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Introduction to chemical construction of immunotoxins and their applications in the treatment of diseases

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Abstract

Immunotoxins are mostly contain a monoclonal antibody part linked to a toxin, generally of bacterial or plant origin. The antibody gives specificity (ability to identify and react with the target), while the toxin gives cytotoxicity (ability to kill the target). In this paper, we will explain about the different parts of immunotoxins, linkage methods of the antibody to the toxin and their application in treatment of cancer and other diseases. This review will help physicians better inform patients about the potential benefits of these experimental treatments.

Introduction

Immunotoxins are mostly contain a monoclonal antibody part linked to a toxin, generally of bacterial or plant origin. The antibody gives specificity (ability to identify and react with the target), while the toxin gives cytotoxicity (ability to kill the target) (1-3). It has been used in both mice and humans to root out autoimmune cells, tumor cells, and virus infected cells (4).

Key point

Immunotoxins are mostly contain a monoclonal antibody part linked to a toxin, generally of bacterial or plant origin. They are made by chemically conjugating an antibody to a toxin and mostly immunotoxins are produced to kill cancer cells and cancer treatment.

Materials and Methods

For this mini-review, we used a diversity of sources by searching through PubMed/Medline, Scopus, EMBASE, EBSCO and directory of open access journals (DOAJ). The search was conducted, using combination of the following key words and or their equivalents; immunotoxins, monoclonal antibody, toxin, chemical cross linkers and cancers.

Immunotoxins and their mechanism of action

The first immunotoxins were prepared in the initial 1980s when monoclonal antibodies (MoAbs) reacting with cancer cells became widely available (5).

Immunotoxins are made by chemically conjugating an antibody to a protein toxin, devoid of its natural binding domain (6,7). Growth factors and cytokines that are immunologic proteins and smaller than MoAbs, have also been genetically fused and chemically conjugated to protein toxins (8). Mechanism of action of immunotoxin is shown in Figure 1. The MoAb-moiety first binds specifically to its antigen expressed on target cells, following which the entire antigen-IT complex is internalized. Once inside the cell, the bond between the MoAb and toxin is broken, thereby releasing free toxin into the cytoplasm. The toxin kill cells by preventing protein synthesis, a distinctive mechanism of action that is toxic to both dividing and non-dividing cells. While, the toxin-moiety is unable to enter the cell autonomously, and while, it is inactive outside the cell, hence, it is only hazardous for cells, which are capable to bind and internalizing the MoAb. The action mechanism of im-

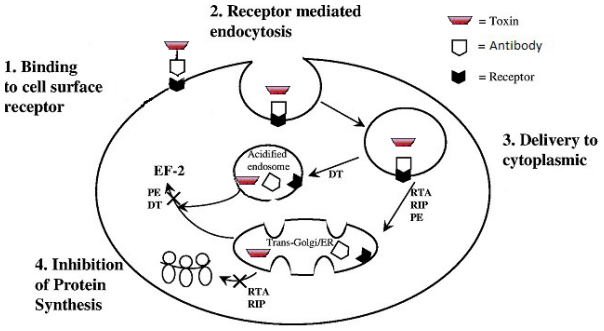

Figure 1. Mechanism of action.

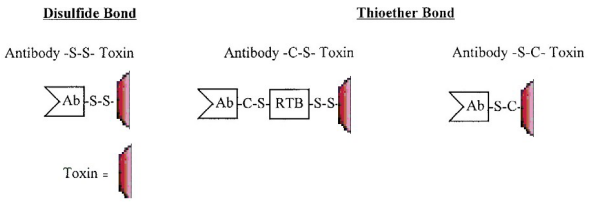

Figure 2. Covalent bonds cross-linking antibody to toxin.

munotoxin depends on the type of toxin. Pseudomonas exotoxin (PE) and diphtheria toxin (DT) deactivate elongation factor-2 (EF-2), whereas plant toxins for example ricin toxin A (RTA) and ribosome-inactivating protein (RIP) deactivate 28S RNA. In either case, the intoxicated cell is unable to synthesize proteins which results in cell death (9).

As mentioned, cells instead of MoAbs, or its fragments can also be under attack by cytokines which receptor is over expressed on the target cell. As an alternative to chemical linkage, MoAb-fragments or cytokines can also be genetically spliced to a toxin, generating a recombinant immunotoxin.

Monoclonal antibody part

Antibodies are proteins constructed by the B lymphocytes of the immune system in response to antigens as foreign factor. According to their chemical and physical properties, antibodies can be divided in IgM, IgG, IgA, IgE, an IgD classes, each having its own function in the host defense system. IgG is the antibody class most widely exploited for clinical use. Antibodies can be considered as bifunctional molecules. Bifunctional antibodies have a variety of potential uses. For example, they have also been used to deliver immunotoxins. In this case, one arm of the bifunctional antibody is connected to the cell-surface antigen on the target cell, while the other is connected to a toxin. The cell are first exposed to bifunctional antibody and then toxin. This work leads to immunotoxin did not lose toxic activity and keeps by internalized antibody toxin complex (10).

