The Biology Behind

Reducing the Immune Response to Immunotoxin


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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 deoxypergualin 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, deoxypergualin, 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 antitumor efficacy with immune modulation. Fourth, clinical testing is warranted with CTLA4Ig, deoxypergualin, 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.
and immunodominant epitope modified.

...immunosuppression or sanctuary sites: chronic lymphocytic leukemia (CLL); post-bone marrow transplantation (Post-BMT); and interstitial therapy with anti-CD4 antibody, and anti-CD20 antibody. 

C. modified immunotoxins as follows: polyethylene glycol (PEG) conjugates, human toxins and ligands, and immunodominant epitope modified.

![Diagram](image)

Fig. 1 Schema of interventions that may reduce immunotoxin immunogenicity. A. patient selection of the following diseases with pre-existing immunosuppression or sanctuary sites: chronic lymphocytic leukemia (CLL); post-bone marrow transplantation (Post-BMT); and interstitial therapy with anti-CD4 antibody, and anti-CD20 antibody. 

**Table 1** "Humanized" Immunotoxins

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>Toxin</th>
<th>Ligand</th>
<th>IC_{50} (nm)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Tf-RNase</td>
<td>Panc</td>
<td>Tf</td>
<td>100</td>
<td>14</td>
</tr>
<tr>
<td>Anti-TIR(Fab')2-Ang</td>
<td>Ang</td>
<td>Chimeric mab</td>
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<td>15</td>
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<td>Panc</td>
<td>EGF</td>
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<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Ang-CD30L</td>
<td>Ang</td>
<td>CD30L</td>
<td>200</td>
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<tr>
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<td>Human F105 Fab</td>
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<td>19</td>
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<tr>
<td>IL2-Onconase</td>
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<td>Humanized LL2 mab</td>
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<tr>
<td>IL2-Bax</td>
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<td>IL2</td>
<td>140</td>
<td>21</td>
</tr>
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</table>

*IC_{50}, 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.*

**References**


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