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AN OVERVIEW: IMMUNOTOXIN

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Abstract:

Immunotoxins are hybrid molecules composed of a monoclonal antibody chemically linked to a biologic toxin. The antibody portion of the molecule directs it to a specific antigenic determinant on a target cell; the molecule is then internalized, and a cytotoxic reaction occurs. First-generation immunotoxins were made by chemically coupling toxins such as ricin (a plant toxin) or diphtheria toxin to MAbs. The early products were highly immunogenic and chemically unstable, sometimes falling apart before reaching the tumor target. These difficulties led to the development of recombinant immunotoxins that were more stably linked to the antibody until bound on the target cell surface.

Key words: *Immunotoxin, Pseudomonous exotoxin, Monoclonal antibody*

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INTRODUCTION:

Immunotoxins are molecules that can bind to specific target cells and selectively kill them. The specificity of the reaction with target cells is dependent on the antibody part and the cytotoxic effect is due to the toxin moiety. Various toxins have been used for this purpose but *Pseudomonas* exotoxin A, plant toxin ricin, and diphtheria toxin are employed most often. As targets for immunotoxins, various surface antigens of tumour cells are applied, including receptors (e.g., interleukin and growth factor receptors), differential antigens, and some others.

The first immunotoxins were complexes of antibody molecules (or their Fab fragments) and purified toxins attached to each other by chemical linkages. The conjugates with whole IgG antibodies have long circulation times in the blood (typically 4–8 hours) and they have shown in clinical trials inhibitory activity against leukemias and lymphomas as well as against solid tumors .More recent constructs are recombinant molecules made from single chain Fv antibody fragments and truncated toxins, which lack the cell-binding domain. They penetrate cells of solid tumors more easily. However, small immunotoxins have short survival times (half-life up to 30 minutes). In some scFv fragments, the peptide link between V_L and V_H can interfere with binding and they are not always stable. Instead, a disulfide bond between residues in the conserved framework, which were mutated to cysteines, was used for the preparation of more stable Fvs (dsFvs).

For wider clinical usage of immunotoxins some important problems must be solved. The immunogenicity of immunotoxins, which is dependent mainly on their toxin part, is one of them. Another problem that has to be overcome is the toxicity of immunotoxins. Ricin-based immunotoxins can cause a capillary leak syndrome and immunotoxins with *Pseudomonas* exotoxin A can damage the liver.

IMMUNOTOXINS

Immunotoxins are a class of proteins that consist of a monoclonal antibody covalently linked to a toxic molecule. The toxic protein may be of plant origin, including ribosome-inactivating
proteins ricin and gelonin. Other toxins widely used include diptheria toxin, pseudomonas exotoxin, and calicheamicin. The FDA-approved immunoconjugate CMA-676, used in the treatment of myeloid leukemia, incorporates calicheamicin, which works by creating double-stranded DNA breaks. Immunotoxins are directed to target antigen in the same manner as native antibodies, which consequently makes immunotoxins susceptible to some of the same mechanisms of resistance affecting unconjugated antibodies. Immunotoxins must be specifically targeted to the surface via monoclonal antibody. Once bound, the immunotoxin will be internalized via receptor-mediated endocytosis and delivered into an endosomal compartment. The immunotoxin will then be trafficked and processed within the cell until the enzymatically active domain is translocated into the cytosol before finally reaching its target. Therefore, mutation, heterogeneity of expression, or shedding of the target antigen may contribute to resistance. Shedding of the antigen target from the surface of the cell or structural changes in the epitope will prevent antigen recognition and inhibit immunotoxin binding.

The development of monoclonal antibodies with unique specificity to tumor antigens has allowed for multiple attempts to use them as a mode of cancer therapy. They may either be used alone to mediate complement-dependent or antibody-dependent cellular cytotoxicity, or they may be linked to therapeutic drugs or toxins so that the conjugate may be targeted specifically to cancer cells. In addition to their relative selectivity and minimal toxicity, they are easily mass produced for widespread application. Their success is unfortunately limited by the relatively low amount of antibody that reaches the tumor and by their limited ability to destroy tumor tissue. Murine monoclonal antibodies are often inactivated by the development of human anti-mouse antibodies, although recombinant chimeric antibodies that combine the constant region of human antibodies with the variable region of mouse antibodies can help overcome this.

