Chapter 16

BOTULINUM TOXIN

ZYGMUNT F. DEMBEK, PhD, MS, MPH*; LEONARD A. SMITH, PhD†; AND JANICE M. RUSNAK, MD‡

*Lieutenant Colonel, Medical Service Corps, US Army Reserve; Chief, Biodefense Epidemiology and Training and Education Programs, Operational Medicine Department, Division of Medicine, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702

†Chief, Department of Molecular Biology, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702

‡Lieutenant Colonel, US Air Force (Ret); Research Physician, Special Immunizations Program, Division of Medicine, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702; formerly, Deputy Director of Special Immunizations Program, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland

INTRODUCTION

The neurotoxins produced by *Clostridia* species are among the most potent toxins known. Because of their extreme toxicity, botulinum (*C botulinum*) neurotoxins were one of the first agents to be considered as a biological weapons agent. Botulinum neurotoxin has been developed as a biological weapon by many countries, including Japan, Germany, the United States, Russia, and Iraq (Figure 16-1). Botulism is a neuroparalytic disease, most commonly caused by foodborne ingestion of neurotoxin types A, B, and E, and is often fatal if untreated.

HISTORY

In the early 1930s, during its occupation of Manchuria, Japan formed a biological warfare command called Unit 731. General Shiro Ishii, the military medical commander of Unit 731, admitted to feeding lethal cultures of *C botulinum* to prisoners.[1] US researchers began working on weaponization of botulinum toxin in the 1940s, and Allied intelligence indicated that Germany was attempting to develop botulinum toxin as a weapon to be used against invasion forces.[2] At the time, neither the composition of the toxic agent produced by *C botulinum* nor its mechanism of injury were fully known. Therefore, the earliest research goals were to isolate and purify the toxin and to determine its pathogenesis. The potential of botulinum neurotoxin as an offensive biological weapon was also investigated[3–5] (the US code name for botulinum neurotoxin was "agent X").

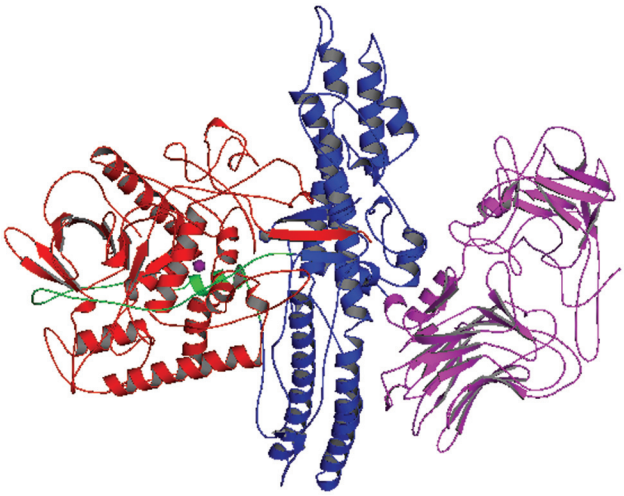

Fig. 16-1. Botulinum neurotoxin A is composed of an ~50 kDa light chain (LC-red) and an ~100 kDa heavy chain linked by a single disulfide bond. The LC functions as a zinc-dependent endopeptidase, whereas the heavy chain contains two functional ~50 kDa domains: a C-terminal ganglioside binding domain (Hc-purple), and an N-terminal translocation domain (Hn-blue). A belt portion of Hn (green) wraps around LC. The active site zinc is shown as a purple sphere. This figure is based on the structure determined by Lacy and colleagues. Data source: Lacy DB, Tepp W, Cohen AC, DasGupta BR, Stevens RC. Crystal structure of botulinum neurotoxin type A and implications for toxicity. *Nat Struct Biol*. 1998;5:898–902.
Courtesy of S Ashraf Ahmed, MD, Integrated Toxicology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

Following President Richard M Nixon's executive orders in 1969–1970, all biological agent stockpiles in the US offensive biological program, including botulinum neurotoxin, were destroyed. The 1975 Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction prohibited the production of offensive toxins.

Although the Soviet Union signed and ratified the convention,[6] its biowarfare program, including botulinum neurotoxin research, weapons development, and production, continued and was even expanded in the post-Soviet era.[7,8] The Soviet Union reportedly tested botulinum-filled weapons at the Soviet site Aralsk-7 on Vozrozhdeniye (Renaissance) Island in the Aral Sea[8,9] and also attempted to use genetic engineering technology to transfer complete toxin genes into other bacteria.[10] In April 1992, President Boris Yeltsin publicly declared that his country had covertly continued a massive offensive biological warfare buildup, which included developing botulinum toxin as a weapon. That same year, Colonel Kanatjan Alibekov (Kenneth Alibek), the former deputy director of Biopreparat (a Soviet agency whose primary function was to develop and produce biological weapons of mass casualties), defected to the United States and described in detail the Soviet biological weapons program.[10]

Iraq, which also signed the 1975 convention, expanded its biowarfare program in 1985. Ten years later, it admitted to the United Nations Special Commission inspection team to having produced 4,900 gallons of concentrated botulinum neurotoxin for use in specially designed missiles, bombs, and tank sprayers in 1989 and 1990.[7,11] Of this preparation, 2,600 gallons were used to fill 13 SCUD missiles with a 600-km range and 100 400-lb (R-400) bombs (each bomb could hold 22 gallons of toxin solution). However, Iraq did not use biological agents during the Persian Gulf War or Operation Iraqi Freedom, and it has maintained that its biological weapon stockpiles were destroyed.[12]

