Review

Immunotoxins and Cancer Therapy

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In the past decade, an increased amount of clinically-oriented research involving immunotoxins has been published. Immunotoxins are a group of artificially-made cytotoxic molecules targeting cancer cells. These molecules composed of a targeting moiety, such as a ligand or an antibody, linked to toxin moiety, which is a toxin with either truncated or deleted cell-binding domain that prevents it from binding to normal cells. Immunotoxins can be divided into two categories: chemically conjugated immunotoxins and recombinant ones. The immunotoxins of the first category have shown limited efficacy in clinical trials in patients with hematologic malignancies and solid tumors. Within the last few years, single-chain immunotoxins provide enhanced therapeutic efficacy over conjugated forms and result in improved antitumor activity. In this review, we briefly illustrate the design of the immunotoxins and their applications in clinical trials. *Cellular & Molecular Immunology*. 2005;2(2):106-112.

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Introduction

The three major forms of cancer treatment are surgery, radiation therapy and chemotherapy. But most cancer patients treated with these three means remain incurable. Chemotherapy selectively kills cells which are more rapidly proliferating, but it has little or no effect on drug-resistant cancer cells. In addition, chemotherapy usually fails in patients whose malignant cells are not sensitive to chemical drugs.

It is clear that other therapeutic approaches are urgently needed. In the past decade, the rapid progress in understanding molecular mechanism of cancer development has made a large impact on the design and evaluation of novel therapeutic strategies. The concept of targeted cancer therapy is so important for improving the therapeutic potential of anticancer agents. This approach takes advantage of some special properties of cancer cells that they usually retain markers on their cell surface (1). It should be possible to use these molecular cell-surface proteins as targets to eliminate the cancer cells in that they are sparing on the normal cells. One of these successful approaches is to generate immunotoxin as a potent targeting cytotoxic agent to cell surface molecules, in which it will be internalized and subsequently result in cell death.

Immunotoxin is composed of a targeting moiety, such as a ligand or an antibody that has cell type selectivity, linked to a protein toxin with extraordinary potency (2). The targeting moiety recognizes and delivers the whole molecule to the specific receptors on cell surface of the malignant cells. The toxin then triggers cell death either by reaching the cytosol and catalytically inactivating vital cell processes or by modifying the tumor cell membrane. Immunotoxins can be divided into two categories: chemical conjugates (or first-generation immunotoxins) and recombinant immunotoxins (or second-generation immunotoxins), both of which contain a toxin with either mutated or deleted cell-binding domain that prevents it from binding to normal cells (3). The truncated toxin can be either fused or chemically conjugated to a ligand or an antibody specific for cancer cells. This review will focus on the design and the development of the immunotoxins in targeted therapy.

Design of immunotoxins

The targeting moiety

As immunotoxins are designed to take advantage of the difference in antigen expression between normal and neoplastic cells, efforts for acquisition of the targeting moiety become so critical for one to design immunotoxin. The application of monoclonal antibody technology has allowed investigators to develop compounds that recognize cell surface molecules expressed in higher extent on neoplastic cells than on normal cells. There are two classes of targeting

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moiety that have been identified binding with high affinity and specificity to these target molecules on the target cancer cells. One group is composed of physiologically important ligands such as growth factors, cytokines, lymphokines and polypeptide hormones; the other consists of monoclonal antibodies (4). Toxins can be chemically conjugated to these two classes of molecules by using either disulfide or thioether bonds, or be linked to these molecules at DNA level. Although most cell surface receptors have unknown functions, the binding of the targeting ligand with these receptors is essentially the first step for these immunotoxins to exert their cytotoxic effect.

Because the target molecules for many toxins are located in the cytosol, receptor-mediated endocytosis is essential for the toxins to perform cytotoxicity. Since transmembrane domain has been removed from most toxin moieties of immunotoxins, the targeting moiety is required to be able to mediate internalization of immunotoxins upon the binding to targes that are present in large amounts on cancer cells. If the ligand-receptor complex is unable to be internalized, the immunotoxin is likely to have a low activity. Once the complex enters the cytosol, the toxin is then released to inhibit protein synthesis and kills the cells (5).

