

The Biology Behind

Reducing the Immune Response to Immunotoxin

Commentary re R. Hassan *et al.*, Pretreatment with Rituximab Does Not Inhibit the Human Immune Response against the Immunogenic Protein LMB-1. *Clin. Cancer Res.*, 10: 16–18, 2004.

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Immunotoxins are hybrid proteins composed of peptide cytotoxins covalently linked to target cell-selective peptide ligands such as monoclonal antibodies, antibody fragments, growth factors, or cytokines. Immunotoxins have been synthesized that kill cells selectively in tissue culture with great potency and have excellent *in vivo* efficacy with short treatment courses in immunocompromised rodents bearing human tumor xenografts. However, clinical benefit from these genetically engineered molecules has been compromised by the strong humoral immune response to these foreign proteins. Humoral immunity reduces the serum half-life and inhibits cytotoxicity (1, 2). A number of approaches have been tested to reduce the immune response (Fig. 1).

Administration of immunosuppressive agents with immunotoxins has been tested in animals and patients. Cyclophosphamide and cyclosporine did not prevent anti-immunotoxin synthesis in patients treated with a mouse monoclonal antibody-ricin A chain conjugate (3, 4). CTLA4Ig was able to block anti-immunotoxin production in rodents and dogs (5), and deoxyspergualin was able to reduce anti-immunotoxin synthesis in rodents and dogs (6) but not substantially in primates (7). Anti-CD4 monoclonal antibodies blocked anti-immunotoxin generation in mice (8). No clinical trials of combined CTLA4Ig, deoxyspergualin, or anti-CD4 antibody and immunotoxin have been reported. Rituximab has been tested in two clinical studies in combination with recombinant *Pseudomonas* exotoxin immunotoxins (9, 10). In a study by Hassan *et al.* (9) reported in this issue of *Clinical Cancer Research*, administration of rituximab beginning 3 days before immunotoxin did not prevent anti-immunotoxin antibody formation. In contrast, Saleh *et al.* (10) found good inhibition of anti-immunotoxin antibody synthesis with rituximab pretreatment for two weeks (four doses) before initiating immunotoxin therapy. It is unclear whether the differences in these two studies are secondary to timing of rituximab or other differences in patient characteristics or immunotoxin properties.

Altering the structure of the immunotoxin is another approach to reduce immunogenicity. Polyethylene glycol modified immunotoxin showed reduced immunogenicity and improved serum half-life and antitumor activity in mice (11, 12). Site-specific mutagenesis of one toxin, *Pseudomonas* exotoxin, has been performed to generate variant toxins with removal of monkey sera-defined immunodominant epitopes (13). No clinical testing has been performed with either polyethylene glycol-modified or immunodominant epitope-modified immunotoxins. “Humanized” immunotoxins have been synthesized by using a human or mammalian toxin (*e.g.*, RNase, protamine/DNA, and Bax) and a human ligand (*e.g.*, monoclonal antibody, cytokine, or growth factor). As shown in Table 1, toxicity to tumor cells was observed, but in most cases, the potency was 1000- to 1 million-fold less than “traditional” immunotoxins. No human studies have been reported with these agents.

A third avenue is to use immunotoxins in a disease setting where there is an impaired immune response. Immunotoxin therapy of chronic lymphocytic leukemia has been associated with lower anti-immunotoxin serum titers (22). Similarly, interstitial brain tumor therapy has yielded lower systemic antibody titers and lack of effect of these antibodies on brain tumor pharmacodynamics (23).

Where do we go from here? First, a quantitative, reproducible assay for humoral immune response will permit more accurate comparison of immune interventions. The assays of Hall *et al.* and Hertler *et al.* provide internal standards and immunoglobulin quantitation (1, 2). Second, patient selection is important. Patients should be stratified for prior exposure to the toxin or ligand moiety. Patients with pretreatment immunity are more likely to mount an amnestic immune response. Third, agents with sufficient clinical activity during the first cycle or month should be selected for tolerance induction. If the agent has little clinical activity in the pre-antibody period, it will be unlikely to show improved anti-tumor efficacy with immune modulation. Fourth, clinical testing is warranted with CTLA4Ig, deoxyspergualin, anti-CD4 and different schedules of rituximab. Several weeks of rituximab may be required to condition patients to tolerate foreign antigens. Fifth, the clinical development of “humanized immunotoxins” may accelerate the field similar to the effects of monoclonal antibody “humanization” on the introduction of monoclonal antibody serotherapy into clinical practice. Although clinical investigators and physicians have waited several decades for the promises of immunotoxin therapy, we can see steady progress in the field and renewed hopes for expanding the role of immunotoxins in the clinical oncology armamentarium.

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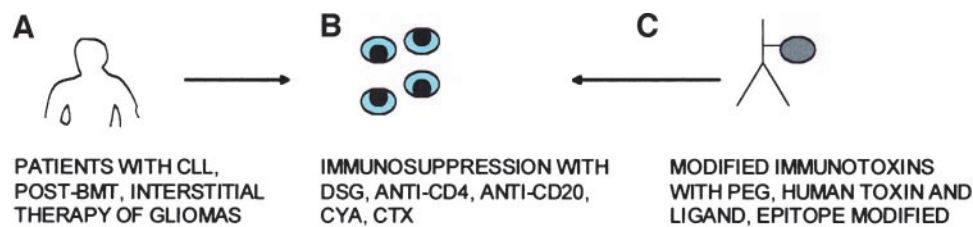


Fig. 1 Schema of interventions that may reduce immunotoxin immunogenicity. **A**, patient selection of the following diseases with pre-existent immunosuppression or sanctuary sites: chronic lymphocytic leukemia (CLL); post-bone marrow transplantation (Post-BMT); and interstitial therapy of gliomas. **B**, immunosuppressive compounds as follows: deoxyspergualin (DSG), CTLA4Ig, cyclosporine (CYA), cyclophosphamide (CTX), anti-CD4 antibody, and anti-CD20 antibody. **C**, modified immunotoxins as follows: polyethylene glycol (PEG) conjugates, human toxins and ligands, and immunodominant epitope modified.

Table 1 "Humanized" Immunotoxins^a

Conjugate	Toxin	Ligand	IC ₅₀ (nM)	Reference
Tf-RNase	Panc RNase	Tf	100	14
Anti-TfR(Fab') ₂ -Ang	Ang	Chimeric mab	100	15
EGF-RNase	Panc RNase	EGF	100	16
IL2-RNase	Panc RNase	IL2	20	17
Ang-CD30L	Ang	CD30L	200	18
anti-gp120-protamine/DNA	PE DNA	Human F105 Fab	100	19
LL2-Onconase	Onconase	Humanized LL2 mab	0.05	20
IL2-Bax	Bax	IL2	140	21

^a IC₅₀, concentration of conjugate producing 50% inhibition of cell viability; Tf, transferrin; TfR, transferrin receptor; Ang, angiogenin; IL2, interleukin-2; Panc, pancreatic; mab, monoclonal antibody; EGF, epidermal growth factor; PE, *Pseudomonas* exotoxin; Fab, antibody fragment. Onconase is derived from *Rana pipiens*.

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