The Aum Shinrikyo, a Japanese cult formed in 1987 by Shoko Asahara, attempted to develop biological weapons after its political party was defeated in the 1990 election campaign. Known for its deadly 1995 sarin attack in the Tokyo subway, Aum Shinrikyo also attempted to produce botulinum neurotoxin. Before the sarin attack, three briefcases containing portable disseminating devices generating water vapor were found in the subway station. At his 1996 trial, Asahara said he believed the cases contained botulinum neurotoxin, although the toxin was not detected in the devices. With 50,000 followers worldwide and an estimated $1 billion in financial resources, the cult had the capability to develop biological toxins for use as weapons, and the intent to do so.[13] Although no cult members were specialists in biological weapons development, microbiologists, medical doctors, and other scientists were among the followers. It is not fully understood why the biological assaults failed, but information from Asahara's trial indicated that the cult's scientists had difficulty overcoming technical barriers in isolating and cultivating *C botulinum*.[13]

A successful bioterrorist attack on large numbers of people with botulinum neurotoxin would likely overwhelm the public health system. The medical intervention required to assist patients with botulism includes mechanical ventilation and urgent attendant healthcare. If the Rajneeshee cult had used a colorless, odorless, and tasteless solution of botulinum toxin instead of *Salmonella typhimurium* on salad bars in its 1984 attack in The Dalles, Oregon,[14–16] many of the 751 persons who contracted *Salmonella* gastroenteritis would likely have died; the neurological sequelae of hundreds of patients with botulinum toxin poisoning would have quickly overwhelmed community medical resources.[17]

In 2005 Wein and Liu[18] described in detail how a bioterrorism attack using botulinum neurotoxin could be perpetrated upon the nations' milk supply. They describe a mathematical model representative of California's dairy industry with milk traveling from cows to consumer in a supply chain: milk is processed from cows; picked up by tanker truck; piped through milk silos; processed via separation, pasteurization, homogenization, and vitamin fortification; and eventually distributed to the public.[18] Naturally occurring salmonellosis outbreaks from milk and milk products affecting over 200,000 persons have already occurred, leading to a realistic assessment of such vulnerability in the national milk distribution system.[19,20] The ability to spread botulinum neurotoxin via a liquid media, if present in sufficient concentration, makes this agent a logical choice for such a scenario. Modeling of botulinum in a liquid dispersal medium is not new, and has been posited for terrorist use in a water fountain,[21] based upon microbiological contamination at a recreational facility[22]; however, Wein and Liu's modeling goes much further than tocsin generation, pinpointing critical entry points of neurotoxin into the milk supply, estimating the amount of toxin required, and pointing out weaknesses in current detection technology.[18] The paper has generated debate.[23] Stewart Simonson, former assistant secretary for public health emergency preparedness at the US Department of Health and Human Services, has regretted the publication decision.[24]

DESCRIPTION OF THE AGENT

Clostridium species bacteria are sporulating, obligate anaerobic, gram-positive bacilli. The spores of *C botulinum* are ubiquitous, distributed widely in soil and marine sediments worldwide, and often found in the intestinal tract of domestic grazing animals.[25–29] Under appropriate environmental or laboratory conditions, spores can germinate into vegetative cells that will produce toxin. *C botulinum* grows and produces neurotoxin in the anaerobic conditions frequently encountered in the canning or preservation of foods. The spores are hardy, and special efforts in sterilization are required to ensure that the spores are inactivated. Modern commercial procedures have virtually eliminated food poisoning by botulinum toxin; most cases today are associated with home-canned foods (particularly vegetables such as beans, peppers, carrots, and corn that are associated with a higher pH) or food items prepared by restaurants.[30,31]

C botulinum produces seven antigenic types of neurotoxins, denoted by the letters A through G. All seven neurotoxins are structurally similar (approximately 150 kd in mass) but immunologically distinct.[32] However, there is some serum cross-reactivity among the serotypes because they share some sequence homology with one another as well as with tetanus toxin.[33] The unique strain *C baratii* produces only serotype F,[34] and the *C butyricum* strain, serotype E.[35]

Botulism is a neuroparalytic disease. Human botulism cases are caused primarily by neurotoxin types A, B, and E,[30] and occasionally by type F.[36] *C argentinense* produces type G, which has been associated with sudden death, but not neuroparalytic illness, in a few patients in Switzerland.[37] Types C and D cause disease in animals. All seven toxins are known to cause inhalational botulism in primates,[38] and therefore could potentially cause disease in humans. Clostridial C2 cytotoxin is an enterotoxin, but not a neurotoxin. It affects multiorgan vascular permeability via cellular

damage from its action on actin polymerization in the cellular cytoskeleton, and has been implicated in a fatal enteric disease in waterfowl.[39,40]

Botulinum Neurotoxin Production

Spore germination and subsequent growth of toxin-producing bacteria occur in improperly preserved foods,[41–48] decaying animal carcasses and vegetable matter,[49–53] and microbiology laboratories.[54–58] A terrorist with the proper expertise and resources could obtain a toxin-producing strain of *C botulinum*. Various scientific journals, textbooks, and Internet sites provide information on how to isolate and culture anaerobic bacteria and, specifically, how to produce botulinum toxin. The major cause of botulism is the ingestion of foods contaminated with *C botulinum* and preformed toxin. The food supply remains vulnerable to a botulinum toxin attack (discussed later in this chapter).

