

RECOMBINANT VACCINE AGAINST BOTULINUM NEUROTOXIN

This application is a divisional application of and claims the benefit of U.S. application Ser. No. 09/910,186 filed Jul. 20, 2001 now U.S. Pat. No. 7,081,529, which is a continuation of U.S. application Ser. No. 09/611,419 filed Jul. 6, 2000 now U.S. Pat. No. 7,214,787, which is a continuation-in-part of U.S. patent application Ser. No. 08/123,975 filed Sep. 21, 1993 (abandoned) and a continuation of International Application No. PCT/US00/12890, which claims the benefit of U.S. Provisional Application Nos. 60/133,866, 60/133,868, 60/133,869, 60/133,865, 60/133,873, 60/133,867, all filed May 12, 1999, and U.S. Provisional Application No. 60/146,192, filed Jul. 29, 1999, all of which are incorporated herein in their entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention is directed to preparation and expression of synthetic genes encoding polypeptides containing protective epitopes of botulinum neurotoxin (BoNT). The invention is also directed to methods of vaccination against botulism using the expressed peptides.

2. Related Art

The sporulating, obligate anaerobic, gram-positive bacillus *Clostridium* produces eight forms of antigenically distinct exotoxins. Tetanus neurotoxin (TeNT) is produced by *Clostridium tetani* while *Clostridium botulinum* produces seven different neurotoxins which are differentiated serologically by specific neutralization. The botulinum neurotoxins (BoNT) have been designated as serotypes A, B, C₁, D, E, F, and G. Botulinum neurotoxins (BoNT) are the most toxic substances known and are the causative agents of the disease botulism. BoNT exert their action by inhibiting the release of the neurotransmitter acetylcholine at the neuromuscular junction (Habermann, E., et al., (1986), "Clostridial Neurotoxins: Handling and Action at the Cellular and Molecular Level," *Cur. Top. Microbiol. Immunol.*, 129:93-179; Schiavo, G., et al., (1992a), "Tetanus and Botulinum-B Neurotoxins Block Neurotransmitter Release by Proteolytic Cleavage of Synaptobrevin," *Nature*, 359:832-835; Simpson, L. L., (1986), "Molecular Pharmacology of Botulinum Toxin and Tetanus Toxin," *Annu. Rev. Pharmacol. Toxicol.*, 26:427-453) which leads to a state of flaccid paralysis. Indeed, only a few molecules of toxin can abolish the action of a nerve cell. Polyclonal antibodies derived for a specific neurotoxin can neutralize the toxic effects of that toxin but will not cross-neutralize another toxin serotype. Thus, to protect against all seven toxins, one needs seven vaccines.

Botulinum neurotoxins are translated as a single 150 kDa polypeptide chain and then posttranslationally nicked, forming a dichain consisting of a 100 kDa heavy chain and a 50 kDa light chain which remain linked by a disulfide bond (DasGupta, B. R., et al., (1972), "A Common Subunit Structure in *Clostridium botulinum* Type A, B, and E Toxins," *Biophys. Res. Commun.*, 48:108-112; DasGupta, B. R., (1989), "The Structure of Botulinum Neurotoxins," *Botulinum Neurotoxin and Tetanus Toxin*, (Simpson, L. L., Ed.), pp. 53-67, Academic Press, New York). Most of the clostridial strains contain specific endogenous proteases which activate the toxins at a protease-sensitive loop located approximately one third of the way into the molecule from the amino-terminal end. Upon reduction and fractionation (electrophoretically or chromatographically), the two chains can be sepa-

rated; one chain has a Mr of ~100 kDa and is referred to as the heavy chain while the other has a Mr ~50 kDa and is termed the light chain.