The toxin moiety

The toxin used to make immunotoxin is a class of protein toxins. The rationale for using protein toxins to kill cancer cells is based on their extreme potency and ability to kill drug-resistant cells (6). Protein toxins are divided into plant and bacterial toxins. The representative of plant toxin is ricin, which is composed of two subunits linked together by a disulfide bond. The chain A exerts the enzymatic activity, which catalyzes the inactivation of ribosomes. Chain A cleaves a glycosidic bond in 28S ribosomal RNA (rRNA), thereby destroying the ability of ribosomes to synthesize protein. The chain B binds to the galactose residues of surface glycoproteins and glycolipids presented on many cells to translocate the toxin across the cell membrane (7). The plant toxins are glycosylated. On the contrary, the bacterial toxins, such as *Pseudomonas* exotoxin A (PE) and diphtheria toxin (DT), are not glycosylated. They are naturally produced by *Pseudomonas aeruginosas* and *Corynebacterium diphtheriae*, respectively. PE is a single polypeptide chain with a size of 66 kD that is arranged into three major structural domains. Crystallographic analysis showed that domain Ia was responsible for the binding of PE to target cells, while domain II was necessary for the translocation of the toxin into the cytosol, and domain III was required for the adenosine diphosphate (ADP) ribosylation and inactivation of elongation factor 2 (EF2). The function of subunit Ib remains unclear, when deleted, the activity of the whole toxin appears unchanged (8, 9).

To make a toxin that selectively kills cancer cells, domain Ia (the binding domain, amino acids 1-1252) is usually removed and replaced by a ligand which binds to a tumor associated antigen. This removal could decrease the hepatotoxicity of PE immunotoxin, which is due to binding of domain Ia to the hapatocytes (10).

The genetic excision of domain Ia results in production of a molecule termed PE40 which retains its translocation function and EF-2 inhibition properties (7, 11). It has extremely low cellular and animal cytotoxicity (12). PE40 has been used to produce conventional immunotoxins by chemically coupling to antibodies. Moreover, it was used to make recombinant chimeric toxin by fusion to DNA fragments encoding growth factors, antibody-binding sites, or other recognition elements.

DT is an extensively studied single chain toxin in which its functional domains are arranged distinctively from PE, with the enzymatic domain located at the amino terminus and the binding domain at the carboxyl terminus (13, 14). DT follows a similar pathway as PE in cell killing. The toxin-receptor complex enters the cells *via* clatherin-coated pits where the toxin is cleaved into two pieces through a proteolytic-step as well as a cleavage of a disulfide bond. The 37 kD fragment derived from the COOH portion, which contains part of domain II and all of domain III is translocated to the cytosol where it inactivates elongation factor 2 and consequently leads to cell death. Despite their similarity in functions, PE and DT share almost no sequence homology (15). The most commonly used version of DT is DT388 or DAB389, both of which contain a methionine followed by the first 388 amino acids of DT (16).

Other toxins, such us saporin and gelonin, which act by irreversibly arresting the synthesis of protein in eukaryotic cells, have been used to make active immunotoxins. However, PE and DT are the most widely used toxins to generate immunotoxins. Although studies on the mechanism of action of DT and ricin were initiated before those on PE, there is currently much more information available about how PE acts than that about the other toxins.

Conjugated immunotoxins

The targeting moieties of first-generation immunotoxins are whole antibodies chemically conjugated to toxins. The coupling could be achieved by yielding a disulfide bond or a thioether bond between two moieties. Although a thioether bond is much more stable in blood and tissue fluids than a disulfide bond, thioether conjugates are less active in killing cells than comparable disulfide conjugates. Hence there is no known enzyme in animals that can cleave a thioether bond. Several conjugated immunotoxins have been launched to clinical trials successfully for different purposes. Anti-CD5 and anti-CD7 linked to ricin or chain A of ricin have been used to eliminate contaminated tumor cells in autologous bone marrow transplantation (17). PE coupled with OVB3, a murine monoclonal antibody that reacts with human ovarian cancer cells, was shown to kill human ovarian cancer cells in cell culture and to prolong survival time of nude mice bearing tumor xenografts (18). In a phase I clinical evaluation, 23 patients who had refractory ovarian cancer with minimal residual peritoneal disease were treated with OVB3-PE. No clear clinical antitumor responses were observed because of inadequate distribution and poor

penetration to bulk disease of OVB3-PE (19). PE was also used to target B-cell malignancies by attaching to the mAb LL2. This complex produced a complete regression of tumors in an *in vivo* pre-clinical model (20).