PATHOGENESIS

The seven neurotoxins have different specific toxicities[59–61] and durations of persistence in nerve cells.[62,63] All botulinum toxin serotypes inhibit acetylcholine release, but they act through different intracellular protein targets, exhibit different durations of effect, and have different potencies.[64] All seven toxins may potentially cause botulism in humans given a large enough exposure. Botulinum neurotoxin can enter the body via the pulmonary tract (inhalational botulism), the gastrointestinal tract (foodborne and infant botulism), and from infected wounds (wound botulism). Upon absorption, the circulatory system transports the toxin to peripheral cholinergic synapses, primarily targeting neuromuscular junctions.[65] The toxin binds to high-affinity presynaptic receptors and is transported into the nerve cell through receptor-mediated endocytosis. In the nerve cell, it functionally blocks neurotransmitter (acetylcholine) release, thereby causing neuromuscular paralysis. Other neurotransmitters co-located with acetylcholine may also be inhibited,[66,67] and noncholinergic cells may also be affected.[68] The estimated human dose (assuming a weight of 70 kg) of type A toxin lethal to 50% of an exposed population (the LD_{50}) is estimated, based on animal studies, to be approximately 0.09 to 0.15 μg by intravenous administration, 0.7 to 0.9 μg by inhalation, and 70 μg by oral administration.[69–72]

CLINICAL DISEASE

Untreated botulism is frequently fatal. The rapidity of the onset of symptoms, as well as the severity and duration of the illness, is dependent on the amount and serotype of toxin.[30,73] In foodborne botulism, symptoms appear several hours to within a few days (range 2 hours to 8 days) after contaminated food is consumed.[30] In most cases the onset of symptoms occurs within 12 to 72 hours postexposure. In one study, the median incubation period for the onset of symptoms from all toxin serotypes was 1 day.[73] However, the median time to onset of symptoms for serotype E was much shorter (range 0–2 days) compared to toxin serotypes A (range 0–7 days) and B (range 0–5 days); most individuals with toxin serotype E had symptoms within 24 hours of ingestion. Symptoms from foodborne botulism from toxin serotype A generally are more severe than from toxin serotypes B and E.[73]

As a neuroparalytic illness, botulism presents as an acute, symmetrical, descending, flaccid paralysis. However, early symptoms may be nonspecific and difficult to associate with botulinum intoxication. Individuals with foodborne botulism often present initially with gastrointestinal symptoms such as nausea, vomiting, abdominal cramps, and diarrhea. Initial neurologic symptoms usually involve the cranial nerves, with symptoms of blurred vision, diplopia, ptosis, and photophobia, followed by signs of bulbar nerve dysfunction such as dysarthria, dysphonia, and dysphagia. Onset of muscle weakness ensues in the following order: muscles involving head control, muscles of the upper extremities, respiratory muscles, and lastly muscles of the lower extremities. Weakness of the extremities generally occurs in a proximal-to-distal pattern, and is generally symmetric.[31] However, asymmetric extremity weakness may occasionally be observed, occurring in 9 of 55 botulism cases in one review.[74] Respiratory muscle weakness can result in respiratory failure, which may be abrupt in onset. In one study, the median time between the onset of intoxication symptoms and intubation was 1 day.[73] Other commonly reported symptoms include fatigue, sore throat, dry mouth, constipation, and dizziness.[74] Botulism is not associated with sensory nerve deficits. However, one review of botulism from toxin serotype A or B showed that 8 of 55 cases reported symptoms of paresthesias.[74] Death is usually the result of respiratory failure or secondary infection associated with prolonged mechanical ventilation. In general, intoxication with toxin serotype A results in a more severe disease, often with bulbar and skeletal muscle impairment, and thus the need for mechanical ventilation.[73–75] Intoxication with toxin serotype B or E is more often

associated with symptoms of autonomic dysfunction, such as internal ophthalmoplegia, nonreactive dilated pupils, and dry mouth.

Paralysis from botulism can be long lasting. Mechanical ventilation may be required for 2 to 8 weeks with foodborne botulism, with paralysis lasting as long as 7 months.[74] Symptoms of cranial nerve dysfunction and mild autonomic dysfunction may persist for more than a year.[76–78]

The following symptom triad should suggest the diagnosis of botulism: (1) an acute, symmetric, descending, flaccid paralysis with prominent bulbar palsies in (2) an afebrile patient with (3) a normal sensorium. The bulbar palsies of botulism consist of the "four Ds": diplopia, dysarthria, dysphonia, and dysphagia. Five classic symptoms have also been used to diagnose botulism: (1) nausea and vomiting, (2) dysphagia, (3) diplopia, (4) dry mouth, and (5) fixed dilated pupils.[74] However, individuals may not exhibit all five symptoms; a recent review from the Republic of Georgia reported that only 2% of patients (13/481) presented with all five criteria.[48]

Although foodborne botulism is the most likely route of exposure for botulism from natural causes or a bioterrorist event, botulism acquired on the battlefield is most likely to occur from inhalation of botulinum toxin, a route of exposure that does not naturally occur. The duration from exposure to the onset of symptoms for inhalational botulism is similar to that observed with ingestion of botulinum toxin, generally ranging from 24 to 36 hours to several days postexposure.[73,79] Clinical symptoms resulting from inhalational intoxication are similar to botulism acquired from ingestion of the toxin.

