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J Moss, … , P H Fishman, S H Richardson

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Research Article

Chemically transformed mouse fibroblasts did not raise their cyclic AMP level in response to Escherichia coli heat-labile enterotoxin. These fibroblasts did, however, incorporate exogenous mono-, di-, and trisialogangliosides. After the uptake of monosialoganglioside galactosyl-N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide (GM1), the cells responded to E. coli heat-labile enterotoxin. The di- and trisialogangliosides were considerably less effective. GM1, the putative cholera toxin (choleragen) receptor, has been implicated previously as the receptor for E. coli heat-labile enterotoxin based on the ability of the free ganglioside to inhibit the effects of toxin. This investigation establishes that the ganglioside, when incorporated into fibroblasts, serves a functional role in mediating the responsiveness to the toxin.

Gangliosides Sensitize Unresponsive Fibroblasts to *Escherichia coli* Heat-Labile Enterotoxin

JOEL MOSS, SAM GARRISON, PETER H. FISHMAN, and STEPHEN H. RICHARDSON,
Laboratory of Cellular Metabolism, National Heart, Lung, and Blood Institute, and Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205; and Department of Microbiology and Immunology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina 27103

ABSTRACT Chemically transformed mouse fibroblasts did not raise their cyclic AMP level in response to *Escherichia coli* heat-labile enterotoxin. These fibroblasts did, however, incorporate exogenous mono-, di-, and trisialogangliosides. After the uptake of monosialoganglioside galactosyl-*N*-acetylgalactosaminyl-[*N*-acetylneuraminyl]-galactosylglucosylceramide (G_{M1}), the cells responded to *E. coli* heat-labile enterotoxin. The di- and trisialogangliosides were considerably less effective. G_{M1}, the putative cholera toxin (choleragen) receptor, has been implicated previously as the receptor for *E. coli* heat-labile enterotoxin based on the ability of the free ganglioside to inhibit the effects of toxin. This investigation establishes that the ganglioside, when incorporated into fibroblasts, serves a functional role in mediating the responsiveness to the toxin.

INTRODUCTION

Certain strains of *Escherichia coli* are believed to be responsible for "traveler's diarrhea" (1–5). In some cases, the symptoms may result in part from production of a heat-labile enterotoxin (LT)[1] (1–5). LT appears to exert its effects through activation of adenylate cyclase (3, 6–9), and in this way it is similar to the enterotoxin from *Vibrio cholerae*, choleragen (10). In addition, both choleragen and LT require NAD for activation of adenylate cyclase in disrupted cells (9, 11, 12) and possess NAD glycohydrolase and ADP-ribosyltransferase activities (13–15). It has been proposed that both toxins exert their effects through the NAD-dependent ADP-ribosylation of either adenylate cyclase itself or of a protein critical to cyclase activation (9, 11, 13–17).

LT cross-reacts immunologically with antisera directed primarily against the B-subunits of choleragen (9, 18, 19). It is through the B-subunit that choleragen binds to specific cell surface receptors believed to be ganglioside G_{M1}[1] (10). Hence, it is possible that LT and choleragen share similar receptors. In fact, G_{M1} can block the biological effects of LT (20, 21), but the affinity of LT for G_{M1} (as well as the specificity for G_{M1} relative to other gangliosides) was lower than that observed with choleragen (20). In addition, choleragenoid, a protein composed of the B-subunits of choleragen, was effective in inhibiting the biological activity of LT in some studies but not in others (20, 22, 23). Thus, it is unclear whether the toxins actually share the same receptors.

We have shown that a chemically transformed line of mouse fibroblasts (National Collection of Type Cultures [NCTC] 2071), which is deficient in gangliosides and lacks G_{M1}, responds to choleragen only after the cells have incorporated exogenous G_{M1} but not other gangliosides (24, 25). In these experiments, we have examined the responsiveness of these cells to LT, before and after incorporation of gangliosides.

METHODS

NCTC 2071 fibroblasts were grown as described previously (24). Gangliosides were purified from brain or erythrocytes

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[1] *Abbreviations and nomenclature used in this paper*: cAMP; cyclic AMP; G_{M1}, galactosyl-*N*-acetylgalactosaminyl-[*N*-acetylneuraminyl]-galactosylglucosylceramide; G_{M2}, *N*-acetylgalactosaminyl - [*N* - acetylneuraminyl] - galactosylglucosylceramide; G_{M3}, *N*-acetylneuraminylgalactosylglucosylceramide; G_{D1a}, *N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl - [*N* - acetylneuraminyl] - galactosylglucosylceramide; G_{D1b}, galactosyl-*N*-acetylgalactosaminyl-[*N*-acetylneuraminyl - *N* - acetylneuraminyl] - galactosylglucosylceramide; G_{T1}, *N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl-[*N*-acetylneuraminyl - *N* - acetylneuraminyl] - galactosylglucosylceramide; LT, heat-labile enterotoxin; NCTC, National Collection of Type Cultures.

TABLE I
Effect of G_{M1} on the Response of Ganglioside-Deficient Fibroblasts to LT

G_{M1} added	cAMP content	
	No LT	Plus LT
	pmol/mg protein	
None	13	15
1 nmol	16	27

Fibroblasts were incubated for 18 h in 10 ml of NCTC 135 medium with or without G_{M1}. The medium was aspirated, and the cells were washed twice with 2 ml of medium. LT (258 μg protein) was then added to 10 ml of NCTC 135 medium where indicated. After 3 h at 37°C, the medium was aspirated, and 2 ml of 5% TCA was added. The cells were harvested and centrifuged. Samples of the supernate were taken for assay of cAMP as described previously (30). Data are means of values from duplicate incubations. The experiment was repeated twice with similar results.

