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Ganglioside and Rabbit Erythrocyte Membrane Receptor for Staphylococcal Alpha-Toxin

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The hemolytic activity of staphylococcal alpha-toxin is inhibited by an *N*-acetylglucosamine-containing ganglioside (GlcNAc-ganglioside) but not by any of the related glycolipids. The GlcNAc-ganglioside also is precipitated with the toxin by a gel-diffusion technique. It is postulated that GlcNAc-ganglioside may be the membrane receptor for the alpha-toxin.

Staphylococcal alpha-toxin has recently been purified in a crystallized state and physicochemically characterized (15, 16). Alpha-toxin has a lytic effect on rabbit erythrocytes, but the mechanism of the cell-membrane interaction of the toxin is unknown. In regard to the inhibitory mechanism of flavin mononucleotide on the hemolytic activity of the alpha-toxin, we have suggested that flavin mononucleotide interacts with specific glycoproteins or glycolipids in rabbit erythrocyte cell membranes, which are the binding sites for alpha-toxin (4).

It is known that tetanus toxin has a particular affinity for certain sialidase-sensitive gangliosides referred to as G_{D1b} and G_{T1} (13, 14). Furthermore, recent work has established that the membrane receptor site for the purified cholera toxin closely resembles a specific sialidase-resistant ganglioside, G_{M1} (3, 14).

The present study demonstrates that prior incubation of alpha-toxin with an *N*-acetylglucosamine-containing ganglioside (GlcNAc-ganglioside) inhibits, in parallel, the ability of the toxin to bind to rabbit erythrocytes and to activate the hemolytic response in the cells, and provides evidence that, at least for the erythrocytes, the GlcNAc-ganglioside may resemble, or be part of, the receptor site for the alpha-toxin.

Crystalline staphylococcal alpha-toxin and ^{125}I-labeled alpha-toxin preparations were obtained by methods described previously (5, 16). Various iodinated alpha-toxin preparations had a specific activity of 8×10^3 to 1.5×10^4 counts/min per μg of protein. GlcNAc-ganglioside(NAN) (1, 7, 11, 17) and hematoside(NAN) (12) were prepared from human erythrocytes. GlcNAc-ganglioside(NGN) (17) and hematoside(NGN) (6, 18) were prepared from bovine and equine erythrocytes, respectively. Paragloboside was prepared by treatment of GlcNAc-ganglioside(NAN) with neuraminidase from *Clostridium perfringens* (Sigma Chemical Co.), and GlcNAc-CTS was prepared by the treatment of paragloboside with jack bean β-galactosidase, a gift from S.-C. Li of the University of Tulane. (For chemical structures, see Table 1.) These glycolipids were purified by column chromatography on diethylaminoethyl-Sephadex and silicic acid according to conventional procedures and analyzed by thin-layer chromatography for homogeneity and by gas chromatography for neutral sugars, hexosamines, and sialic acid (M. Naiki, J. Hong, R. Ledeen, and D. M. Marcus, Biochemistry, in press). The preparations and the sources of the other glycolipids were described previously (8-10).

Table 1 shows the relative order of inhibitory potency of the glycosphingolipids studied, in addition to the approximate concentration required to obtain half-maximal inhibition of hemolysis and the specific binding of ^{125}I-labeled alpha-toxin to rabbit erythrocytes. The most potent inhibitor of both the hemolysis by alpha-toxin and binding of ^{125}I-labeled alpha-toxin to the erythrocytes is GlcNAc-ganglioside(NAN), which is effective in final concentrations as low as 10 ng/ml. The quantitative analyses (Fig. 1) indicated that 10 ng of GlcNAc-ganglioside(NAN) inactivated approximately 10 hemolytic units of alpha-toxin as compared with 10 ng of crystallized preparation inactivating 1 hemolytic unit (15). Thus, 1 weight unit of the ganglioside could inactivate up to approximately 10 weight units of toxin, which corresponds to a molar proportion of 2:1 since the molecular weight of GlcNAc-ganglioside(NAN) is 1,600 and that of toxin is 36,000. The gangliosides and related neutral glycosylceramides were tested by the double-diffusion-in-agar technique for the capacity to fix and precipitate

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TABLE 1. *Effect on staphylococcal alpha-toxin of gangliosides and allied neutral glycosylceramides*[a]