Debinski W et al. constructed two immunotoxins by chemically coupling the monoclonal antibody C242 to PE or a modified form of PE, NlysPE40 (21). Monoclonal antibody C242 recognizes a specific sialylated carbohydrate epitope of glycoprotein presented on cell membrane of human colon, pancreatic, and cervical cancers. C242-PE and C242-NlysPE40 were quite cytotoxic for Colo205 cells expressing this specific antigen with a LD_{50} at 0.2 ng/ml and 6.0 ng/ml, respectively. These immunotoxins also exhibited a strong antitumor effect on a human colon cancer xenograft inoculated in nude mice. The specificity and efficacy of these two C242-related immunotoxins warrant their therapeutic promise for the treatment of cancers.

Conjugated immunotoxins have not been further developed because of the difficulty in maintaining the activity of both moieties during their production. Additionally, the application of conjugated immunotoxins appears to have some problems such as nonspecific toxicity, e.g., the dose-limiting neurotoxicity of central nervous system resulted from the reactivity of OVB3-PE with normal brain tissue. Furthermore, some relatively large conjugated proteins (200 kD) in the plasma lead to the vascular leak syndrome (VLS) due to their long circulating lifetimes (22). In addition, tight junctions between tumor cells with high interstitial pressure limit the success of whole antibody-toxin conjugates for the therapy of solid tumors (23). Hence the extravasations of these conjugates may be largely restrained due to their relatively large sizes.

Recombinant immunotoxins

Second-generation immunotoxins overcome many problems of the conjugated immunotoxins. The development of advanced technologies to produce recombinant-proteins enables the large-scale production of recombinant immunotoxins with high purity and quality for clinical use in sufficient quantities to perform clinical trials. Most of these recombinant immunotoxins with clinical approval consist of whole IgG molecules, which are quite stable during prolonged storage. However, the presence of the Fc domain usually leads to the binding to certain proteins (opsonins) in plasma, which increases the undesired toxicity of immunotoxins to the liver and spleen (24). Since the binding sites for antigens are on the variable regions of antibodies (Fv), elimination of Fc domain by antibody engineering will not affect the affinity of antibodies. This technology enables the generation of many kinds of recombinant antibody fragments including single-chain Fv (ScFv), disulfide-stabilized Fv, bivalent disulfide-stabilized Fv and single-chain disulfide-stabilized Fv (SdsFv) (25). Among these fragments, ScFv appears to be the best candidate as targeting domain in fusion protein because of its readiness to produce and decreased toxicity to normal tissues.

Immunoglobulin heavy and light chain variable domains (V_H and V_L) of most ScFv-toxins are covalently connected by a flexible peptide linker, which contains 15 amino acids or more. This structure can retain the binding specificity and affinity compared with the monovalent Fab fragment. A truncated toxin is then fused to the free C-terminus of the ScFv. Although ScFvs have reduced binding avidity compared with the whole antibodies, their small sizes improve their tumor penetration ability *in vivo* (26).

Recombinant immunotoxins against solid tumors

The treatment of solid tumors with immunotoxins is quite challenging due to their physiological nature. Tight junctions between tumor cells, high interstitial pressure, and heterogeneous blood supply are main hurdles determining the effectiveness of immunotoxin treatment (27). Thus, it would be critical for immunotoxins to exert their effects by penetrating into the tumor cells. The use of recombinant fragments is especially useful for this purpose.

In recent years, several recombinant immunotoxins that target solid tumors such as breast, lung, gastric, bladder, and central nervous system cancers have been developed (28). These immunotoxins are investigated at different levels, some of which are already employed in clinical trails (7, 29, 30).

Non-tissue-restricted immunotoxin

LMB-1 (B3-LysPE38) is an immunotoxin composed of the tumor-reactive monoclonal antibody B3 and a genetically engineered form of *Pseudomonas* exotoxin (PE). B3 recognizes antigen Le^Y presented on many human solid tumors (e.g., breast, ovarian, and lung cancers). In an animal study, LMB-1 was reported to have an antitumor activity in rats bearing Le^Y-positive tumors (31). In a phase I trial, Pai LH et al. conducted a study of 38 patients with Le^Y-positive tumors (32), who previously failed the conventional therapies. LMB-1 showed antitumor activities and produced several clinical responses including one CR in a patient with metastatic breast cancer and one PR in a patient with colon cancer. To our knowledge, this is the first clinical report of immunotoxin against epithelial tumors.

