

# Ganglioside and Rabbit Erythrocyte Membrane Receptor for Staphylococcal Alpha-Toxin

IWAO KATO\* AND MASAHARU NAIKI<sup>1</sup>

*Institute of Medical Science, University of Tokyo, Tokyo, Japan*

Received for publication 20 August 1975

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 <sup>125</sup>I-labeled alpha-toxin preparations were obtained by methods described previously (5, 16). Various iodinated alpha-toxin preparations had a specific activity of  $8 \times 10^3$  to  $1.5 \times 10^4$  counts/min per  $\mu\text{g}$  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  $\beta$ -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 <sup>125</sup>I-labeled alpha-toxin to rabbit erythrocytes. The most potent inhibitor of both the hemolysis by alpha-toxin and binding of <sup>125</sup>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

<sup>1</sup> Present address: Department of Biochemistry, the Faculty of Veterinary Medicine, University of Hokkaido, Sapporo 060, Japan.

TABLE 1. Effect on staphylococcal alpha-toxin of gangliosides and allied neutral glycosylceramides<sup>a</sup>

Compounds tested	Chemical structure	Concn required for half-maximal inhibition <sup>b</sup> (μg/ml)	% Binding of toxin <sup>c</sup>
None			100
GlcNAc-ganglioside(NAN)	NAN $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-Cer	0.01	2
GlcNAc-ganglioside(NGN)	NGN $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-Cer	5	28
G <sub>M1</sub>	Gal $\beta$ 1-3GalNAc $\beta$ 1-4(NAN $\alpha$ 2-3)Gal $\beta$ 1-4Glc-Cer	10	46
G <sub>M1</sub>	GalNAc $\beta$ 1-4(NAN $\alpha$ 2-3)Gal $\beta$ 1-4Glc-Cer	15	67
G <sub>D1a</sub>	NAN $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\beta$ 1-4(NAN $\alpha$ 2-3)Gal $\beta$ 1-4Glc-Cer	15	71
Paragloboside	Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-Cer	17	79
GlcNAc-CTS	GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-Cer	20	95
Globoside	GalNAc $\beta$ 1-3Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc-Cer	20	96
Forsman	GalNAc $\beta$ 1-3GalNAc $\beta$ 1-3Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc-Cer	>20	100
Hematoside(NAN)	NAN $\alpha$ 2-3Gal $\beta$ 1-4Glc-Cer	>20	100
Hematoside(NGN)	NGN $\alpha$ 2-3Gal $\beta$ 1-4Glc-Cer	>20	100

<sup>a</sup> 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).

<sup>b</sup> 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.

<sup>c</sup> <sup>125</sup>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).

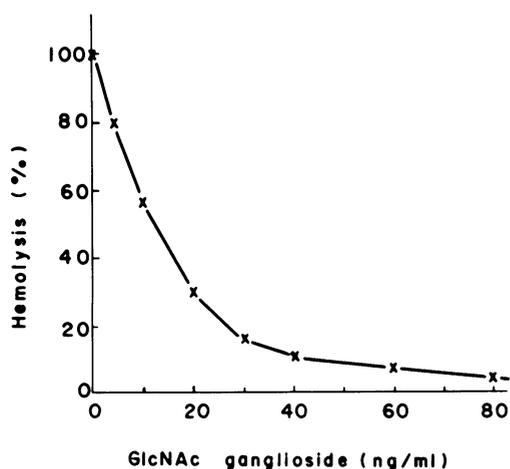


FIG. 1. Dose-response curve of inactivation of staphylococcal alpha-toxin by GlcNAc-ganglioside(NAN) in rabbit erythrocytes suspended in solution. The various concentrations of GlcNAc-ganglioside(NAN) were preincubated with alpha-toxin (10 hemolytic units per ml) for 30 min at 37 C. The hemolytic assays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

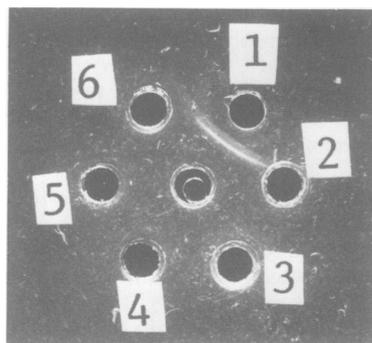


FIG. 2. Specific precipitation of GlcNAc-ganglioside(NAN) and alpha-toxin in gel-diffusion analyses. Toxin (3 μg) (center well) tested with GlcNAc-ganglioside(NAN) (1), paragloboside (2), G<sub>M1</sub> (3), G<sub>M2</sub> (4), G<sub>D1a</sub> (5), and GlcNAc-ganglioside(NGN) (6) (6 μg each).

