

## Evaluation of Neutralizing Antibodies to Type A, B, E, and F Botulinum Toxins in Sera from Human Recipients of Botulinum Pentavalent (ABCDE) Toxoid

LYNN S. SIEGEL

*Department of Toxinology, Pathology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-5011*

Received 6 March 1989/Accepted 2 May 1989

**Twenty-five serum specimens from personnel immunized with botulinum pentavalent toxoid (ABCDE) had titers of neutralizing antibodies to type A (5.7 to 51.6 IU/ml), type B (0.75 to 18 IU/ml), and type E (0.61 to 10 IU/ml) botulinum toxins. Titers for one type could not be used to predict titers for another type in individuals receiving the toxoid. Cross-neutralizing antibodies to type F botulinum toxin were not detected (<0.0125 IU/ml).**

There are seven types of *Clostridium botulinum*, designated A to G, each type producing a pharmacologically similar but immunologically distinct neurotoxin. Immunization with botulinum toxoid has been used for over 40 years to protect laboratory personnel at risk for botulism due to contact with the neurotoxins. The botulinum toxoid currently distributed by the Centers for Disease Control is pentavalent, containing Formalin-inactivated botulinum toxins of type A, B, C, D, and E adsorbed to aluminum phosphate (4). Toxoids against types F and G are not currently available for human use. However, it has been reported that large quantities of antibody to type E neurotoxin can neutralize small quantities of type F neurotoxin (2, 3, 12, 15). This is of particular interest because type F botulinum neurotoxin causes human disease, both food-borne (11, 12) and infant (10) botulism. Furthermore, there are single strains of *C. botulinum* that produce type F along with another type of neurotoxin: A + F (7), including a strain isolated from a patient with food-borne botulism (5), and B + F, isolated from a case of infant botulism (9). We therefore investigated the possibility that sera from individuals receiving the pentavalent toxoid and having high titers of neutralizing antibodies to type E neurotoxin could also neutralize type F neurotoxin.

As previously described (13), sera obtained from individuals immunized with botulinum pentavalent (ABCDE) toxoid were tested for neutralizing antibodies to type A or B botulinum toxin by using a mouse bioassay. Serum samples that had high A and B titers (arbitrarily defined as >5 and >0.7 IU/ml, respectively) were assayed for neutralizing antibodies to type E and type F botulinum toxins. (One international unit is defined as the amount of antibody neutralizing 10,000 mouse intraperitoneal 50% lethal doses [LD<sub>50</sub>s] of type A, B, C, D, or F botulinum toxin or 1,000 mouse intraperitoneal LD<sub>50</sub>s of type E toxin [14].) For comparison, note that the Centers for Disease Control recommends against administration of a booster immunization to any individual having a titer of approximately 0.25 IU/ml (1:16) or greater for the types of botulinum toxin for which he or she is at risk (4).

The concentration of type E or F toxin used in the assay was that which was neutralized by 0.0125 IU/ml of the homologous type of World Health Organization Interna-

tional Standard antitoxin. For type E, fourfold dilutions of serum samples (1/16 to 1/1,024) were mixed with an equal volume of standardized toxin. In the type F neutralization test, sera were initially tested without dilution and at 1/4 to 1/64 dilutions, but in subsequent assays only undiluted serum was used. For both E and F, the toxin-serum mixtures were incubated for 1 h at room temperature, and then 0.2 ml was injected intraperitoneally into each of eight mice. The animals were observed for 4 days for deaths. The concentration of neutralizing antibodies in the serum was calculated relative to the homologous World Health Organization International Standard antitoxin (equine for E, rabbit for F) which was included in each test, and results are reported in international units per milliliter. Undiluted sera that did not protect mice from death are reported as having neutralizing titers of <0.0125 IU/ml for type F.

Results are shown in Table 1. In this group of 25 serum specimens, the A titers ranged from 5.7 to 51.6 IU/ml and the B titers ranged from 0.75 to 18 IU/ml, but the E titers were lower than anticipated, 0.61 to 10 IU/ml (Table 1). Ranking the sera in ascending order by type A titer did not demonstrate a corresponding increase in titer for type B or type E. For the 25 serum samples assayed, there was no correlation between the neutralizing antibody titers for type A, B, and E botulinum toxins, and the titer to one toxin type could not be used to predict that to another type.