LMB-7 (B3(Fv)-PE38) is a single-chain immunotoxin constructed from mAb B3 and PE38. It showed considerable activity against human cancer xenografts in mice, but with unexpectable low activity in a phase I clinical trial. It was evident that LMB-7 became aggregated and rapidly lost their activity when incubated at 37ºC. Pastan I et al. found that intrathecal administration of LMB-7 was effective in the treatment of carcinomatous meningitis in rats (33). Benhar I et al constructed a chimeric immunotoxin containing the V_H of B3 and the V_L of B5 (34), which recognizes a carbohydrate epitope on human carcinoma cells. This chimera had an improved stability, a higher antigen binding affinity and cytotoxic activity compared with LMB-7. When tested in an *in vivo* model system, the chimera had an improved antitumor activity in a human xenograft mouse model.

LMB-9 (B3(dsFv)-PE38) contains the light and the heavy chains of the Fv portion of B3, which is linked by a disulfide

bond. It has an improved stability over LMB-7 without aggregation at 37ºC for several days. Therefore it can be used as a continuous-infusion model in mice bearing human-tumor xenografts (35). Currently a phase I study of LMB-9 is under investigation in patients with advanced pancreatic, esophageal, stomach, colon or rectal cancer.

Immunotoxin against colon carcinoma

Shinohara H et al. determined the antitumor efficacy of HB21(Fv)PE40, a single-chain immunotoxin made by fusing a Fv directed at the human transferrin receptor with a truncated mutant of PE. *In vitro* study indicated that human colon carcinoma cell line, KM12L4, could be lysed by HB21(Fv)PE40. An animal model revealed that systemic administration of HB21(Fv)PE40 eliminated liver metastasis of KM12L4 and delayed the growth of tumors inoculated subcutaneously (36).

Immunotoxin against breast cancer and sarcoma

The 8H9 monoclonal antibody is highly reactive with a cell surface glycoprotein expressed on human breast cancers, childhood sarcomas, and neuroblastomas but not that on normal human cells. This specific reactivity suggests that MAb 8H9 may be useful for targeted cancer therapy. To explore this possibility, Onda M et al. generated a recombinant immunotoxin, 8H9(scFv)-PE38 (37), which showed specific cytotoxicity on nine different cancer cell lines derived from breast cancer, osteosarcoma, and neuroblastoma. The antitumor activity of 8H9(scFv)-PE38 was evaluated in severe combined immunodeficient mice bearing MCF-7 breast cancer or OHS-M1 osteosarcoma.

Immunotoxin against glioma & head and neck squamous cell carcinoma

Receptors for interleukin 4 (IL-4R) are overexpressed on the surface of various human solid tumors including renal cell carcinoma, glioblastoma, Kaposi's sarcoma, and head and neck squamous cell carcinoma (SCCHN) (38). IL-4-PE38-KDEL is an immunotoxin targeting IL-4R expressed on malignant glioma cells. PE38KDEL is a genetically engineered PE molecule. It has amino acids 253-364 linked to amino acids 381-608 which in turn are fused to KDEL (an endoplasmic retaining sequence) at position 609-612 (39). In an *in vitro* study, IL-4-PE38KDEL was highly cytotoxic to glioma cells overexpressing IL-4R while less cytotoxic or nontoxic to hematopoietic and normal brain cells that express IL-4R at regular extent. In a nude mouse model, IL-4-PE38KDEL showed a significant antitumor activity with partial or complete regression of human glioblastoma tumors. Based on this pilot study, an extended phase I/II clinical trial is currently ongoing to determine the safety, tolerability, and efficacy of IL-4-PE38KDEL when stereotactically injected into the recurrent glioma by convection enhanced delivery (40). Furthermore, a circularly permuted IL-4-targeted cytotoxin, IL-4 (38-37)-PE38KDEL, was developed. Protein synthesis inhibition assays demonstrated a growth inhibition effect of IL-4 (38-37)-PE38KDEL on two cell lines of SCCHN in a dose-dependent fashion. In two SCCHN murine models, intraperitoneal and intratumoral injection of IL-4 (38-37)-PE38KDEL mediated tumor regression without visual toxicity. This study indicated that IL-4 (38-37)-PE38KDEL was effective in mediating significant antitumor effects in SCCHN and may represent an attractive therapeutic option for patients with advanced cancers of the upper aerodigestive tract (38).

