animal models, and in patients. The most useful of these agents appear to be the small recombinant fusion toxins that contain either growth factor or Fv fragments as ligands. Future development may include combinations of immunotoxins with other anticancer therapies in order to overcome problems of tumor penetration, toxicity, and immunogenicity. A successful immunotoxin therapy is obtained by the careful study and analysis of biology of tumour cells, choice of the ligand and toxin and their mode of delivery.

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