The only reported inhalation-acquired botulism in humans occurred in 1962 in a German research laboratory.[80] Three laboratory workers experienced symptoms of botulinum intoxication after conducting a postmortem examination of laboratory animals that had been exposed to botulinum toxin type A. Hospitalized 3 days after their exposure, the workers were described as having (*a*) a "mucous plug in the throat," (*b*) difficulty in swallowing solid food, and (*c*) "the beginning of a cold without fever." The symptoms had progressed on the 4th day, and the patients complained of "mental numbness," extreme weakness, and retarded ocular motions. Their pupils were moderately dilated with slight rotary nystagmus, and their speech became indistinct and their gait uncertain. The patients were given antibotulinum serum on the 4th and 5th days. Between the 6th and 10th days after exposure, the patients experienced steady reductions in their visual disturbances, numbness, and difficulties in swallowing. They were discharged from the hospital less than 2 weeks after the exposure, with a mild general weakness as their only remaining symptom.[80]

DIAGNOSIS

The differential diagnosis of botulism includes other diseases with symptoms of paralysis:

- Guillain-Barré syndrome (usually ascending paralysis, paresthesias common, elevated cerebrospinal fluid (CSF) protein [may be normal early in illness], electromyogram findings). Note: The CSF findings are usually normal in botulism, but mild elevation of CSF protein between 50 and 60 mg/dL has been noted in a minority of botulism patients.[74]
- Myasthenia gravis (dramatic improvement with edrophonium chloride, autoantibodies present, electromyogram findings). Note: Botulism cases may have a positive response to edrophonium chloride (26%), but the response is generally not dramatic.[74]
- Tick paralysis (ascending paralysis, paresthesias common, usually does not involve cranial nerves; detailed exam often shows presence of tick).
- Lambert-Eaton syndrome (commonly associated with carcinoma, particularly lung carcinomas; deep tendon reflexes absent; usually does not involve cranial nerves; electromyogram findings similar to botulism).
- Stroke or central nervous system mass lesion (paralysis usually asymmetric, brain imaging abnormal).
- Paralytic shellfish poisoning (history of shellfish ingestion; paresthesias of mouth, face, lips, and extremities common).
- Belladonna toxicity, such as atropine (history of exposure, tachycardia, and fever).
- Aminoglycoside toxicity (drug history of aminoglycoside therapy).
- Other neurotoxins, such as snake toxin (history of snake bite, presence of fang punctures).
- Chemical nerve agent poisoning (often associated with ataxia, slurred speech, areflexia, Cheyne-Stokes respiration, and convulsions).

The clinical presentation of an afebrile patient with an acute, symmetric, descending, flaccid paralysis (without sensory deficits) with a normal sensorium suggests the diagnosis of botulism. Any occurrence of botulism requires notification of public health officials and an epidemiological evaluation. Electrophysiological studies

are helpful in distinguishing botulism from other causes of acute flaccid paralysis, and support a presumptive diagnosis of botulism.[81–83] An electromyogram with repetitive nerve stimulation at 20 to 50 Hz showing facilitation (an incremental response to repetitive stimulation), usually occurring only at 50 Hz, may be helpful in distinguishing botulism from Guillain-Barré syndrome or myasthenia gravis, but not from Eaton-Lambert syndrome.[31] Electrophysiological testing in botulism may also demonstrate a small evoked muscle action potential response to a single supramaximal nerve stimulus, with normal sensory nerve function and nerve conduction velocity test results. However, electrophysiological tests may be normal in botulism. Approximately 15% of patients with botulism may have normal muscle action potential amplitudes, and as many as 38% of patients may not exhibit facilitation.[74] CSF findings are usually normal in botulism, and abnormal findings should suggest another diagnosis. However, mild elevation of CSF protein (between 50 and 60 mg/dL) has been reported in 3 of 14 patients (17%) who had spinal fluid analysis performed.[74] Laboratory findings, such as complete blood count, chemistries, liver and renal function tests, and electrocardiogram are normal in botulism, unless a complication (eg, secondary infection, respiratory failure) has occurred.

Foodborne Botulism

In foodborne botulism, a confirmatory diagnosis can often be made by demonstrating the presence of toxin in patient specimens, such as the serum, stool, gastric aspirate, or vomitus, using mouse bioassays. Mouse bioassays are performed by injecting mice intraperitoneally with the specimen sample suspected to contain toxin (with and without antitoxin). If toxin is present in the specimen, mice injected with the specimen alone (without antitoxin) will usually die from botulism within 6 to 96 hours, but mice injected with the specimen treated with antitoxin will survive. Specimens for mouse bioassays may be sent to the Centers for Disease Control and Prevention (CDC) or other designated state or municipal public health laboratories.

Diagnosis can also be achieved by anaerobic culture and isolation of *Clostridium* species toxigenic strains from clinical specimens, including fecal specimens, gastric aspirates, vomitus, or infected wounds. The organism or toxin can also be isolated from the suspect food to help support the diagnosis.