Previous investigations with animal sera (2, 3, 12, 15) demonstrated that large quantities of type E antitoxin would neutralize small quantities of type F toxin. Moller and Scheibel (12) reported that equine antitoxin from the Microbiological Research Establishment, Porton, England, neutralized 3,200 mouse minimal lethal doses (MLD) of type E toxin and 2 MLD of type F toxin (Langeland strain). Antitoxins from the Pasteur Institute, Paris, produced analogous results (12). A quantity of E antitoxin (equine) that neutralized 4,000 MLD of type E toxin also neutralized 10 MLD of type F Langeland (2). Two to three MLD of type F toxin from strain 202F, a nonproteolytic strain isolated from marine sediments on the Pacific Coast of the United States, was neutralized by a volume of type E antitoxin from the Centers for Disease Control which neutralized 1,000 MLD of type E toxin (3). Rabbit antitoxin prepared against purified type E neurotoxin which neutralized 2,000 LD<sub>50</sub>s of type E

TABLE 1. Neutralization titers to type A, B, E, and F botulinum toxins for 25 individuals

Neutralization titer (IU/ml) to toxin type <sup>a</sup> :			
A	B	E	F
5.74	1.28	2.53	<0.0125
5.74	4.07	9.16	<0.0125
6.01	2.32	9.16	<0.0125
6.45	2.18	2.26	<0.0125
7.24	5.12	2.26	<0.0125
9.12	3.23	9.16	<0.0125
10.2	2.03	0.69	<0.0125
11.5	2.18	5.65	<0.0125
12.0	3.46	6.73	<0.0125
12.9	2.03	0.62	<0.0125
12.9	2.87	2.76	<0.0125
14.5	0.75	1.10	<0.0125
14.5	6.64	2.85	<0.0125
16.3	3.23	2.26	<0.0125
18.2	2.87	3.20	<0.0125
18.2	2.87	6.40	<0.0125
22.2	4.07	1.74	<0.0125
23.0	3.23	1.74	<0.0125
25.6	2.75	6.40	<0.0125
35.2	3.23	6.40	<0.0125
41.0	3.23	0.61	<0.0125
41.0	4.18	7.07	<0.0125
41.0	5.75	10.0	<0.0125
41.0	5.75	10.0	<0.0125
51.6	18.2	7.07	<0.0125

<sup>a</sup> One international unit is the amount of antibody neutralizing 10,000 mouse intraperitoneal LD<sub>50</sub>s of type A, B, or F botulinum toxin or 1,000 mouse intraperitoneal LD<sub>50</sub>s of type E toxin.

toxin also neutralized 5 LD<sub>50</sub>s of type F toxin (15). Thus, the type E/type F neutralization ratios previously reported are 1,600/1 for two equine samples (12) and 400/1 for sera from an equine source (2), a rabbit source (15), and an unknown animal source (3). In this study for human immune sera, titrations to determine the exact number, if any, of LD<sub>50</sub>s of type F toxin that the sera could neutralize were precluded by the lack of sufficient volumes of sera. However, our purpose was to determine whether immunization of personnel with the pentavalent toxoid had elicited levels of cross-neutralizing antibody to type F neurotoxin that could be considered protective.

The correlation between the level of neutralizing antibody in the serum and the ability to withstand an exposure to botulinum toxin is, of course, not known for humans. Values used as "satisfactory" titers in humans for types A, B, C, D, and E have been extrapolated from animal studies and were established to indicate that an individual had responded to the pentavalent immunogen (6). These satisfactory levels are twice the lowest titer that can be measured by using the mouse bioassay (1). Using purified type F monovalent toxoid to immunize guinea pigs, Hatheway demonstrated a relationship between antibody levels and the ability of the animals to survive challenge with 10<sup>5</sup> LD<sub>50</sub>s of type F toxin (8). All guinea pigs having antibody concentrations greater than 0.04 U/ml survived challenge, as did 50 to 100% of the animals with titers of 0.01 to 0.04 U/ml. However, groups of animals with antibody levels that were undetectable (<0.01 U/ml) had survival rates of less than 50% after challenge. Thus, antibody levels in guinea pigs that are protective for type F toxin are comparable to those previously established for types A, B, C, D, and E. Similar extrapolation of these data for type F to humans would indicate that 0.025 IU/ml is a satisfactory titer.

Neutralizing antibodies to type F were not detected (<0.0125 IU/ml) in each of the sera (Table 1). Thus, human sera at 10 IU/ml for type E (1 ml of serum can neutralize 10,000 LD<sub>50</sub>s of type E botulinum toxin) failed to neutralize 125 LD<sub>50</sub>s of type F botulinum toxin. Since we tested undiluted serum for its ability to neutralize only one concentration of type F toxin, it is possible that a lower level of immunity to type F exists in personnel immunized with the pentavalent toxoid. However, if 1 ml of undiluted serum had indeed neutralized 125 mouse LD<sub>50</sub>s of type F toxin, then, assuming a plasma volume of 3,000 ml, this individual would be capable of neutralizing 3.75 × 10<sup>5</sup> mouse LD<sub>50</sub>s of type F toxin. Immunity and protection must be considered in the context of a potential laboratory exposure of at-risk personnel. Since growing cultures of type F *C. botulinum* produce 10<sup>5</sup> to 10<sup>6</sup> mouse LD<sub>50</sub>s of type F toxin per ml of culture fluid (8), we would not consider this level of immunity adequate.

Of the 25 serum specimens tested (Table 1), 14 were from individuals who had received five or more annual boosters of the pentavalent toxoid. Therefore, it is unlikely that additional immunizations with this product could be used to increase titers to type F. A monovalent type F botulinum toxoid for human use is currently being developed, as is a monovalent type G toxoid.

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