MoAbs are a type of monospecific and bifunctional antibodies which are made of identical immune cells that are all clones of a unique parent cell, the antibodies of monoclonal have monovalent affinity, in that, they attach to the same epitope (the part of an antigen that is identified by the antibody) (11). The Moab's binding specificity is the major property determining its suitability as atoxin transporter. Ideally, the MoAb binds an antigen that is expressed solely on thosecells that are to be eliminated.

Mechanism of action as is that moAb recognize and bind to specific proteins created by cells that is epitope. Each monoclonal antibody recognizes one specific protein. Some moAb have attached to drugs, toxin or radiation. The MoAb capable finds the cancer cells and sends the drug, toxin or radiation directly to them. These are named conjugated MoAb. They work in diverse ways depending on the protein they are targeting. Thus different moAb have to be prepared to target different types of cancer.

Various kinds of moAb are now available to treat cancer. Some are licensed to treat specific types of cancer. Some newer types are in clinical trials yet. Various kinds of moAb lead to different side effects. It can take a long time to progress this type of treatment because design moAb can be very complicated (12)

Toxins

The toxins most commonly applied for the construction of immunotoxins are derived from either bacteria or plants and have in common that they catalytically and irreversibly disrupt protein synthesis, typically at picomolar concentration. The bacterial toxins most extensively studied so far are PE and DT. The plant toxins, also referred to as ribosome inhibitory proteins (RIP), can be divided into holotoxins (type II RIP), including abrin, mistletoe lectin, modeccin, and ricin, and hemitoxins (type I RIP), such as gelonin, pokeweed antiviral protein (PAP), and saporin (13).

The linkage of the antibody to the toxin

Conventional immunotoxins are produced by linking a toxin and cell binding ligand using one of two general methods, chemical or genetic. Two types of chemical bonds can be used to production of immunotoxins; thioether bonds (14) and disulfide bonds (15) (Figure 2). Disulfide bonds are sensitive to reduction in the target cells cytoplasm there with releasing the toxin so that it can keep its inhibitory activity only in the cells binding the antibody moiety (16). Disulfide bonds has been used to make immunotoxins containing single-chain plant toxins (pokeweed antiviral protein [PAP], ricin A chain (RTA), saporin, gelonin, and so forth). Because mammalian enzymes cannot break thioether bonds, thioether-linked conjugates of toxins and antibodies are not cytotoxic to target cells (17). But there are two exceptions. The first exception is an immunotoxin with the intact ricin toxin (RT). RT is formed of two polypeptide chains linked by a disulfide bond. If the antibody is bound to the toxin through the RTB, in the target cell cytosol the toxic chain can be released by reduction of the interchain disulfide bond (18). The second exception is an immunotoxin produced with PE. it can be joined to antibody by a thioether link, since this toxin has

a protease-sensitive peptide bond that is cleaved intracellularly to prepare a toxic type bound to the remains of the molecule by a disulfide bond (Figure 2).

Used of immunotoxins in treatment of diseases

Mostly, immunotoxins are produced to kill cancer cells and cancer treatment. Other applications for immunotoxins is immune regulation and the treatment of parasitic or viral diseases.

Used in the treatment of hairy cell leukemia

Hairy cell leukemia (HCL) is a seldom seen cancer of cells identified as B-lymphocytes. The new experimental therapy includes the injection of an immunotoxin produced to destroy the cancerous cells. This immunotoxin known as LMB-2, is prepared by using recombinant DNA technology to attach part of an antibody molecule (designed torecognize a material called CD25) to the toxin created by bacteria called pseudomonas (19).

Immunotoxins in the treatment of acute myelogenous leukemia

In research, an immunotoxin is developed as a conjugate of a monoclonal antibody that joins CD33, commercially this drug immunotoxin is named as Mylotarg. Mylotarg is the first immunotoxin to show promise in the fight against cancer. Mechanism of action as is that a cell-surface molecule expressed by the cancerous cells in acute myelogenous leukemia (AML) a complex oligosaccharide that lead to double-stranded breaks in DNA but not found on the normal stem cells needed to repopulate the bone marrow and calicheamicin (20).

Immunotoxins in the treatment of lymphomas

A conjugate of monoclonal antibody against the CD22 is developed and made by a molecule found on the surface of some lymphomas and leukemias with pseudomonas exotoxin, a bacterial product that prevents protein synthesis in cells is expanded. Commercially this immunotoxin has been named as BL22. Since immunotoxins are often studied as cancer therapy agents, other uses have been suggested and evaluated. These contain modulating immune responses: such as removing T-cells from grafts (21) or the elimination T-regulatory cells (22) and preventing graft versus host disease (23). Some development has been prepared also in producing immunotoxins with anti-viral (24) or anti-parasitic activity (25).

Conclusion

Immunotoxins are proteins that contain a monoclonal antibody part or growth factor linked to a toxin, mostly of bacterial or plant origin. The antibody gives specificity (ability to identify and react with the target), while the toxin gives cytotoxicity (ability to kill the target). Immunotoxins are a novel class of antibody-based therapeutics currently in clinical development. This review will help physicians better inform patients about the potential benefits of these experimental treatments.