The mechanism of nerve intoxication is accomplished through the interplay of three key events, each of which is performed by a separate portion of the neurotoxin protein. First, the carboxy half of the heavy chain (fragment C or H_C) is required for receptor specific binding to cholinergic nerve cells (Black, J. D., et al., (1986), "Interaction of ¹²⁵I-botulinum Neurotoxins with Nerve Terminals. I. Ultrastructural Autoradiographic Localization and Quantitation of Distinct Membrane Acceptors for Types A and B on Motor Nerves," *J. Cell Biol.*, 103:521-534; Nishiki, T.-I., et al., (1994), "Identification of Protein Receptor for *Clostridium botulinum* Type B Neurotoxin in Rat Brain Synaptosomes," *J. Biol. Chem.*, 269:10498-10503; Shone, C. C., et al., (1985), "Inactivation of *Clostridium botulinum* Type A Neurotoxin by Trypsin and Purification of Two Tryptic Fragments. Proteolytic Action Near the COOH-terminus of the Heavy Subunit Destroys Toxin-Binding Activity," *Eur. J. Biochem.*, 151:75-82). There is evidence suggesting that polysialogangliosides (van Heyningen, W. E., (1968), "Tetanus," *Sci. Am.*, 218:69-77) could act as receptors for the toxins but the data supporting a specific receptor remains equivocal (Middlebrook, J. L., (1989), "Cell Surface Receptors for Protein Toxins," *Botulinum Neurotoxins and Tetanus Toxin*, (Simpson, L. L., Ed.) pp. 95-119, Academic Press, New York). After binding, the toxin is internalized into an endosome through receptor-mediated endocytosis (Shone, C. C., et al., (1987), "A 50-kDa Fragment from the NH₂-terminus of the Heavy Subunit of *Clostridium botulinum* Type A Neurotoxin Forms Channels in Lipid Vesicles," *Euro. J. Biochem.*, 167:175-180). The amino terminal half of the heavy chain is believed to participate in the translocation mechanism of the light chain across the endosomal membrane (Simpson, 1986; Poulain, B., et al., (1991), "Heterologous Combinations of Heavy and Light Chains from Botulinum Neurotoxin A and Tetanus Toxin Inhibit Neurotransmitter Release in *Aplysia*," *J. Biol. Chem.*, 266:9580-9585; Montal, M. S., et al., (1992), "Identification of an Ion Channel-Forming Motif in the Primary Structure of Tetanus and Botulinum Neurotoxins," *FEBS*, 313:12-18). The low pH environment of the endosome may trigger a conformational change in the translocation domain, thus forming a channel for the light chain. The final event of intoxication involves enzymatic activity of the light chain, a zinc-dependent endoprotease (Schiavo, 1992a; Schiavo, G., et al., (1992b), "Tetanus Toxin is a Zinc Protein and its Inhibition of Neurotransmitter Release and Protease Activity Depend on Zinc," *EMBO J.*, 11:3577-3583), on key synaptic vesicle proteins (Schiavo, 1992a; Oguma, K., et al., (1995), "Structure and Function of *Clostridium botulinum* Toxins," *Microbiol. Immunol.*, 39:161-168; Schiavo, G., et al., (1993), "Identification of the Nerve Terminal Targets of Botulinum Neurotoxin Serotypes A, D, and E," *J. Biol. Chem.*, 268:23784-23787; Shone, C. C., et al., (1993), "Proteolytic Cleavage of Synthetic Fragments of Vesicle-Associated Membrane Protein, Isoform-2 by Botulinum Type B Neurotoxin," *Eur. J. Biochem.*, 217:965-971) necessary for neurotransmitter release. The light chains of BoNT serotypes A, C₁, and E cleave SNAP-25 (synaptosomal-associated protein of M25, 000), serotypes B, D, F, and G cleave VAMP/synaptobrevin (synaptic vesicle-associated membrane protein); and serotype C₁ cleaves syntaxin. Inactivation of SNAP-25, VAMP, or syntaxin by BoNT leads to an inability of the nerve cells to release acetylcholine resulting in neuromuscular paralysis and possible death, if the condition remains untreated.