The targeting moiety

The targeting moiety should be able to differentiate neoplastic cell based on the antigen expression pattern. There are two classes of targeting moiety. One group is composed of physiologically important ligands such as growth factors, cytokines, lymphokines and poly peptide hormones are other group consist of monoclonal antibodies.

The toxin moiety

The toxin used to make immunotoxin is a class of protein toxin. They have extreme potency and ability to kill drug resistant cell. Plant toxin and bacterial

toxin are used as protein toxin. The toxin which used in brain tumor destruction is pseudomonas exotoxin (PE), which is a bacterial toxin.

Early Immunotoxin Development

Thorpe et al. set the stage for immunotoxin development by confirming that protein toxins could be redirected to kill selected cell types over ystander cells [2]. However, their result was achieved with a poorly defined antibody preparation. Using the same concept but with the benefit of Kohler and Milstein's monoclonal antibody technology [3,73], well defined immunotoxins of a single specificity were produced. These included, ricin-, DT- and PE-derived immunotoxins. Besides antibody and toxin selection, other steps in the manufacture of immunotoxins included the use of different chemical "glues" (called cross linkers) to join the two molecules in a manner that kept both parts functional [74,75]. Early on it was appreciated that antibodies alone were rarely cytotoxic. This fueled research into making antibodies more potent by attaching protein toxins to them. Potency depended not only on internalization but also on the "correct" internal conditions within the cell. For instance, in the case of early immunotoxins to CD5 made with the T101 antibody, neutralization of acidic pH was deemed important for optimal killing [76]. In other immunotoxins, disulfide linkers allowed for cytotoxic activity while thioether linkers did not, confirming the need for the appropriate reducing environment to allow separation of toxin from antibody [75,77].

For PE the first immunotoxins were made via thioether linkage from an intact monoclonal antibody to the native intact toxin (Figure 2B). When the functions of the toxin's structural domain were discovered, it made sense to delete the receptor binding domain, producing a molecule termed PE40-based on its molecular weight. However, the deletion of the N-terminal domain (harboring many lysine residues for chemical conjugation) created a problem of how to attach PE40 to antibodies. This was solved by the introduction of a novel lysine residue near the terminus of PE40, producing Lys-PE40 (Figure 2C). Together, these chemical conjugates made up first and second generations of immunotoxins. Toxins2013, 51491

Figure 2. Immunotoxin construction-from oldest to newest. First generation immunotoxins were constructed by using chemical crosslinking agents to attach intact toxins to intact antibodies. Second generation immunotoxins used modified toxins lacking receptor-binding domains. Third generation molecules used cloned antibody fragments fused to modified toxin genes; allowing for the recombinant production of homogeneous protein. Further improvements of the third generation olecule might include the removal of immunogenic amino acids including (as shown) much of the multi-helical domain of PE.