Authors' contribution

KH and AH prepared primary draft. SSBM, MRA and MRT edited the manuscript. All authors read and signed the final manuscript.

Conflicts of interest

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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References

1. Ghetie V, Vitetta ES. Immunotoxins in the therapy of cancer: from bench to clinic. Pharmacol Ther. 1994;63:209-34.
2. Thrush GR, Lark LR,Vitetta ES. Immunotoxins. In: Austen KF, Burak off SJ, Rosen FS, Strom TB, eds. Therapeutic Immunology, Boston: Blackwell Science; 1996:385-97.
3. Pai LH, Pastan I. Immunotoxin therapy for cancer. JAMA. 1993;269:78-81.
4. Frankel AE, Tagge EP, Willingham MC. Clinical trials of targeted toxins. Semin Cancer Biol. 1995;6:307-17.
5. Antignani A, Fitzgerald D. Immunotoxins: the role of the toxin. Toxins. 2013;5:1486-502.
6. Moolten FL, Cooperband SR. Selective destruction of target cells by diphtheria toxin conjugated to antibody directed against antigens on the cells. Science. 1970;169:68-70.
7. Krolick KA, Villemez C, Isakson P, Uhr JW, Vitetta ES. Selective killing of normal or neoplastic B cells by antibodies coupled to the A chain of ricin. Proc Natl Acad Sci U S A. 1980;77:5419-23.
8. Cawley DB, Herschman HR, Gilliland DG, Collier RJ. Epidermal growth factor-toxin A chain conjugates: EGF-ricin A is a potent toxin while EGF-diphtheria fragment A is nontoxic. Cell. 1980;22:563-70.
9. Thrush GR, Lark LR, Clinchy BC, Vitetta ES. Immunotoxins: an update. Annu Rev Immunol. 1996;14:49-71.
10. Nolan O, O'Kennedy R. Bifunctional antibodies: concept, production and applications. Biochim Biophys Acta. 1990;1040:1-11.
11. Campbell NA. Biology. 4th ed. San Francisco, CA: The Benjamin/Cummings Publishing Co; 1996;862-9.
12. Breedveld FC. Therapeutic monoclonal antibodies. Lancet. 2000;355:735–40.
13. Antignani A, FitzGerald D. Immunotoxins: the role of the toxin. Toxins. 2013;5:1486-502.
14. Brinkley MA. A survey of methods for preparing protein conjugates with dyes, haptens and crosslinking reagents. Bioconjug Chem. 1992;3:2-13
15. Carlsson J, Drevin H, Axen, R. Protein thiolation and reversible protein–protein conjugation N-succinimidyl 3-(2-pyridyldithio) propionate, a new heterobifunctional reagent. Biochem J. 1978;173:723-37.
16. Thorpe PE, Wallace PM, Knowles PP, Relf MG, Brown AN, Watson GJ. Improved anti-tumor effects of immunotoxins prepared with deglycosylated ricin A chain and hindered disulfide linkages. Cancer Res. 1988;6396-403.
17. FitzGerald D, Idziorek T, Batra JK, Willingham M, Pastan I. Antitumor activity of a thioether-linked immunotoxin: OVB3-PE. Bioconjug Chem. 1990;1:264-8.
18. Lambert JM, Goldmacher VS, Collinson AR, Nadler LM, Blattler WA. An immunotoxin prepared with blocked ricin: a natural plant toxin adapted for therapeutic use. Cancer Res. 1991;51:6236-42.
19. Kreitman RJ, Wilson WH, Robbins D. Responses in refractory

hairy cell leukemia to a recombinant immunotoxin. Blood. 1999;94:3340-8.
20. Uckun F. Immunotoxins for the treatment of leukaemia. Br J Haematol. 1993;85:435-8.
21. Thompson J, Hu H, Scharff J, Neville DM. An anti-CD3 single-chain immunotoxin with a truncated diphtheria toxin avoids inhibition by pre-existing antibodies in human blood. J Biol Chem. 1995;270:28037-41.
22. Martin PJ, Pei J, Gooley T, Anasetti C, Appelbaum FR, Deeg J, et al. Evaluation of a CD25-specific immunotoxin for prevention of graft-versus-host disease after unrelated marrow transplantation. Biol Blood Marrow Transplant. 2004;10:552-60.
23. Stong RC, Uckun F, Youle RJ, Kersey JH, Vallera DA. Use of multiple T cell-directed intact ricin immunotoxins for autologous bone marrow transplantation. Blood. 1985;66:627-35.
24. Ashorn P, Moss B, Weinstein JN, Chaudhary VK, Fitz GD, et al. Elimination of infectious human immunodeficiency virus from human T-cell cultures by synergistic action of CD4-Pseudomonas exotoxin and reverse transcriptase inhibitors. Proc Natl Acad Sci U S A. 1990;87:8889-93.
25. Li H, Gu C, Ren Y, Dai Y, Zhu X, Xu, J, et al. The efficacy of NP11-4-derived immunotoxinsc Fv-artesunate in reducing hepatic fibrosis induced by Schistosomajaponicum in mice. J Biomed Res. 2011;25:148-54.