Toxin Assays in Foodborne Botulism

Toxin assays of specimens from cases of foodborne botulism from 1975 to 1988 showed the presence of toxin in specimens from various sites as follows: sera, 37% (126/240); stool, 23% (65/288); and gastric aspirate, 5% (3/63). Specimens were more likely to be positive if obtained soon after toxin ingestion. Toxin assays of sera were positive in more than 60% of specimens obtained within 2 days after toxin ingestion, in 44% of specimens obtained within 3 days of toxin ingestion, but in only 23% of specimens obtained at day 4 or later.[73] Toxin assays of sera were more likely to be positive in intoxications from toxin serotype A than from toxin serotypes B and E. Toxin assays of the stool were positive in 50% of specimens obtained within 1 day following toxin ingestion, in 39% of specimens obtained within 3 days of ingestion, but in less than 20% of specimens obtained at day 5 or later.[73]

Cultures in Foodborne Botulism

Stool and gastric aspirate cultures for *C botulinum* resulted in a higher yield of diagnosis than toxin assays.[73] Gastric aspirates were positive in 45% of specimens (35/78). Nearly 80% of stool cultures were positive at day 2 postingestion of toxin, with nearly 40% of specimens remaining positive at 7 to 9 days after ingestion. However, in this cohort of patients, laboratory confirmation of botulism could not be obtained in 32% of patients. This reflects the insensitivity of the diagnostic testing, especially when specimens are obtained more than 3 days after toxin ingestion. In these patients, the diagnosis must be based on clinical history, physical examination, electromyography results, epidemiological history (including food consumption), and tests on ingested food samples from epidemiologically linked food. Epidemiological history of injection of black tar heroin (wound botulism), laboratory work with botulinum toxins, or therapeutic use (eg, for cervical dystonia or cosmetic purposes) of botulinum neurotoxin preparations not approved by the Food and Drug Association (FDA) may also support the diagnosis of botulism.[84]

Inhalation-acquired Botulism

Laboratory confirmation of botulism acquired by inhalation may be difficult, because toxin acquired by inhalational exposure is not generally identifiable in the serum or stool, as in foodborne botulism.[85,86] In inhalational exposures to botulinum toxin, the toxin may be detected in the nares for up to 24 hours after exposure, using either an enzyme-linked immunosorbent assay or polymerase chain reaction test of a nasal mucosal swab.[86,87] However, these tests have limited validation in botulism diagnosis, and they may not be as sensitive as mouse neutralization assay in the detection of toxin. Antibody titers also have limited

use in the diagnosis of botulism, because individuals may not develop an antibody response to the small quantity of toxin protein required to cause symptoms. Additionally, cultures of *C botulinum* are not helpful for inhalation of toxin preparations that do not contain spores of the organism.

TREATMENT

The current recommended treatment for botulism, although limited, includes antitoxin therapy and supportive care as needed, including mechanical ventilation. If ingestion of the implicated food has been recent, removal of unabsorbed toxins may be hastened with cathartic agents or enemas, provided ileus is not present. Surgical debridement and antibiotic therapy are recommended for the treatment of wound botulism. Because respiratory failure may begin suddenly, individuals with suspected botulism should be closely monitored, with frequent assessment of the vital capacity and maximal inspiratory force.[88]

Antitoxin

Mortality from botulism before 1950 was approximately 60%.[31] In the early 1970s, therapy with equine antitoxins was introduced, and the botulism case fatality rate dropped to about 23%. The evidence for efficacy of botulinum antitoxin in humans is based on retrospective analyses of small numbers of patients and on animal studies. In one study, type A botulism was associated with a mortality rate in humans of 10% (3/30) with antitoxin therapy, versus 46% (6/13) without antitoxin therapy.[75] The fatality rate of botulism from toxin serotype E in humans was associated with a mortality rate of 3.5% with antitoxin therapy, versus 28.9% without antitoxins.[89] Although the evidence is limited, it is believed that early treatment, especially within 24 hours, is most effective in preventing progression of paralysis. Because antitoxin cannot neutralize toxin once it has bound to the nerve receptors, the antitoxin cannot reverse paralysis; it can only prevent paralysis progression. Symptoms may often progress for up to 12 hours after antitoxin administration before an effect is observed. With adequate ventilatory assistance, tracheostomy, and improved intensive care support, fatality rates from botulism are now less than 5% to 10%.

Individuals suspected to have been exposed to botulinum toxin should be carefully monitored. If a person begins to develop symptoms of botulism, botulinum antitoxin should be administered. Because most antitoxin preparations are equine products, there is a risk of hypersensitivity reactions. Skin testing must be performed before administering equine antitoxins, as described in the package insert. The bivalent botulinum equine antitoxin (serotypes A and B) is the only FDA-approved antitoxin preparation currently available for adults, and may be obtained from the CDC (contact the local health department or, if it is unavailable, the CDC at 770-488-7100). The CDC also has an investigational equine antitoxin product for toxin serotype E. The trivalent equine botulinum antitoxin product (A, B, E) is no longer available at the CDC because of declining antitoxin titers to toxin serotype E in the product. An investigational human botulinum immune globulin against toxin serotype E is also available at the California Department of Health Services (510-231-7600).