IMMUNOTOXIN DEVELOPMENT

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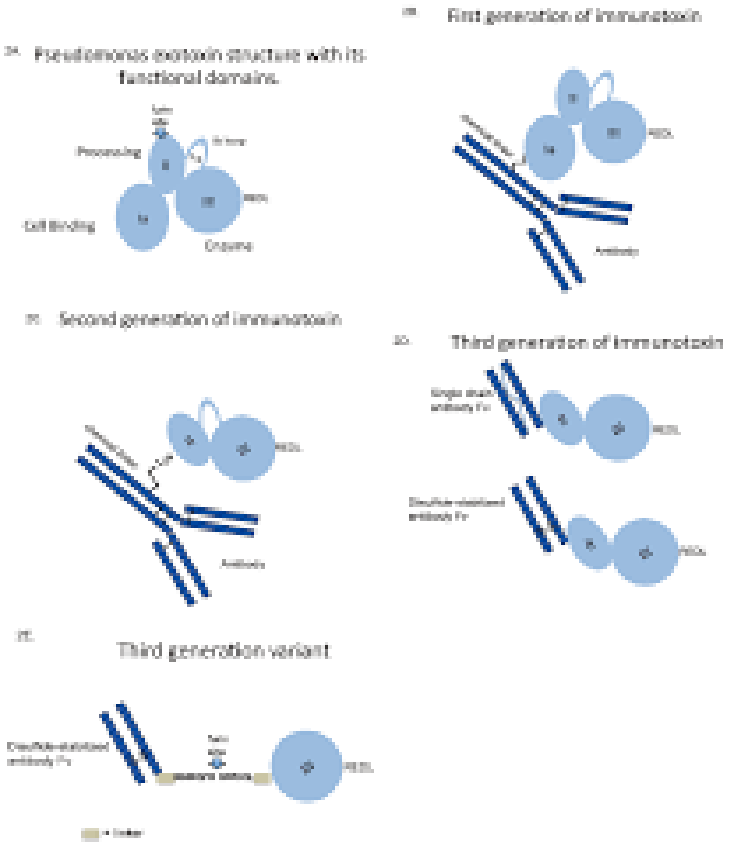


Immunotoxins are basically composed of two functional moieties: one is a MAb or Fv portions of an antibody; another is a plant or bacterial toxin. MAbs are known to be the most specific agent against an antigen expressed by cancer cells, while the toxin part is among the most potent agents against cancer cells. One single IT molecule can inactivate over 200 ribosomes or elongation factor-2 molecules per minute and is potent enough to kill a cell as compared to 10^4–10^5 molecules of a chemotherapeutic drug that are needed to kill one cell .

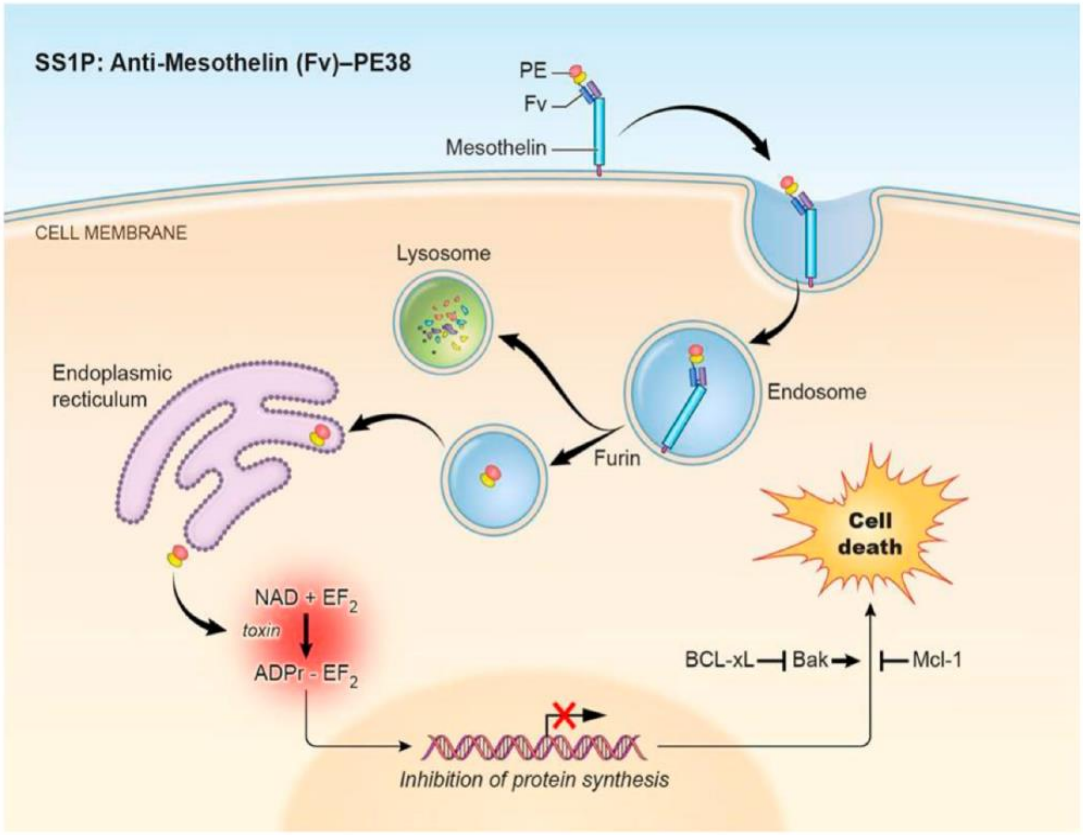
Development of ITs evolves with time and technology. The first generation of ITs was generated by coupling a native toxin with a MAb through a crosslinking reagent that forms disulfide bonds between the toxin and antibody moieties. However, native toxins induce severe side effects when given to humans due to their non-specific binding to normal cells. Native toxins are commonly composed of three domains: one is the receptor binding or cell recognition domain that enables the toxin to bind to the cell surface; one is the translocation domain that helps translocation of the A chain into cytosol; and the third one is the catalytic domain (also called activity domain or A chain) that exerts cytotoxic effects on cells upon translocation to the cytosol compartment. The binding domains of different toxins recognize various receptors ubiquitiously on normal cells. The non-specific binding compromises the specificity of ITs, and induces severe systemic side effects. Thereby, toxins were deglycosylated and the binding domain was deleted when conjugated to MAbs, which led to the development of second generation of ITs. As expected, this approach significantly reduces the non-specific toxicities of ITs, allowing more ITs to be given to humans. Although the results were encouraging, some problems for the second generation ITs persisted, including: 1) poor stability due to the chemical crosslinking between antibody and toxin moieties; 2) heterogeneous composition and reduced binding affinity caused by the random conjugation; 3) poo3r penetration to solid tumor mass because of the large molecular size (>190 kDa); 4) immunogenicity; and 5) limited production.

