New *Clostridium difficile* recombinant toxin for safe vaccine development

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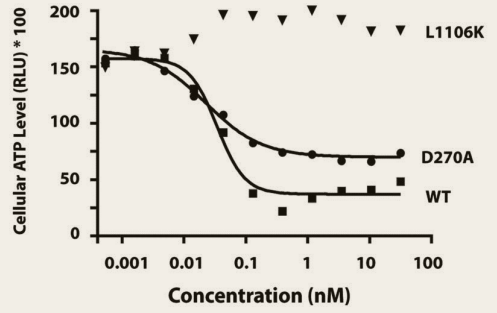
Summary

A structural biology approach has identified a conserved region common to multiple *Clostridium* toxins. Specific mutations of the protein sequence in this region prevent the toxins from entering into intestinal cells, thereby preventing widespread tissue damage. These recombinant *Clostridium* toxins may be used to create a multivalent vaccine to protect against multiple species of *Clostridium*. Furthermore, the recombinant toxin may be used as a safer alternative to the native toxins in vaccine manufacturing. This discovery stems from a collaboration between the laboratories of Dr. Borden Lacy of Vanderbilt University and Dr. Roman Melnyk of the Hospital for Sick Children.

Characterization of defective purified TcdB mutants



Effect of pore formation on enzyme-independent cytotoxicity. The high-dose acute cytotoxicity of purified WT TcdB, a glucosyltransferase-defective mutant (D270A), and a pore-formation defective mutant (L1106K). Constructs were tested on human IMR-90 fibroblasts under necrosis-like conditions.

Commercial Applications and Key Advantages

- The creation of a recombinant toxin with alterations in the conserved region critical for cytotoxicity may be used for development of vaccines protective against a specific *Clostridium* toxin. In addition, other *Clostridium* toxins can be mutated in this region to create safe antigens for a multivalent vaccine.
- The recombinant toxin eliminates all cytotoxicity, hence bypassing the need for formalin treatment to generate safe vaccine antigens, which is customary for vaccines currently in development.
- Enables development of a neutralizing antibody against the common toxin variants, TcdA and TcdB produced by *C. diff*, as well as against toxins produced by other *Clostridium* species.

Technology Description

Despite efforts by multiple research groups to construct recombinant *C. diff* toxins based upon knowledge of protein sequence, none have been found to lack toxicity. Therefore, antigens used for *C. diff* vaccines currently under development require formalin-treatment for inactivation in order to more safely produce the vaccine product. This technology expanded the search for the elusive safe antigen by the addition of a third dimension, literally, by solving the 3D structure of the *C. diff* toxin TcdA. Not surprisingly, this new method of investigation yielded the identification of previously undiscovered structural regions of the protein critical for toxicity. Importantly, this allows for safer production of vaccines because the antigen no longer acts as a toxin. See figure on left for the effect of mutating part of this region in TcdB as an example.

This region is conserved across six other homologous toxin variants, including the highly variable TcdB and four other toxins from rarer *Clostridium* species. This now enables the development of a new multivalent vaccine with improved efficacy, as it could effectively target all of these toxin variants (a "pan-Clostridium vaccine").

Additionally, the knowledge of the conserved region required for toxicity sets the stage for neutralizing antibody discovery. Both a vaccine and a neutralizing antibody therapy would fulfill important unmet medical needs, as *C. diff* is among the most common hospital-acquired-infections in the United States, affecting nearly half-a-million people each year and causing close to 30,000 deaths.

Intellectual Property Status

A patent application has been filed.

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