Immunotoxin against prostate and lung cancer

AR209 (e23(Fv)PE38KDEL) was developed to treat neoplasia that expresses the c-erbB-2 protein product p185(erbB-2). This immunotoxin has unusual specificity in that it does not bind to most normal tissues including peripheral nerve or renal tissue. The AR209 compound contains a single-chain antibody domain specific for p185(erbB-2), coupled with PE38KDEL. The treatment effect of AR209 on prostate cancer was conducted using DU-145 and PC-3 cell lines, which are p185erbB-2 positive. The results revealed that xenografted tumor could be effectively reduced in size. This study provides preliminary evidence for the utility of a recombinant oncotoxin in the treatment of prostate carcinoma (41). Kasprzyk PG et al. indicated that overexpression of erbB-2 in lung cancer is also frequent (42). Their study demonstrated that AR-209 reacted with lung cancer cells with varying levels of erbB-2 expression in the presence and absence of gene amplification. To evaluate the efficacy of AR209 on lung cancer treatment, Skrepnik N et al. inoculated nude mice with 201T, a cell line derived from a lung adenocarcinoma expressing elevated levels of p185(erbB-2) (43). Mice treated with *i.v.* injections of AR209 had reduced tumor size and 20% of cases showed no evidence of tumor growth after the treatment. These data indicated that AR209 may be an effective agent for patients with non-small cell lung cancers that express p185(erbB-2).

Recombinant immunotoxins target hematological tumors

Compared with solid tumors, hematological tumors are more sensitive to immunotoxin therapy. In consistency with that to solid tumors, small-sized recombinant immunotoxins are better agents to target antigens on hematological tumors than those with whole antibody structures. Because such small molecules can approach cells out of the circulation. This could eliminate easily accessible tumors as well as less accessible malignancies.

Recombinant immunotoxins targeted at leukemia and lymphoma antigens have been made from antibody fragments specific for the subunit of the IL-2 receptor (CD25) and for CD22. In addition, growth-factor fusion proteins have been produced targeting the receptors of IL-2, IL-4, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF).

Immunotoxins targeting CD25

Overexpression of the α subunit of IL-2 receptor is observed in many hematological tumors such as cutaneous T-cell lymphoma, B-cell non-Hodgkin lymphoma, hairy cell leukemia, chronic lymphocytic leukemia and acute myelo-

blastic leukemia (44-46). Anti-Tac is a murine monoclonal antibody binding to human CD25 (47). Anti-Tac(Fv)-PE38(LMB-2), a molecular drug developed at clinical stage, has been produced to target CD25 for the treatment of hematologic malignancies. Phase I clinical trials with LMB-2 have shown promising results. The immunotoxin was administered to 35 patients with CD25 (+) hematological tumors, who failed standard and salvage therapies. Intravenous administration of LMB-2 at dose 2 to 63 μg/kg resulted in complete disease remission in a patient with hairy cell leukemia (HCL) for 20 months. Three other HCL patients had 98% to 99.8% reductions of malignant circulating cells. Seven partial responses were also observed in patients with cutaneous T-cell lymphoma, chronic lymphocytic leukemia, Hodgkin's disease, and adult T-cell leukemia. The overall response rate was 24%. The response rate of 20 patients, who received more than 60 μg/kg of LMB-2, was 40% (CR + PR) (48, 49).

Many mutated forms of LMb-2 are still currently at preclinical stage, some of which display potential effect. An *ex vivo* experiment showed that anti-Tac(Fv)-PE38KDEL, which contains the sequence KDEL at the carboxyl terminus of PE38, was very cytotoxic toward fresh cells obtained from patients with chronic lymphocytic leukemia (CLL) (46, 50).

DAB(389)IL-2 (denileukin diftitox, ONTAK) is a cytokine-targeted fusion protein that delivers the catalytic domain of diphtheria toxin (DT) to lymphoma cells expressing the interleukin-2 receptor (IL-2R). In a phase I trial, 73 patients with cutaneous T-cell lymphoma (CTCL), non-Hodgkin's lymphoma (NHL), or Hodgkin's disease (HD) were treated with ONTAK. There were 5 complete (CR) and 8 partial (PR) remissions in patients with CTCL, 1 CR and 2 PR occurring in NHL. The median time to response was 2 months and the duration of response was 2 to 39+ months. No response was documented in patients with HD (51). In a phase III study, 71 patients with cutaneous T-cell lymphoma (CTCL) were assigned to one of two dose levels (9 or 18 mg/kg·d) of ONTAK. The patients were received ONTAK for 5 consecutive days every 3 weeks for up to 8 cycles. Overall, 30% of the patients had an objective response (20% PR, 10% CR) (52). Steroid premedication such as dexamethasone or prednisone significantly improved the tolerability of ONTAK without compromising the clinical response. When ONTAK was used with dexamethasone at 8 mg/d, response was seen in 12 of 20 CTCL patients (53). Based on these trials, FDA approved ONTAK as the first immunotoxin for treatment of advanced CTCL in 1999.