To improve the pharmacokinetics and reduce the side effects of ITs, great efforts have then been made to generate the third generation ITs which is called recombinant ITs (RITs). Development of RITs is driven by the ability to genetically design and express the antibody fragments and toxins with recombinant DNA techniques. Generally speaking, development of RITs involves two critical steps: 1) design and

construct the recombinant antibody fragments and mutated PE or DT; and 2) expression and purification of the constructed products.

IMMUNOTOXINS AND THEIR WORKING MECHANISM

The dimeric its consist of toxins and monoclonal antibodies, either as unmodified or modified molecules, linkes together by a peptide sequence or by a disulfide bond. The plant and bacterial toxins used in immunotoxins kill cells byhalting cellular protein synthesis. Intracellular delivery to the cytosol is required for antitumor activity. After the immunotoxin targeting moiety binds to the cancer cell surface,the molecule is internalised to the endocytic compartment. Processing and trafficking of these molecules is target and toxin specific but ultimately results in delivery of the enzymatically active portion of the toxin to the cytosol. The bacterial toxins, diphtheria toxins (DT) and pseudomonous exotoxins (PE) irreversibily modify and inactivate Eukariyotic elongation factor 2 (eEF2), a critical component of the protein synthesis machinery. Plant toxins such ar gelonin and recin also arrest protein synthesis but do so by inactivating the ribosome instead eEF2. These toxins mediated modification in stimulate the apoptotic pathway, leading to cell death.



Immunotoxin on melanoma cells is a targeted approach, theoretically leaving normal cells unaffected. Even developed to target melanoma cells demonstrate advantages for several reasons, IT binding to antigens over expressed though it has not been investigated, this mechanism of cell killing may be independent of mutations and alterations in melanoma cells, which drive the proliferation and cause resistance to therapy.

CONCLUSION:

An immunotoxin is a human made protein that consists of a targeting protein linked to a toxin. When the protein binds to the cell, it is taken in through endocytosis and the toxin kills the cell without harming normal cells. Imunotoxin based on the concept of targeting, cancers or tumor cells using an antibody-toxin conjugate.

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