Editorial

Increased Sophistication of Immunotoxins

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The article by Salvatore et al. (1) in this issue of Clinical Cancer Research describes the synthesis and biological activity of an improved, recombinant immunotoxin targeted to the CD22 B-cell antigen. This particularly well-performed study is one of a large series of significant innovations in the field by this and other groups of investigators in the last few years. To appreciate more fully the work in this issue and the recent developments in immunotoxin pharmacology, some historical perspective is useful.

Immunotoxins consist of cell-selective ligands (usually monoclonal antibodies, antibody fragments, or cytokines) linked covalently to modified peptide toxins. The ligand binds cell surface receptors and triggers internalization. In defined intracellular vesicle compartments, the toxin moiety escapes to the cytosol, where it catalytically alters critical cell functions leading to cell death. Because immunotoxins kill by distinct mechanisms (e.g., inactivation of protein synthesis or signal transduction) than standard chemotherapy agents, which damage DNA or cell proliferation, it was envisioned that they would be active on chemoresistant malignancies. In addition, because immunotoxins possess a cell targeting function, the molecule should have distinct, nonoverlapping toxicities from chemotherapy drugs. Finally, immunotoxins may have additive or synergistic efficacy in combination with the standard chemotherapy agents.

In the 1980s, clinical studies were conducted with antitumor monoclonal antibodies linked to protein synthesis inactivating toxins. The toxins (ricin and Pseudomonas exotoxin) had been either chemically or genetically altered to reduce normal tissue binding. Studies were done in patients with non-Hodgkin’s lymphoma, chronic lymphocytic leukemia, cutaneous T-cell lymphoma, breast cancer, ovarian cancer, adult T-cell leukemia, colon cancer, peripheral T-cell lymphoma, and melanoma (2). However, few durable remissions were observed.

Among the most active of these early immunotoxins was an anti-CD22 monoclonal antibody (RFB4) chemically linked to deglycosylated ricin A chain (3).

In the 1990s, genetic engineering was used to fuse the catalytic and translocation domains of toxins (diphtheria toxin and Pseudomonas exotoxin) to cytokines and single chain Fvs, and these were administered to patients. These second generation immunotoxins were called fusion toxins or fusion proteins. In addition, several peptide toxins with absent normal tissue binding domains (gelonin, pokeweed antiviral protein, and saporin) were conjugated to monoclonal antibodies and tested in patients. Finally, a growth factor was coupled to a binding site mutant diphtheria toxin and infused directly into patients’ brain tumors. Improved remission rates were seen with DAB389 IL2 in cutaneous T-cell lymphoma, anti-Tac(Fv)-PE38 and anti-CD22 (dsFv)-PE38 in hairy cell leukemia, and transferrin-CRM107 and IL-4 (38–37)-PE38KDEL in high-grade glioma (4).

Several persistent problems have impeded the wider application of this technology. These include difficulties in immunotoxin production, rarity of high affinity, tumor selective ligands, heterogeneity of antigen/receptor expression on tumor cell surfaces, nontargeted toxicities, and immunogenicity. Third generation immunotoxins, such as those described in this issue, are being developed to address these problems and will hopefully lead to agents with higher response rates in a broader range of malignancies.

Immunotoxin production problems occur because of misfolding of denatured and renatured inclusion bodies (5) or poor yields of secreted immunotoxins from transfected toxic-resistant eukaryotic cells (6). One possible solution may be to revisit chemical conjugation techniques with site-modified toxins and ligands.

Identification of high-affinity, adequately tumor-selective ligands has been challenging, particularly for epithelial cancers. The article by Salvatore (1) showed a method to enhance the affinity of selective ligands. They modified an anti-CD22 sFv by hot spot mutagenesis and phage display. For epithelial tumors, several groups have targeted the tumor vascular endothelium with vascular endothelial growth factor or urokinase ligands (7, 8). These may yield an amplified killing effect by causing tumor tissue hypoxia. Specific receptors on epithelial cancers do not need to be targeted. Another approach has been to enhance specificity by adding a second tumor-specific step in the cell intoxication process. Anthrax protective antigen has been genetically modified so that the furin/PACE4 cleavage site has been replaced with tumor-selective urokine or metalloprotease cleavage sequences (9, 10). Combined with reengineering the receptor binding domain, highly specific targeting molecules may be synthesized. Such a polypeptide may be combined with any fusion protein containing the NH2-terminal 255 amino acid residue domain of lethal or edema factor to deliver cytotoxic materials to tumor cell cytosols (11). Alternatively, Vallera and Chen (12, 13) have expressed fusion proteins in tumor-selective T cells and achieved antitumor activity with less selective ligands.