Compounds tested	Chemical structure	Concn required for half-maximal inhibition[b] (μg/ml)	% Binding of toxin[c]
None			100
GlcNAc-ganglioside(NAN)	NANα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer	0.01	2
GlcNAc-ganglioside(NGN)	NGNα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer	5	28
G_{M1}	Galβ1-3GalNAcβ1-4(NANα2-3)Galβ1-4Glc-Cer	10	46
G_{M1}	GalNAcβ1-4(NANα2-3)Galβ1-4Glc-Cer	15	67
G_{D1a}	NANα2-3Galβ1-3GalNAcβ1-4(NANα2-3)Galβ1-4Glc-Cer	15	71
Paragloboside	Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer	17	79
GlcNAc-CTS	GlcNAcβ1-3Galβ1-4Glc-Cer	20	95
Globoside	GalNAcβ1-3Galα1-4Galβ1-4Glc-Cer	20	96
Forssman	GalNAcβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-Cer	>20	100
Hematoside(NAN)	NANα2-3Galβ1-4Glc-Cer	>20	100
Hematoside(NGN)	NGNα2-3Galβ1-4Glc-Cer	>20	100

[a] Abbreviations used are: Cer, ceramide; CTS, ceramide trisaccharide; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcNAc, *N*-acetylglucosamine; NAN, *N*-acetylneuraminic acid; and NGN, *N*-glycolylneuraminic acid. The ganglioside nomenclature is according to Svennerholm (12).

[b] Each glycolipid preparation was diluted with 0.05 M phosphate-buffered saline solution (pH 7.0; PBS buffer). Each preparation was incubated with 10 hemolytic units of alpha-toxin per ml for 30 min at 37 C. Rabbit erythrocytes (2%) were then incubated with the reaction mixture at 28 C for 30 min. Hemolytic assays were performed by the method of Bernheimer (2). The values were obtained from inhibition curves, all showing hyperbolic shapes.

[c] ^{125}I-labeled alpha-toxin (1 μg/ml) was preincubated at 37 C for 30 min in 0.5 ml of PBS buffer (pH 7.0) containing the glycolipid preparation (10 μg/ml). A 0.5-ml volume of 2% rabbit erythrocytes suspended in PBS buffer was then added to the incubated mixture. After 10 min at 20 C the specific binding of the labeled toxin to the cells was determined as described previously (5).

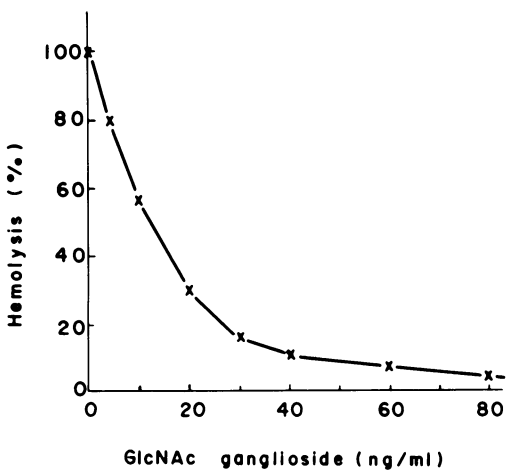

FIG. 1. *Dose-response curve of inactivation of staphylococcal alpha-toxin by GlcNAc-ganglioside(NAN) in rabbit erythrocytes suspended in solution. The various concentrations of GlcNAc-ganglioside(NAN) were preincubated with alpha-toxin (10 hemolytic units per ml) for 30 min at 37 C. The hemolytic assays were performed as described in footnotes b and c of Table 1.*

alpha-toxin. Only the GlcNAc-ganglioside-(NAN) was reactive with toxin, giving a fine precipitation line (Fig. 2). The precipitate formed between the toxin and the ganglioside was not due to unspecific precipitation of pro-

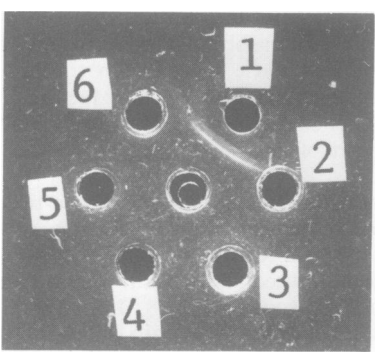

FIG. 2. *Specific precipitation of GlcNAc-ganglioside(NAN) and alpha-toxin in gel-diffusion analyses. Toxin (3 μg) (center well) tested with GlcNAc-ganglioside(NAN) (1), paragloboside (2), G_{M1} (3), G_{M2} (4), G_{D1a} (5), and GlcNAc-ganglioside(NGN) (6) (6 μg each).*

tein, since no precipitate was formed between GlcNAc-ganglioside(NAN) and normal rabbit serum, bovine serum albumin, and human gamma globulin.

Even minor changes in these glycolipid structures severely affected the inhibitory capacities of hemolysis and toxin binding. Both GlcNAc-ganglioside(NGN), in which the terminal Gal is linked to a NGN, and paragloboside, which is devoid of the terminal NAN, had an affinity for alpha-toxin about 500- and 1,700-fold, respectively, lower than that of GlcNAc-ganglioside(NAN). It may be concluded, therefore, that in the GlcNAc-ganglioside the position NAN-Gal-GlcNAc- is the critical region for fixation and inactivation of alpha-toxin.

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