Immunotoxins targeting CD22

RFB4(dsFv)-PE38 (BL22) is a fusion protein consists of anti-CD22 variable domain (Fv) and PE38. CD22 is a B-cell specific transmembrane protein, which is expressed in both mature and malignant B cells and involved in antibody responses and the lifespan of B cells (54, 55). BL22 has high cytotoxic activity on cultured tumor cells as well as in animal models. In phase I trial, BL22 was tested in 16 patients with purine, analogues resistant hairy-cell leukemia (HCL), and the results were quite encouraging. BL22 could induce complete remissions in almost 70% of patients with HCL. The dose level was 3 to 50 μg/kg intravenously administered every other day. There were 11 CR and 2 PR. The 3 nonresponders either received a low dose of BL22 or had pre-existing toxin neutralizing antibodies before therapy was started. The remissions were durable with only 3 out of 11 responders relapsing after 10 to 23 months. Retreatment for these 3 patients produced complete remissions (56). These results demonstrated that BL22 is particularly effective in patients with chemotherapy-refractory HCL patients.

There are several other immunotoxins that have been tested in phase I-III clinical trials for treating Hematological neoplasm, including B43-PAP, SPV-T3a-dgA, WT1-dgA, DT388GMCSF, etc. The number of approved recombinant toxins for the treatment of cancer is expected to increase in the coming years.

Current problems and future direction of recombinant immunotoxins

Although some of the problems such as design, large-scale production and stability associated with the initial recombinant immunotoxins have been solved, specificity, toxicity and immunogenicity are major factors that will determine the usefulness and success of recombinant immunotoxins.

Specificity of the recombinant immunotoxins is determined by the distribution location and expressing extent of the targeted antigens. In some instances, the targets are presented on normal tissues as well as tumor cells, which could induce side effects by nonspecific binding. To overcome this problem, new specific targets and novel agents against the cancer antigens must be developed. The phage-display approach has been used to isolate a scFv that binds with high affinity to a mutant form of the EGF receptor in which a deletion of a portion of the extracellular domain of the receptor generates a tumor-specific epitope (57).

Toxicity is usually due to nonspecific uptake of immunotoxins by non-targeted cells (58). For example, LMB-7 binds Le^Y antigen on normal stomach mucosa, which resulted in nausea, vomiting and diarrhea (59). In addition, ligand-independent toxicities have been observed in most clinical studies. These consist of either hepatocyte injury causing abnormal liver function, or vascular endothelial damage with resultant vascular-leak syndrome (VLS). Reaction of immunotoxins with macrophages, lymphocytes, or endothelium may lead to cytokine release followed by consequent symptoms and elevated circulating cytokines. The hepatotoxicity may be directly due to the binding of immunotoxin to macrophages or secondary release of cytokines from macrophages (60). VLS induced by many immunotoxins is mediated indirectly by the activation of vascular endothelial cells and/or macrophages *via* elevated circulating cytokines such as TNF-α and IFN-γ (61). Recombinant immunotoxins in smaller sizes appear to have a lower incidence of VLS, possibly due to their shorter

circulating half-lives. VLS can be inhibited in future trails by anti-inflammatory agents to block cytokine action, or by mutation of receptors or administration of peptide inhibitors that will prevent the binding of the immunotoxin to endothelial cells (1).

Immunogenicity is another major barrier to the clinical application of immunotoxins. Kreitman et al. pointed out that the presence of existing toxin-neutralizing antibodies could be the possible reason for the non-response of 3 patients in a clinical trial involving BL22-toxin (56). Several approaches have been taken to reduce this adverse effect. One is to minimize the size of immunotoxins to 60-65 kD, which appears to be less immunogenic in patients than the larger molecules (200 kD) (29); another attempt is to use immunosuppressive agents such as deoxyspergualin (62) or CTLA4 Ig (63), an inhibitor of the costimulation pathways required for helper T-cells through the CD28/CTLA4-CD80/86 complex. The last approach is to use the nonimmunogenic agent such us Rituximab, which induces B-cell depletion in the majority of patients (64).

Recombinant immunotoxins with improved properties have shown great promise as an alternative treatment for cancer therapy. The Fv fragments should be more thermally stable and of sufficiently high affinity. Excellent activity and specificity can be observed from many recombinant immunotoxins in *in vitro* assays using cultured cancer cells as well as in animal tumor models. The outcome of ongoing clinical trials will provide sufficient data to demonstrate whether recombinant immunotoxins will be successful in cancer treatment in the future.

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