Heterogeneity of antigen expression predisposes to treat-
ment failure because of escape of antigen-negative tumor cells. One approach has been to target oncogene products or differentiation antigens necessary for tumor cell survival on all of the malignant cells. The oncogene protein-directed immunotoxin, anti-EGF receptor III-PE38 (14), and the growth factor fusion protein, DAB389IL7 (15), are conjugates in development for gliomas and lymphomas, respectively, that may target critical tumor cell proteins. Alternatively, as noted above, damage to tumor endothelium may lead to death of oxygen-deprived heterogeneous tumor cells. Finally, Gorgun et al. (16) have used differentiation modulators, such as phenylbutyrate and bexarotene, to up-regulate receptor expression on tumor cells to improve fusion protein sensitivity.

Nontargeted toxicities have been dose limiting for most immunotoxins. The VLS is associated with hypoalbuminemia, edema, weight gain, hypotension, and, at times, dyspea. Experimental models suggest immunotoxin-mediated direct endothelial injury (17). Baluna et al. (18) hypothesized a VDL-related peptide binding motif with endothelial integrin receptor binding function as necessary for toxin-induced VLS. They have recently produced recombinant ricin conjugates lacking these sequences, and the molecules show less VLS in their animal models (19). Renal injury, sometimes associated with a hemolytic uremic syndrome, occurs in patients treated with some immunotoxins. We recently found evidence of immunotoxin micro-aggregates measurable by static and dynamic light scattering that may be linked to cases of renal injury (20). Careful formulation and 0.2-μm filtration immediately before immunotoxin administration may prevent this complication. Liver injury from immunotoxins has been seen frequently and may be multifactorial in origin. Some conjugates, such as DAB389EGF and anti-EGF receptor (Fv)-PE38, may directly bind and intoxicate hepatocytes (21, 22). Pseudomonas exotoxin conjugates may bind toll receptors on Kupffer cells, triggering prostanoid and tumor necrosis factor-α release (23). These inflammatory products cause secondary hepatic injury. Kreitman et al. (24) have blocked this injury both in animal models and patients by prophylaxis with cox-2 inhibitors and antibody to tumor necrosis factor-α. The diphtheria fusion protein directed to the GMCSF receptor, DT388GMCSF, also produces dose-limiting liver damage (20). Again, DT388GMCSF may react with liver macrophages rather than hepatocytes. Thus, hepatocyte apoptosis is likely secondary to release of products from activated macrophages. The chloride-channel blocker glycine is being tested as prophylaxis to prevent the DT388GMCSF-induced transaminasemia (25). Finally, intratumoral infusions of transferrin-CRM107 have been associated with cortical necrosis because of brain capillary binding and intoxication (26). This injury may be prevented by chloroquine prophylaxis, which will selectively block normal brain endothelial endosome acidification and diphtheria toxin translocation to the cytosol (27).

A major limitation to retreatment with immunotoxins is the development of a humoral immune response to the foreign bacterial or plant polypeptides. Because chronic lymphocytic leukemia patients are unable to mount effect humoral immune responses, these patients are an excellent cohort for immunotoxin clinical trials. Tsutsumi et al. (28) have also derivatized immunotoxins with polyethylene glycol to reduce immunogenicity. Finally, Rybak et al. (29, 30) have fused human toxins, including RNases, to human-derived peptide ligands. These immunotoxins should be less immunogenic and permit repetitive dosing.

The numerous preclinical manipulations that have been undertaken recently to improve the therapeutic index of immunotoxins should lead to exciting new clinical trials in the next decade. We hope to provide reports in Clinical Cancer Research that will document advances, improve understanding, and speed applications of these novel and potentially effective anticancer agents.

References
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