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Human milk SIgA binds to botulinum type B 16S toxin and limits toxin adherence on T84 cells

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Abstract

Botulinum neurotoxin produced by *Clostridium botulinum* type B is in the form of a complex of 12S and 16S toxins. Food-borne botulism is caused by these complex toxins which are ingested orally and absorbed from the digestive tract. Here, we show that the human milk SIgA binds to the type B16S toxin. The binding of SIgA to 16S toxin and HA was inhibited by carbohydrates such as galactose, suggesting that the interaction of carbohydrate side chain of the SIgA with the HA of the 16S toxin is important for SIgA–16S complex formation. We also demonstrate that SIgA inhibits the attachment of 16S toxin to intestinal epithelial cells. These data suggest that the interaction of antigen nonspecific SIgA with 16S toxin has a large influence on the absorption of 16S toxin from the intestinal epithelium, and that SIgA may provide insight into developing a therapeutic agent for type B food-borne botulism.

Keywords: *Clostridium*; Botulinum; Botulism; Hemagglutinin; Neurotoxin; Toxin; Secretory IgA; Immunoglobulin; Galactose; Innate immunity

Botulinum neurotoxin (7S toxin, M_r ~150 kDa, types A–G) is a metalloprotease toxin produced by *Clostridium botulinum*. This neurotoxin binds specifically to neuronal cells, enters the cytoplasm, then cleaves the core proteins involved in the vesicular fusion machinery, thereby blocking the release of neurotransmitter (reviewed in [1]). When produced by the bacterium, the neurotoxin is found as complex forms (progenitor toxins) associated with nontoxic components (reviewed in [2,3]). The *C. botulinum* type B strains produce two different forms of progenitor toxins; 12S and 16S toxins. 12S toxin is composed of a neurotoxin and an NTNH (~130 kDa). 16S toxin is composed of a neurotoxin, an NTNH, and two hemagglutinin (HA) components. The HA component has four different subunits; HA1, HA2, HA3a, and HA3b.

Food-borne botulism is caused by these complex toxins, which are ingested orally and absorbed from the digestive tract. To cause disease, the toxin must penetrate the mucosal barrier lining the gut and enter the blood-stream. Although the mechanism by which this large protein toxin crosses the mucosal barrier is still poorly understood, it has been demonstrated that bare neurotoxin is able to pass through the intestinal epithelial monolayer via transcytosis (reviewed in [4]). On the other hand, we have found that the HA component of the 16S toxin has a selective binding activity to intestinal epithelial cells via cell surface glycoconjugates, which may increase the efficiency of the neurotoxin absorption across the epithelial barrier (reviewed in [5]).

The epithelial monolayer covering the mucosal surface of the intestine is constantly exposed to an abundant variety of pathogens, such as bacteria, viruses, and toxins. Thus, the epithelial monolayer is equipped with efficient defense sytems against these pathogens through a

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combination of innate or constitutive immunity (e.g., intercellular tight junctions, mucins, defensins, trefoil peptide, and filamentous brush border glycocalyx (FBBG)) and by induced specific immune responses such as the secretion of secretory Immunoglobulin A (SIgA) (reviewed in [6]). SIgA is the major immunoglobulin found in gastrointestinal secretions, with the concentration estimated to exceed hundreds of micrograms per milliliter. SIgA exists mostly as a dimer complexed with the J chain and the secretory component (SC), in contrast to serum IgA, which is predominantly monomeric and lacks the J chain and SC. SIgA protects the host by neutralization of microbes, enzymes, and toxins, and inhibition of their attachment to the epithelium. The antimicrobial properties of SIgA have been attributed to the specificity of the antigen-binding site for these pathogens. In addition to its well-known role as an adaptive mucosal defense factor, there is accumulating evidence that SIgA also contributes to innate immune systems via their carbohydrate side chains (reviewed in [7]). The SC and H chain of SIgA is highly glycosylated (reviewed in [8]), and these glycans interact with bacteria such as *Escherichia coli* [9–12] and *Helicobacter pylori* [13,14], and toxins such as *Clostridium difficile* toxin A [15] and ricin [16], and limits their adherence on the epithelium.