(26) and appeared to be at least 99% pure when separated by thin-layer chromatography and visualized with resorcinol reagent (27). LT was purified by procedures described earlier (28). The low molecular weight LT isolated by this procedure is enterotoxic in infant rabbits and adult ligated ileal loops.[2] All assays comparing the relative effectiveness of gangliosides were performed in the same experiment. Protein was determined by the method of Lowry et al. (29).

RESULTS AND DISCUSSION

When grown in ganglioside-free chemically defined medium, the transformed fibroblasts did not respond to LT even after 3 h (Table I). Cells exposed to G_{M1} and then to LT accumulated cyclic AMP (cAMP); G_{M1} by itself had no effect. There was a delay before cAMP levels began to rise in G_{M1}-treated cells exposed to LT (Fig. 1); maximal accumulation occurred by 2 h. These results are similar to those reported previously with choleragen (24).

We examined the effects of several gangliosides on the responsiveness of NCTC 2071 fibroblasts to LT (Table II). G_{M1} was clearly the most effective followed by G_{D1a}[1] and G_{T1}; only a slight response was observed with G_{M2} and G_{D1b} and none with G_{M3}. G_{D1a} and G_{M2} were shown previously to sensitize NCTC 2071 cells to choleragen but were much less effective than G_{M1} (25). The cells contain a highly active sialidase that converts exogenous G_{D1a} to G_{M1} (25); under conditions where G_{D1a}-treated cells became responsive to choleragen, ≅25% of the G_{D1a} taken up by the cells was converted to G_{M1} (25). The effects observed with G_{T1} and G_{D1b} may also be a result of the enzymatic breakdown of these gangliosides to G_{M1}. When the fibroblasts

[2] Personal communication with Dr. D. G. Evans.

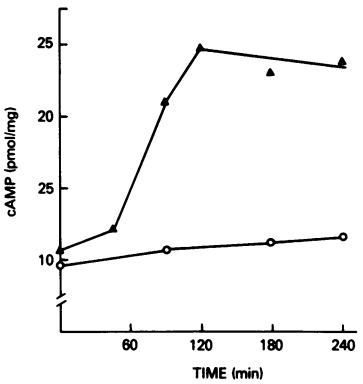

FIGURE 1 Effect of time of incubation with LT on the cAMP content of fibroblasts. Fibroblasts were incubated with (▲) or without (○) G_{M1} (1 nmol/10 ml) for 18 h at 37°C. The ganglioside was removed, the cells washed twice with 2 ml of NCTC 135 medium, and 10 ml of NCTC 135 medium was added. LT (172 μg) was added where indicated (▲). At the chosen times, the medium was aspirated, 2 ml of 5% TCA was added, and intracellular cAMP content was determined. Points are the average of duplicate incubations.

were exposed to G_{D1b}, they became responsive to choleragen (Table III), but again G_{M1} was more effective. Although the gangliosides used in these studies appear homogeneous, we cannot rule out the possibility that some of the effects observed with other gangliosides might be a result of the presence of trace amounts of G_{M1} or conversion to G_{M1} by the cells.[3] It also is conceivable that the higher ganglioside homologues do act directly as receptors for LT.

These studies demonstrate that a line of transformed mouse fibroblasts deficient in gangliosides responds to

[3] Both the choleragen and LT preparations used in these studies were assayed for sialidase activity as previously described (25). No activity was detected.

TABLE II
Effect of Mono-, Di-, and Trisialogangliosides on the Response of Transformed Fibroblasts to LT

Ganglioside added	cAMP content
	pmol/mg protein
None	6
G_{M3}	6
G_{M2}	10
G_{M1}	21
G_{D1a}	14
G_{D1b}	9
G_{T1}	13

Cells were incubated with gangliosides and LT (258 μg), and cAMP was determined as described in Table I. Assays are the average of duplicate incubations. The cAMP content of cells incubated without gangliosides or LT was 6 pmol/mg protein.

TABLE III

Effect of G_{D1b} on the Response of the Fibroblasts to Choleragen

Ganglioside added	cAMP content	
	No CT*	Plus CT
	pmol/mg protein	
G_{M1}	4	15
G_{D1b}	2	9

Cells were incubated with 25 pmol of ganglioside, as described in Table I, and subsequently without or with choleragen, 10 μg/10 ml of Hanks' solution for 3 h at 37°C. cAMP was determined as described previously. Data are means of values for duplicate incubations.

* Choleragen.

E. coli LT only after they have been incubated with certain gangliosides. Of these, G_{M1} is clearly the most effective. These results are similar to those reported previously on the effect of G_{M1} on choleragen responsiveness and indicate that G_{M1} can serve as the functional receptor for both toxins in these cells. Thus, although the effects of choleragenoid and gangliosides on the responsiveness of cells to LT were reported to differ from those noted with choleragen (20, 22), it would appear from this study that G_{M1} can mediate the action of LT on animal cells. Whether G_{M1} is the native receptor for LT in other cells, especially intestinal cells, has yet to be determined.

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