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.

#### LITERATURE CITED

1. Ando, S., K. Kon, M. Isobe, and T. Yamakawa. 1973. Structural study on tetraglycosyl ceramide and gangliosides isolated from human red blood cells. *J. Biochem.* 73:893-895.
2. Bernheimer, A. W., and L. L. Schwartz. 1963. Isolation and composition of staphylococcal alpha-toxin. *J. Gen. Microbiol.* 30:455-468.
3. Holmgren, J., I. Lonnroth, and L. Svennerholm. 1973. Tissue receptor for cholera exotoxin: postulated structure from studies with G<sub>M1</sub> ganglioside and related glycolipids. *Infect. Immun.* 8:208-214.
4. Kato, I., K. Sakoda, M. Saito, Y. Suzuki, and M. Watanabe. 1975. Inhibitory effect of flavin mononucleotide on the hemolysis of rabbit erythrocytes by staphylococcal alpha-toxin. *Infect. Immun.* 12:696-697.
5. Kato, I., and A. Watanuki. 1970. Effect of diphtheria toxin on lysosome activity in leukocytes and Ehrlich ascites tumor cells. *Jpn. J. Exp. Med.* 40:87-100.
6. Klenk, E., and G. Padberg. 1962. Gangliosides from horse erythrocytes. *Z. Physiol. Chem.* 327:249-255.
7. Li, Y.-T., J.-E. Mansson, M.-T. Vanier, and L. Svennerholm. 1973. Structure of the major glucosamine-containing ganglioside of human tissues. *J. Biol. Chem.* 248:2634-2636.
8. Naiki, M., H. Kamimura, T. Taketomi, and R. Ichikawa. 1972. Chemical, physicochemical and morphological properties of Forssman hapten purified from caprine erythrocytes. *Jpn. J. Exp. Med.* 42:205-219.
9. Naiki, M., and D. M. Marcus. 1974. Human erythrocyte P and P<sup>a</sup> blood group antigens. *Biochem. Biophys. Res. Commun.* 60:1105-1111.
10. Naiki, M., D. M. Marcus, and R. Ledeen. 1974. Properties of antisera to ganglioside G<sub>M1</sub> and asialo G<sub>M1</sub>. *J. Immunol.* 113:84-93.
11. Siddiqui, B., and S. Hakomori. 1973. A ceramide tetrasaccharide of human erythrocyte membrane reacting with anti-type XIV pneumococcal polysaccharide antiserum. *Biochim. Biophys. Acta* 330:147-155.
12. Svennerholm, L. 1963. Isolation of the major ganglioside of human spleen. *Acta Chem. Scand.* 17:860-862.
13. van Heyningen, W. E. 1963. The fixation of tetanus toxin, strychnine, serotonin and other substances by ganglioside. *J. Gen. Microbiol.* 31:375-387.
14. van Heyningen, W. E., C. C. J. Carpenter, N. F. Pierce, and W. B. Greenough III. 1971. Deactivation of cholera toxin by ganglioside. *J. Infect. Dis.* 124:415-418.
15. Watanabe, M., and I. Kato. 1974. Purification and some properties of staphylococcal alpha-toxin. *Jpn. J. Exp. Med.* 44:165-178.
16. Watanabe, M., and I. Kato. 1974. Chemical structure and biochemical action of staphylococcal alpha-toxin. *Toxicon* 13:129-130.
17. Wiegandt, H., and H. W. Bücking. 1970. Carbohydrate components of extraneuronal gangliosides from bovine and human spleen and bovine kidney. *Eur. J. Biochem.* 15:287-292.
18. Yamakawa, T., and S. Suzuki. 1951. The chemistry of the lipids of posthemolytic residue or stroma of erythrocytes. I. Concerning the ether-insoluble lipids of lyophilized horse blood stroma. *J. Biochem.* 38:119-121.