In October 2003 the FDA approved human botulism immune globulin (BabyBIG), a significant advancement in the treatment of infantile botulism. BabyBIG is a human botulism immune globulin derived from pooled plasma of adults immunized with pentavalent botulinum toxoid, with subsequent development of high titers of neutralizing antibodies against toxins serotypes A and B. Because it is derived from humans, BabyBIG does not have the high risk of anaphylaxis observed with the equine products, nor the risk of lifelong hypersensitivity to equine antigens. Infantile botulism occurs primarily in newborns less than one year of age, caused by toxin production from intestinal colonization and growth of *C botulinum*, with approximately 100 cases diagnosed per year in the United States.[90] Use of BabyBIG is anticipated to save about $70,000 per case in hospital costs.[91,92] BabyBIG can be obtained from the California Department of Health Services.

Additionally, two equine antitoxin preparations against all seven toxin serotypes, developed by the US Army Medical Research Institute of Infectious Diseases, are available as investigational drugs for treating botulism: (1) botulinum antitoxin, heptavalent, equine, types A, B, C, D, E, F, and G (HE-BAT) and (2) botulinum antitoxin, $F(ab')_2$ heptavalent, equine toxin neutralizing activity types A, B, C, D, E, F, and G (Hfab-BAT). These products are "despeciated" equine antitoxin preparations, made by cleaving the Fc fragments from the horse immunoglobulin G molecules to reduce the side effects such as serum sickness and hypersensitivity reactions, leaving only the $F(ab')_2$ fragments. Although the species-specific antigens have been removed, there is still a reduced risk for hypersensitivity reactions because 4% of horse antigen molecule remains in the preparation. The HE-BAT heptavalent product, when administered to an individual as a single vial of 10 mL, was formulated

to provide more than 4,000 IU of serotypes A, B, C, E, and F, and more than 500 IU of serotypes D and G antitoxin. One IU (international unit) of antitoxin, by definition, is the amount of antitoxin that will neutralize 10,000 LD_{50} of toxin serotypes A, B, C, D, F, and G, respectively, and 1,000 LD_{50} of toxin serotype E. These investigational products would be considered for treatment of botulism in the event of biowarfare or bioterrorism, which may involve the use of any of the seven toxin serotypes.

Animal studies show that the heptavalent antitoxin products are protective in both mice and nonhuman primates. The products were shown to neutralize each of the botulinum toxin serotypes in vitro; mice injected with a mixture of heptavalent antitoxin and a specific toxin serotype did not develop symptoms of botulism. The Hfab-BAT product, given to asymptomatic mice within a few hours after aerosol challenge with approximately 10 LD_{50} of serotype A, was protective, even with a dose as low as one tenth of one human dose. This dose resulted in low levels of antitoxin titers, 0.02 IU/mL or lower.[72] The product was also protective against aerosol challenge to toxin serotype A at a dose of approximately 2,000 mouse intraperitoneal LD_{50}/kg, when given to nonhuman primates immediately prior to exposure (protection in 5/5 animals), and when given 48 hours after inhalational exposure (protective in 3/5 monkeys).

If antitoxin was given at the onset of respiratory failure, the Hfab-BAT product was not protective in the mouse model against aerosol exposure or intraperitoneal exposure, even with a dose that was 3-fold greater than the recommended human-equivalent dose. The ineffectiveness of delayed antitoxin administration in mice may be because the majority of toxin is no longer present in the circulation at the time of the antitoxin administration (ie, it is already bound to nerve terminals). Respiratory failure in mice occurred within 1 to 3 hours, and death within 2.8 to 11 hours postexposure, much earlier than observed in humans and nonhuman primates, in which death generally does not occur until 2 to 3 days postexposure. In one review of foodborne botulism in humans, shortness of breath at presentation was also identified as a poor prognostic factor for survival, even with antitoxin therapy; it was noted in 94% (50 of 55) of the deaths.[48]

Clinically Relevant Signs of Bioterrorist Attack

The first evidence of a bioterrorist attack with botulinum toxin would likely be reports from hospitals and urgent care medical facilities as they begin to receive victims with symptoms suggestive of botulism. Because antitoxin therapy must be given early to have a beneficial effect, the initial diagnosis of botulism is clinical, with confirmation by laboratory findings afterwards. Neurological signs and symptoms resulting from a toxin-induced blockade of neurotransmission at voluntary motor and cholinergic junctions dominate the clinical manifestation of botulism.[73,93,94] A diagnosis of botulism is suggested in individuals presenting with an acute onset of cranial nerve weakness (ie, diplopia, ptosis, dysphonia, dysphagia, and dysarthria). In mild cases, no further symptoms may develop. In more severe cases, individuals may progress and develop descending symmetrical weakness and flaccid paralysis. Because mechanical ventilation may be required for individuals with respiratory failure resulting from paralysis of the respiratory muscles, hospital bioterrorism plans should include contingency plans for additional ventilatory and intensive care unit support for mass intoxication. Antitoxin therapy is indicated in cases of suspected botulism, to inactivate and clear toxin from the circulatory system before it can enter peripheral cholinergic nerve cells.

