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Serum Antibody Response to *Clostridium botulinum* Toxin in Infant Botulism

LORRY G. RUBIN,[1]* MANUCHEHR DEZFULIAN,[2] AND ROBERT H. YOLKEN[1]

Eudowood Division of Infectious Diseases, Department of Pediatrics,[1] and Division of Infectious Diseases, Department of Medicine,[2] Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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A serum antibody response has not been previously demonstrated after infection with *Clostridium botulinum*. We developed an enzyme immunoassay for measuring serum antibody to *C. botulinum* toxins A, B, and E. This assay system detected a specific immunoglobulin G and immunoglobulin M antibody response to *C. botulinum* toxin in two patients with infant botulism.

Infant botulism, recognized as a distinct clinical entity since 1976 (5), is a neuromuscular disorder affecting infants less than 1 year of age which is being diagnosed with increasing frequency (2). The pathogenesis differs from that of classical food-borne botulism in that patients with infant botulism become colonized with *Clostridium botulinum* organisms. The subsequent intestinal absorption of toxin produced in vivo results in the neurological symptoms (1). Infant botulism is usually associated with toxin types A and B. The laboratory diagnosis is difficult to establish since it depends upon the demonstration of neutralizable toxin in a mouse lethality bioassay (3). A serological response to *C. botulinum* toxins has not been demonstrated in patients after either infection with *C. botulinum* or ingestion of *C. botulinum* toxin (4). This report describes the development of a solid-phase immunoassay for detecting immunoglobulin G (IgG) and IgM antibody to *C. botulinum* and documents a serum antibody response in two patients with infant botulism.

The first patient was a 4-month-old female infant who presented with constipation, poor feeding, and respiratory failure. *C. botulinum* type B was isolated from multiple stool specimens, and type B toxin was identified in the stool by the mouse neutralization assay. The infant required ventilatory support for 7 weeks and has subsequently made a full recovery. The second patient was a 3-week-old male infant with hypotonia, constipation, and lethargy. *C. botulinum* type A as well as type A toxin was identified in a stool specimen. He also required ventilatory support and is currently convalescing from his illness. Neither patient received antitoxin.

A solid-phase enzyme immunoassay was performed in a manner analogous to other systems for measuring IgG and IgM antibody (6). Type A, type B, type E, or pentavalent (A, B, C, D, and E) botulism toxoid (Michigan Department of Health, Lansing, Mich.) was adsorbed to polyvinyl microtiter plates (model 220-24; Dynatech Laboratories, Inc., Alexandria, Va.). After the removal of unbound toxoid, serum specimens from the patients were diluted in phosphate-buffered saline containing 0.5% Tween 20 and 0.5% gelatin and were then reacted with the bound toxoid. Antibody reacting with the bound toxoid was then quantitated by reaction with alkaline phosphatase-conjugated goat anti-human IgG (Miles-Yeda, Elkhart, Ind.) or alkaline phosphatase-conjugated anti-human IgM (Dynatech). The substrate (*para*-nitrophenylphosphate, Sigma 104 phosphatase substrate tablets; Sigma Chemical Co., St. Louis, Mo.) was added, and the colorometric endpoint was read by an automated spectrophotometer. Positive samples were defined as those having optical densities two standard deviations greater than the mean of the blank specimens. The amount of nonspecific binding to the solid phase was quantitated by reacting the specimens with uncoated wells.

The results are shown in Table 1. Patient 1 developed a serum antibody response to both type A and type B but not to type E toxoid; she also demonstrated an IgM response to both type B (not shown) and to the pentavalent toxoids. Patient 2 had a higher titer of antibody to the type A toxoid than to the type B toxoid preparation and no antibody to type E toxoid; IgM antibody to the pentavalent toxoid was also detected in this serum. Acute and convalescent serum from an adult with *Clostridium difficile*-associated colitis showed no increase in antibody to the types A, B, or E toxoids and no IgM antibody response.

TABLE 1. Serum antibody to *C. botulinum* toxin in infant botulism

Serum source and diagnosis	Reciprocal dilution of antibody titer[a] to toxoid:			Reciprocal dilution of IgM antibody titer[a] to pentavalent (A,B,C,D,E) toxoid
	Type A	Type B	Type E	
Case 1: infant botulism, type B				
Acute (day 4)[b]	<40	40	40	40
Convalescent (day 64)	160	640	<10	40
Case 2: infant botulism, type A				
Convalescent (day 31)	640	160	<10	160
Positive control: immunized adult	>5,120	>5,120	2,560	40
Negative control: *C. difficile*-associated colitis				
Acute (day 3)	<40	160	40	<10
Convalescent (day 12)	<40	<40	40	<10

[a] Determined by enzyme immunoassay (see text).
[b] Numbers in parentheses show days after the onset of illness.

The specificity of these assays is not fully defined. The assay demonstrates some cross-reactivity between type A and B toxoids. This may reflect reaction with other antigens of *C. botulinum* or shared antigenic sites on these two toxins or both. The lack of seroconversion in a patient with a different clostridial infection, namely *C. difficile*-associated colitis, indicates some specificity. Antibody seroconversion and the detection of IgM antibody to type B toxin in patient 1 clearly demonstrate a host immune response to this toxin in infant botulism. Once the specificity of these antibody titers is confirmed and more serum samples are available for testing, this assay may provide a simplified means of confirming the diagnosis of infant botulism, and the assay for IgM antibody may provide a means of rapid diagnosis. Determinations of the prevalence of seropositivity in individuals in infancy, childhood, and adulthood may lend further insight into the host response and the pathogenesis of this infection and may further define the clinical spectrum of this disorder. Comparisons of rates of seropositivity in developing countries with the rate in the United States may provide clues to the worldwide public health significance of infant botulism.

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