DETECTION, ISOLATION AND PURIFICATION OF *CLOSTRIDIUM DIFFICILE* TOXIN A WITH TOXIN RECEPTORS

REFERENCE TO GOVERNMENT GRANT

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FIELD OF THE INVENTION

The invention relates generally to the detection, isolation, and purification of *Clostridium difficile* toxin A. The invention also relates to the detection, isolation and purification of materials containing the human I, X or Y antigens which act as receptors for toxin A.

DEFINITIONS

The following sugars are represented by their customary abbreviations:

Gal	galactose
Glc	glucose
GlcNAc	N-acetyl glucosamine
Fuc	fucose
Man	Mannose
LNF III	Lacto-N-fucopentose
LNnH	Lacto-N-neo-hexose

BACKGROUND OF THE INVENTION

Clostridium difficile causes disease within the gastrointestinal tract, usually following an alteration of the intestinal microecology. The pathogenic role of toxigenic *C. difficile* in antibiotic-associated pseudo-membranous colitis in humans is well established. Disease symptoms vary with the source of the pathogen, antibiotic exposure, toxin production, and patient age.

C. difficile produces two toxins, designated A and B. The toxins are implicated in the etiology of the disease. Toxin A is primarily an enterotoxin with slight cytotoxic activity, whereas toxin B is a potent cytotoxin. Toxin A causes extensive damage to the gut mucosa, as well as accumulation of fluid in the intestinal tract. It is believed that the primary event in the mechanism of *C. difficile* infection involves the specific binding of toxin to receptors on the intestinal cell surface.

C. difficile-associated intestinal disease has been reported in infants, and in adults in the absence of antibiotic therapy. Moreover, *C. difficile* is one of the most common bacterial enteropathogens found in stool specimens in hospitals. The organism has been reported to be one of the most commonly detected bacterial pathogens of enteric disease.

C. difficile causes pseudomembranous colitis in humans as a result of the elimination of the normal flora of the colon by antibiotic usage, and growth of this toxin-producing bacterium. The disease usually occurs in hospitalized patients where it causes a massive diarrhea with extensive inflammation of the colon. Mortality rates as high as 44% have been reported for this disease. Treatment is based on a proper diagnosis which is accomplished by establishing the presence of the toxin and demonstrating the characteristic lesions in the colon.

One method for detecting pathogenic *C. difficile* involves the culture of human feces, which requires specialized facilities for a long period of incubation. This test suffers from interference by non-pathogenic *C. difficile* strains, namely strains not producing toxin. The test is costly, time-consuming, and can only be performed in larger, well-equipped hospitals or in private laboratories.

Specific antisera to toxin A have been used to detect toxin A by means of an enzyme-linked immunosorbent assay (ELISA). Lyerly, et al., *J. Clin. Microbiol.* 17, 72–78 (1983). Similarly, the resulting immobilized antibody agglutinates soluble toxin A. The agglutination of the large latex beads may be visualized, indicating the presence of toxin A in the sample.

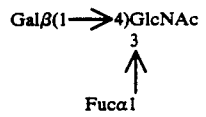
Lyerly et al., *J. Clin. Microbiol.* 21, 12–14 (1985) disclose a monoclonal antibody, affinity-purified polyclonal antibody and monospecific antiserum against toxin A as possible immunodiagnostic reagents for *C. difficile* disease.

U.S. Pat. No. 4,863,852 describes a method for isolating, detecting and purifying *C. difficile* toxin A utilizing a biological receptor for toxin A comprising a reagent containing the terminal oligosaccharide sequence Galα(1→3)Galβ(1→4)GlcNAc. The carbohydrate does not exist on human cells. Maximal binding of the toxin A to the Galα(1→3)Galβ(1→4)GlcNAc receptor occurs at 4° C. At 37° C., binding is not detected.

SUMMARY OF THE INVENTION

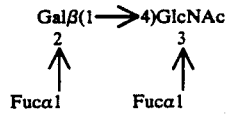
We have found that in addition to binding the non-human receptor Galα(1→3)Galβ(1→4)GlcNAc, toxin A is capable of specific reversible binding to human antigens X, Y and I, none of which contain the α(1→3)-linked terminal galactose of the animal receptor.

Thus, *C. difficile* enterotoxin (toxin A) may be detected and/or purified by contacting a sample suspected of containing the toxin with a reagent comprising one or more of the terminal non-reducing structures which characterize antigens X, Y or I. The terminal non-reducing portion of the X antigen recognized by toxin A, which antigen is also known as "LNF III" or "Le^X", comprises the trisaccharide:



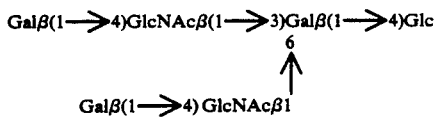
(I)

The terminal non-reducing structure characterizing the Y antigen, which antigen is also known as "Le^Y", comprises the branched tetrasaccharide:



(II)

The terminal non-reducing structure of the I antigen, which antigen is also known as "LNnH", comprises the branched hexasaccharide:



(III)

Quantitative binding of toxin A to these structures occurs rapidly, specifically, reliably, and reversibly in a