Recently, it has been reported that type A and type B botulinum 16S toxin binds to lactose (Galβ1-4Glc) via the HA1 subunit [17–19], and the carbohydrate-binding domain of the HA1 is similar to those of the ricin B subunit in the amino acid sequence [20,21] and in the crystal structure [22,23]. In this report, we investigated the interaction of the type B 16S toxin with SIgA, and found that SIgA binds to the 16S toxin in an antigen-nonspecific manner. We also demonstrated that interaction of the 16S toxin with SIgA attenuates the binding activity of the toxin to intestinal epithelial cells. These findings suggest that the antigen–nonspecific interaction of SIgA with the 16S toxin has a large influence on the absorption of 16S toxin from the intestinal epithelium, and that SIgA may provide insight into developing a therapeutic agent for type B food-borne botulism.

Materials and methods

Preparation of toxins and nontoxic components. 16S toxin, 12S toxin, and free-HA were purified from culture fluids of *C. botulinum* type B strain Lamanna as described [19].

Antibodies. Human secretory IgA (from pooled colostrum) was purchased from Sigma. Human serum IgA (from pooled serum, almost in monomeric form) was obtained from Calbiochem. Anti-type B HA rabbit pAb was obtained by immunizing rabbits with the free-HA. To reduce the nonspecific reaction to the neurotoxin and T84 cell surface, the antiserum was pre-cleared by the 12S toxin immobilized on an Affi-gel 15 (Bio-Rad) and a T84 membrane fraction. Anti-type B neurotoxin rabbit pAb was generated by immunizing rabbits with neurotoxin derived from the 16S toxin. To reduce the nonspecific reaction to the nontoxic components (NTNH + HA) and T84 cell surface, the antiserum was pre-cleared by the nontoxic components immobilized on a lactose gel (EY Laboratories, Inc.) and a T84 membrane fraction.

ELISA for IgA–toxin interaction. Plates were coated with SIgA or serum IgA at a final concentration of 1.0 μg/ml in PBS (pH 7.4) for overnight at 4 °C. The wells were washed three times with PBS-0.05% Tween 20 (pH 6.0), blocked with 2% BSA–PBS–Tween, and incubated with toxins for 1 h at room temperature. After washing, rabbit anti-neurotoxin or anti-HA was added and plates were incubated for 1 h at room temperature. After washing, HRP-labelled anti-rabbit IgG (Jackson

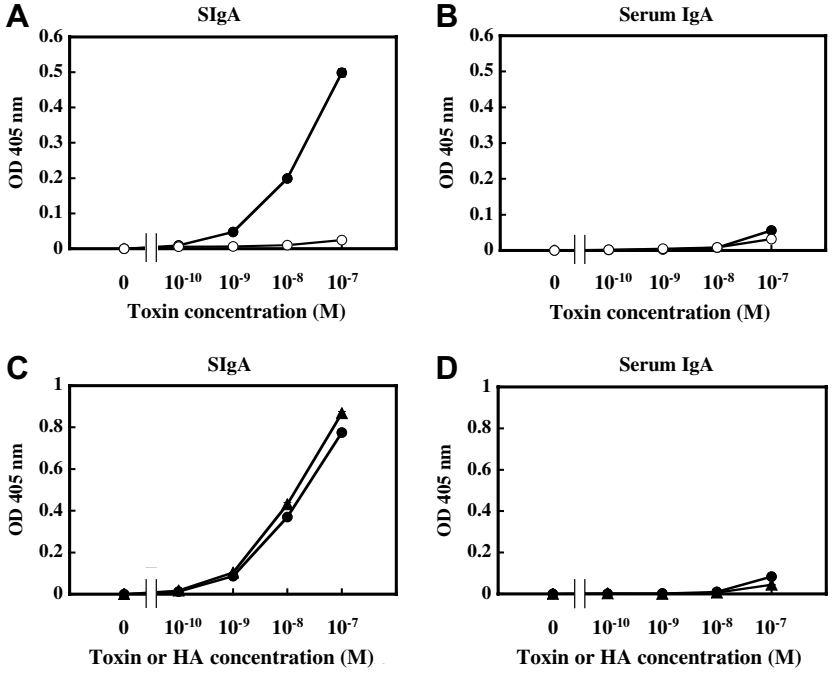

Fig. 1. Binding of *botulinum* type B 16S toxin, 12S toxin, and HA to human SIgA and serum IgA. (A,B) 16S (●) or 12S (○) toxins were added to plates coated with SIgA (A) or serum IgA (B). Bound toxins were detected by using anti-neurotoxin as primary antibodies. (C,D) 16S (●) or HA (▲) were added to plates coated with SIgA (C) or serum IgA (D). Bound toxins were detected by using anti-HA as primary antibodies. Values are means ± SD for triplicate cultures. Representative of three independent experiments.