An outbreak of botulism in 2004 illustrates the vulnerability of readily accessible bulk botulinum toxin. Four cases of botulism resulted from use of toxin serotype A for cosmetic purposes. A vial of raw bulk botulinum toxin (a non-FDA approved formulation) containing between 20,000 and 10 million units of botulinum toxin (a vial of FDA-approved BOTOX [Allergan, Inc, Irvine, Calif] contains only 100 units of toxin) was used by an unlicensed physician for cosmetic injections into three patients and himself.[95,96] The four individuals were subsequently admitted to medical facilities with symptoms of botulism and faced a long-term recovery.[97]

Preexposure and Postexposure Prophylaxis

Although passive antitoxin prophylaxis has been effective in protecting laboratory animals from toxin exposure, the limited availability and short-lived protection of antitoxin preparations makes preexposure or postexposure prophylaxis with these agents impractical for large numbers of persons.[85,98] Administration of equine antitoxin is not recommended for preexposure prophylaxis, due to the risk of anaphylaxis from the foreign equine proteins, particularly with repeated doses. These products are not generally recommended for use in asymptomatic persons. In asymptomatic persons with known exposure to botulinum toxin, the risk of anaphylaxis from the equine antitoxin must be weighed against the risk of disease from botulinum toxin. However, botulinum immune globulin is most effective when administered within 24 hours of a high dose aerosol exposure to botulinum toxin.

There are currently no FDA-approved vaccines for the prevention of botulism. However, an investigational product, the pentavalent botulinum toxoid (PBT) against botulinum toxin serotypes A through E, has been used since 1959 for persons at risk for botulism (ie, laboratory workers)[99–101] and is available as an investigational product on protocol through the CDC. PBT is a toxoid (toxin that has been inactivated) derived from formalin-inactivated, partially purified toxin serotypes A, B, C, D, and E, which was developed by the Department of Defense at Fort Detrick and originally manufactured by Parke-Davis and Company (Detroit, Mich). Each of the five toxin serotypes was propagated individually in bulk culture and then underwent acid precipitation, filtration, formaldehyde inactivation, and adsorption onto an aluminum phosphate adjunct. The five serotypes were then blended together to produce the end product, in a formulation based on concentrations that induce protective immunity in guinea pigs against a lethal challenge of 10^5 mouse intraperitoneal LD_{50} of each of the respective toxin serotypes. The Michigan Department of Community Health is responsible for formulation of current lots of PBT. The final product is bottled in 5-mL multidose vials, each containing 0.22% formaldehyde as a stabilizer and thimerosal in a 1:10,000 ratio as a preservative. Each 0.5-mL dose of vaccine contains 7 mg of aluminum phosphate and approximately 5 μg of inactivated toxin.

PBT has been found to be protective in animal models against challenge with botulinum toxin serotypes A through E,[102] including protection in nonhuman primates against aerosol challenge to toxin serotype A.[103] At-risk laboratory workers in the US offensive biological warfare program at Fort Detrick were immunized with a bivalent botulinum toxoid (serotypes A and B) beginning in 1945, and then with PBT beginning in 1959.[101] Between 1945 and 1969, 50 accidental exposures to botulinum toxins (24 percutaneous, 22 aerosol, and 4 ingestion) were reported, but no cases of laboratory-acquired botulism occurred, possibly because of the toxoid immunizations.

PBT was originally given as a primary series of three subcutaneous injections (0.5 mL at 0, 2, and 12 weeks), a booster dose at 12 months, and annual booster doses thereafter.[104] Since 1993, and until recently, booster doses subsequent to the 12-month booster were administered only for declining titers (no detectable titer on a 1:16 dilution of serum, corresponding to approximately 0.25 IU/mL for toxin serotype A).[105] The PBT dosing schedule was changed in 2004 due to (*a*) a recent decline in PBT immunogenicity and potency noted on the yearly potency testing, and (*b*) data from a 1998–2000 PBT study found a decrease in antitoxin titers by week 24 (6 months) in approximately two thirds of vaccinees.[106,107] The protocol for PBT (for current lots produced in the 1970s, which are now 30 years of age) now requires a primary series of four injections (0.5 mL at 0, 2, 12, and 24 weeks), followed by a booster dose at 12 months (because the 1998–2000 PBT study showed that antitoxin titers after the 24-week dose declined again by month 12), and booster doses annually thereafter.[106-108]

Results of the potency testing are consistent with results of antitoxin titers obtained at the US Army Medical Research Institute of Infectious Diseases from 1999 to 2001. The PBT showed continued induction of antitoxin titers to toxin serotype A (≥ 0.02 IU/mL) in nearly all vaccinees (30/32, or 94%) at 28 to 56 days after completion of the initial three doses of PBT.[105] Titers to toxin serotype A even lower than 0.02 IU/mL have been shown to provide protection in nonhuman primates.[103] However, only one of seven persons had detectable antitoxin titers to toxin serotype E between day 28 and 56 after completion of the initial three PBT doses.[105] Additionally, although PBT booster doses resulted in higher titers to toxin serotype A (≥ 0.32 IU/mL) in 96% (47/49) of the vaccinees at 28 days after the booster, as well as persistent titers—95% (35/37) of vaccinees had detectable titers (> 0.02 IU/mL) and 76% (28/37) had higher titers (≥ 0.32 IU/mL) at 6 to 12 months—detectable titers to toxin serotype E after a booster dose were observed in only 42% (10/24) of vaccinees at 6 to 12 months.[105]

