



Atoxic Recombinant Holotoxins of *Clostridium Difficile* as Immunogens

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Background and Summary: *Clostridium difficile* is a gram positive, spore forming anaerobic bacillus that produces two exotoxins: toxin A and toxin B. Many strains of this species have acquired resistance to a majority of commonly used antibiotics. The reduction of commensal microflora as an effect of use of antibiotics allows *C. difficile* to grow and to produce harmful toxins in the intestine, without nutritional competition from normal bacterial flora. Transmitted primarily through contact with contaminated surfaces, *C. difficile* is a common cause of nosocomial antibiotics-associated diarrhea (CDAD) and pseudomembranous colitis. Infection caused by *C. difficile* accounts for millions of patient cases and billions of dollars yearly in treatment in hospitals, nursing homes and other care centers. *C. difficile* –associated disease is mediated mainly by exotoxins A (TcdA) and B (TcdB), which disrupts the epithelial barrier and cause intestinal inflammation. TcdA and TcdB are similar in sizes and structures and share putative receptor binding, transmembrane, and enzymatic domains.

The diagnosis of *C. difficile* infection remains a challenge. The current diagnostic modalities mainly consist of the detection of the *C. difficile* organisms and of their toxins in fecal samples which are labor-intensive and time-consuming. Standard therapy depends on antibiotic treatment, which in recent years has become less effective. Many patients who initially appear to have been cured suffer multiple relapses. In recent years, *C. difficile* –associated disease has emerged as the leading cause of one of the most widespread and potentially serious health care-associated infections acquired during a stay in a hospital or long-term care facility mostly due to the widespread use of broad-spectrum antibiotics and emergence of hypervirulent strains. .

Our scientists have developed a rapid cell-based test to detect the function of *C. difficile* Toxins A and B. This test is highly sensitive and can detect a trace amount of *C. difficile* toxins, as low as 1pg/ml within 3 hours. Recent development has been focused on generation of inactive forms of TcdA and TcdB and chimera proteins for therapeutic purposes. Multiple mutations in the conserved domains were created to ensure a complete loss of toxicity while the native confirmation remains intact. As a result, our scientists have successfully created a vaccine composition for the treatment of *C. difficile* –associated disease. Preliminary animal studies have demonstrated that this immunogenic vaccine composition can be used in effective immunization against *C. difficile* infection.

Market and Applications: The growing incidence and severity of *C. difficile* –associated disease indicate a need for development of new diagnostic assay and treatment tools. The present inventions provide improved methods of the diagnosis and therapeutics of *C. difficile* –associated disease. Further efforts are under way to study the efficacy of the vaccine composition in patients infected with *C. difficile*.

Product Advantages:

- We have developed the cell-based rapid test which is highly sensitive and more efficient than the currently available diagnostic tools.
- The proposed vaccine composition is consisted of atoxic mutant forms of *C. difficile* Toxins A and B and their chimera proteins with intact native protein confirmation, thus providing a more powerful and practical approach to successful immunization against the infection.
- The mutations were created in the key amino acids known to be responsible for the toxicity and not in the receptor binding domains. Therefore these atoxic Toxins A and B can be internalized without toxicity.
- We have strong IP position including composition of matter and method of use claims.

Licensing Opportunity: An IP portfolio supporting the discoveries is available for licensing.

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