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Short Communication

A Botulism Case of a 12-Year-Old Girl Caused by Intestinal Colonization of *Clostridium botulinum* Type Ab

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SUMMARY: We encountered a 12-year-old girl, who had contracted food-borne botulism, and subsequently suffered from obstinate constipation for more than half a year. Even on hospital day 122, *Clostridium botulinum* and its toxin were detected in her stool specimens. The potency of the toxin of the blood serum sampled before treatment was 20 mouse minimum lethal dose per ml. The toxin in the blood had a molecular size equivalent to that of type A botulinum neurotoxin. On hospital day 250, the patient's serum detoxified type A neurotoxin. We confirmed that the patient had food-borne botulism caused by *C. botulinum* type Ab, followed by intestinal colonization-type botulism.

It is believed that *Clostridium botulinum* fails to germinate or grow in the human intestines except in those of infants. Botulism has been classified into three types: adult food-borne botulism caused by ingestion of toxin in food, infant botulism caused by colonization of ingested spores, and wound botulism caused by wound infection with *C. botulinum* spores. Recently, a few cases of the fourth type, known as the intestinal colonization type, have been reported (1-5), in that *C. botulinum* colonized in the intestines for a long period after consumption of contaminated food. A 12-year-old girl suffered from acute flaccid quadriplegia with respiratory insufficiency after consuming vacuum-sealed food in August 1999 in Japan. Subsequently, the patient experienced a long period of obstinate constipation. Before this case, we had encountered patients of food-borne botulism (especially type A or B) in which symptoms did not improve over months. This report describes the laboratory analyses of a case of stubborn constipation in a 12-year-old girl suffering from botulism.

A 12-year-old girl, who was born underweight (1.1 kg) but who nevertheless developed normally, consumed a vacuum-packed hashedbeef in August 1999. The next morning (18-19 h after the meal), she experienced vertigo with nausea and paralysis that resulted in astasia. When hospitalized, she had flaccid quadriplegia and difficulty in communication. A few minutes later, she suffered respiratory arrest, requiring ventilatory support. Although she had systemic paralysis of the skeletal muscles including respiratory muscles, we were able to communicate with her by having her move her left toe in response to questions. On hospital day 5, her light reflexes and movement of the limbs disappeared and we could no longer communicate with her. Guillain-Barré syndrome (6-8) and myasthenia gravis were ruled out, because electromyography showed that only motor neurons were affected, and due to the absence of anti-acetylcholine-receptor antibodies in the serum and negative tensilon test. She was administered 400 mg/kg of gamma globulin with cefazoline for 5 days, but no improvement was seen. To confirm botulism, the patient's blood serum and stool specimens on hospital day 7 were administered to mice by intraperitoneall injection resulting in typical botulism symptoms. The titer of the toxin in her blood collected on hospital day 1 was estimated at 20 MLD/ml (mouse minimum lethal dose per ml). The toxin was detected also both from the patient's stool and the incriminated food. The molecular size of the toxin appearing in her blood was equivalent to that of botulinum type A neurotoxin, having a molecular weight about 150,000 (data not shown). Similar result had been reported previously in rabbits (9). *C. botulinum* was isolated from the patient's stool and the incriminated food by culturing in cooked meat medium (Difco Laboratories, Detroit, Mich., USA) containing 0.3% glucose and 0.2% soluble starch. The *C. botulinum* isolates from the stool and the food gave the same DNA pattern by pulsed-field gel electrophoresis (data not shown). PCR analysis (10) of the isolates and the neutralization test by botulinum antitoxin (types A-G) of the toxin in her blood identified it as *C. botulinum* type Ab (producing a large amount of type A toxin and a small amount of type B toxin). On hospital day 7, 20 ml of polyvalent horse antitoxic serum (Chiba Serum Institute, Chiba: type A; 500 IU/ml, B; 500 IU/ml, E; 500 IU/ml, and F; 200 IU/ml) was administered three times. On hospital day 11, the patient became able to move her left toe, nevertheless her paralysis improved very slowly. On hospital day 161, she was released from intratracheal intubation using NPPV (non-invasive positive pressure ventilation), and on hospital day 228 she was able to breathe by herself without any help. During this period, she had obstinate constipation. On hospital day 425, although she still had mild constipation, she was discharged from the hospital. She could stand up, walk by herself, but could not run. She also could speak, and eat without help.

On hospital day 122, *C. botulinum* type Ab was isolated from the stool specimen, and the toxin was detected from the

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Table 1. Determination of antitoxic activity of the serum collected from the patient on hospital day 250

Serum and serum mixture	The remaining toxicity of the toxin	
	LD_{50}/ml	(%)
Control	85,000	(100)
Healthy boy (5-y-old)	85,000	(100)
Patient	57,000	(67)
Adult immunized with toxoid	65,000	(77)
Type A anti-toxin serum; 0.450 IU	33,000	(39)
Type A anti-toxin serum; 0.090 IU	65,000	(77)
Type A anti-toxin serum; 0.045 IU	85,000	(100)

The neutralizing activity of the patient serum after hospital day 250 against type A neurotoxin was estimated by titrating a mixture of the patient serum and type A toxin. A portion of type A neurotoxin or phosphate buffered (pH 7.4) saline for control was added to the serum of the patient, a healthy boy (5-year-old), or an adult (56-year-old) who was immunized with botulinum toxoid 28 years before. The mixtures were incubated at 37°C for 90 min. Each of four mice (ddy: 20 g) was injected intravenously (iv) with 0.1 ml of the mixture. The death time in minutes from iv injection to death was converted to ip LD50 values of type A neurotoxin (9). A portion of purified type A neurotoxin (9) (85,000 LD_{50}/ml) was mixed with each serum sample and the remaining toxicity was titrated by mouse iv injection (12).

stool sample, but was no longer detected in her blood serum. On hospital day 208, *C. botulinum* or the toxin was no longer detected in her stool specimens. The serum from the patient on hospital day 250 detoxified type A toxin (Table 1).

In the present case, the botulism of a 12-year-old girl caused by food-borne *C. botulinum* type Ab subsequently became intestinal colonization-type botulism due to continuously discharged toxin from the bacteria. The present case was the first one of the intestinal colonization type of food-borne botulism by *C. botulinum* type Ab in Asia. The stubborn constipation lasting over 425 days may have been caused by the action of the nerves controlling bowel movement being inhibited by direct attack of the toxin in the intestines, or the recuperation of the nerves damaged by the toxin taking a long time. The colonizing *C. botulinum* in the intestines had been producing the toxin, but the toxin absorbed to the circulation was detoxified immediately by the antitoxin produced by the patient. Similar enteral immunization was observed also by Griffin et al. (11). Eight kinds of antibiotics were administered from hospital days 1 to 74 during mechanical ventilation. Her intestinal flora were not normal during the period from hospital days 38 to 55 after the antibiotic treatment. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated from her stool specimens during the above period. The relationship between the intestinal colonization of *C. botulinum* and the effects of the antibiotic treatments is unknown. We emphasize that it is necessary to follow up food-borne botulism patients for at least one year after the illness because the colonized *C. botulinum* might continuously produce the toxin or the spores.

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