PBT has been administered to thousands of at-risk persons, and clinical experience has shown the toxoid to be safe and immunogenic. The vaccine has mainly been used for laboratory workers who work directly with botulinum toxin. Approximately 8,000 service members also received the toxoid between January 23 and February 28, 1991, as part of the US force deployed to the Persian Gulf War.[99] The main adverse event has been local reactions. Adverse events passively reported to the CDC between 1970 to 2002 for over 20,000 vaccinations included moderate local reactions (edema or induration between 30 to 120 mm) in 7% of vaccinees, and severe local reactions (reaction size > 120 mm, marked limitation of arm movement, or marked axillary node tenderness) in less than 1%.[108]

PBT is not useful or recommended for postexposure prophylaxis because measurable antitoxin titers do not usually occur until a month after the third dose of the vaccine (4 months after the first vaccine dose).[104,105,109,110] PBT may be considered for preexposure prophylaxis in at-risk persons (ie, laboratory workers or military troops at high risk of a biowarfare exposure), but not in the general population, for whom the risk of botulinum intoxication is low. Additionally, the requirement

of multiple doses to maintain titers, the status of the vaccine as an investigational new drug, and the limited supply of the vaccine make the product difficult to use in large numbers of persons in an emergency setting. Today nearly all stocks of these products are held either by the US Army or the CDC for the pharmaceutical strategic national stockpile.

New Vaccine Research

Vaccine candidates include formalin-inactivated toxoids (tetravalent [ABEF] and monovalent [F] products) made nearly the same way as formalin-inactivated PBT, with the goal of FDA approval.[111,112] Production of formalin-inactivated toxoids requires handling biohazards, and there is a possibility of toxin reactivation in vivo.[111] However, the risk of reactivation may not be expected to be different from other toxoids, such as tetanus and diptheria toxoids, that are FDA approved. Production also requires partially purified culture supernatants to be exhaustively treated with formaldehyde, which must be performed by a highly trained staff and within a dedicated high-containment laboratory space.[113] Furthermore, the resulting PBT is relatively impure, containing only 10% neurotoxoid (90% is irrelevant material). This impurity may contribute significantly to the occurrence of local reactions and to the need for multiple injections to both achieve and sustain protective titers.

The use of pure and concentrated antigen in recombinant vaccines could offer the advantages of increased immunogenicity and a decrease in reactogenicity (local reactions at the injection site) over formalin-inactivated toxoids.[114] Recombinant techniques use a fragment of the toxin that is immunogenic, but does not have the capability of blocking cholinergic neurotransmitters. Both *Escherichia coli* and yeast expression systems have been used in the production of recombinant fragments, mainly the carboxy-terminal fragment (H_c) of the heavy chain of the toxin. Vaccine candidates using recombinant fragments of botulinum toxins against serotypes A, B, C, E, and F were protective in mice.[115–123] A vaccine recombinant candidate for serotype A was protective in mice against intraperitoneal challenge and produced levels of immunity similar to that attained with PBT, but with an increase in safety and decrease in cost per dose.[113] Recombinant vaccines given by inhalational route are also being investigated.[124,125] Work at the US Army Medical Research Institute of Infectious Diseases led to the development of a new bivalent recombinant botulinum vaccine (toxin serotype A and B) that is currently undergoing phase I trials in humans.[126] The vaccine is administered as two doses (at 0 and 6 weeks). Preliminary review of the safety and immunogenicity data suggests that phase II trials with this vaccine may soon be proposed.

A candidate vaccine that involves the insertion of a synthetic carboxy-terminal fragment (H_c) gene of the heavy chain of toxin serotype A into the vector system of the Venezuelan equine encephalitis virus is also being evaluated.[119] The vaccine induced a strong antibody response in the mouse model, and remained protective in mice against intraperitoneal challenge at 12 months.

SUMMARY

The neurotoxins produced by *Clostridia* species are among the most potent toxins known. Botulinum toxin has been studied and developed as a biological weapon by many countries, and it should be considered as a bioterrorism threat agent. A mass casualty event caused by botulinum toxin, which has been depicted by a mathematical model, has the potential to cause great harm. Botulism is a neuroparalytic disease, most commonly caused by foodborne ingestion of neurotoxin types A, B, and E, and is often fatal if untreated. Intoxication with neurotoxin type A may result in a more severe disease than from toxin serotypes B and E. Paralysis from botulism can be long-lasting, with concomitant demanding supportive care requirements. Clinicians should be able to recognize the classic symptoms of botulinum intoxication. Various laboratory assays for botulinum toxin are available for clinical specimens, but patient treatment is initiated in the absence of laboratory confirmation, given an index of suspicion for botulism. Antitoxin therapy and supportive care are important for treating botulism patients. Bivalent (AB) botulinum equine antitoxin is the sole FDA-approved antitoxin for adults. Human botulism immune globulin (BABY BIG) has recently been approved by the FDA and is available for the treatment of infantile botulism. PBT has been available for over 45 years as an investigational product for immunological protection against botulinum toxin; and two despeciated equine antitoxin preparations for toxin serotype A-G, an equine antitoxin for serotype E, and a human botulinum antitoxin against toxin serotype E are available as investigational drugs. Future vaccine research could lead to a new class of recombinant vaccines to protect against botulism.

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