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

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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ABSTRACT

Twenty-five sera from personnel immunized with botulinum pentavalent toxoid (ABCDE) had titers of neutralizing antibodies to type A [5.7-51.6 international units (IU)/ml], type B (0.75-18 IU/ml), and to type E (0.61-10 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). (F10)

There are seven types of Clostridium botulinum, designated A-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 types 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 does cause 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, using a mouse bioassay. Serum samples that had a high A and B titer (arbitrarily defined as > 5 and > 0.7 international units/milliliter, respectively) were assayed for neutralizing antibodies to type E and to type F botulinum toxins. (One international unit [IU] is defined as the amount of antibody neutralizing 10,000 mouse intraperitoneal 50% lethal doses [LD_{50}] of type A, B, C, D, or F botulinum toxin or 1,000 mouse intraperitoneal LD_{50} of type E [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 to which he or she is at risk (4). Typically, the antibody response to the B component of the toxoid is the poorest (1, 6).

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 International 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, 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 four 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 as IU/ml. Undiluted sera that did not protect mice from death are reported as <0.0125 IU/ml for type F.

Results are shown in Table 1. In this group of 25 sera, the A titers ranged from 5.7 to 51.6 IU/ml and the B titers 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 types 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 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 (Connaught, 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 LD₅₀ of type E toxin also neutralized 5 LD₅₀ of type F toxin (15).

In the study reported here for human immune sera, titers for type E ranged from 0.61 to 10 IU/ml. However, neutralizing antibodies to type F were not detected (<0.0125 IU/ml) in each of the sera. Thus, human sera at 10 IU/ml for type E (1 ml of serum can neutralize 10,000 LD₅₀ of type E botulinum toxin) failed to neutralize 125 LD₅₀ of type F botulinum toxin. Titrations to determine the exact number of LD₅₀ of type F toxin that the sera could neutralize, if any, 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 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 10^5 LD₅₀ 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 a survival rate 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.

The neutralization of small amounts of type F botulinum toxin by type E antitoxin (2, 3, 12, 15) is important biochemically because it indicates that the two neurotoxic proteins share some common epitopes, and therefore have a degree of structural similarity. However, this cross-neutralization is of little practical significance.

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

Neutralization Titer (IU ^a /ml)			
Type A	Type B	Type E	Type 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

^a One International Unit (IU) is the amount of antibody neutralizing 10,000 mouse intraperitoneal 50% lethal doses of type A, B, or F botulinum toxin, or 1,000 mouse intraperitoneal 50% lethal doses of type E.