The Role of Sphingolipids in the Maintenance of Fibroblast Morphology

THE INHIBITION OF PROTRUSIONAL ACTIVITY, CELL SPREADING, AND CYTOKINESIS INDUCED BY FUMONISIN B₁ CAN BE REVERSED BY GANGLIOSIDE GM₃*

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Previous studies demonstrated that inhibition of sphingolipid synthesis by the mycotoxin fumonisin B₁ (FB₁) disrupts axonal growth in cultured hippocampal neurons (Harel, R., and Futerman, A. H. (1993) J. Biol. Chem. 268, 14476–14481) by affecting the formation or stabilization of axonal branches (Schwarz, A., Rapaport, E., Hirschberg, K., and Futerman, A. H. (1995) J. Biol. Chem. 270, 10990–10998). We now demonstrate that long term incubation with FB₁ affects fibroblast morphology and proliferation. Incubation of Swiss 3T3 cells with FB₁ resulted in a decrease in synthesis of ganglioside GM₃, the major glycosphingolipid in 3T3 fibroblasts and of sphingomyelin. The projected cell area of FB₁-treated ganglioside GM₃ even in the presence of FB₁, whereas inhibitors, were all disrupted by FB₁. All the effects of FB₁ on cell morphology could be reversed by addition of ganglioside GM₃ even in the presence of FB₁, whereas the bioactive intermediates, sphinganine, sphingosine, and ceramide, were without effect. Finally, FB₁ blocked cell proliferation and DNA synthesis in a reversible manner, although ganglioside GM₃ could not reverse the effects of FB₁ on cell proliferation. Together, these data suggest that ongoing sphingolipid synthesis is required for the assembly of both new membrane and of the underlying cytoskeleton.

Sphingolipids (SLs)¹ are almost ubiquitous components of eukaryotic cell membranes where they play a variety of roles (1–3). An important approach to defining the precise roles of SLs is to inhibit their synthesis, by either genetic approaches, such as the production of mutants defective in SL synthesis (4), or by specific chemical inhibitors (5, 6). Unfortunately, little information is available using the first approach due to the lack of success in purifying the enzymes of SL synthesis, and the small number of mutants that have been obtained. However, specific inhibitors of SL synthesis have become available recently (5), including the mycotoxin, fumonisin B₁ (FB₁) (7). FB₁ inhibits acylation of the sphingoid long chain bases sphinganine (dihydro sphingosine) and sphingosine to dihydroceramide and ceramide, respectively.

A number of studies have examined the effects of FB₁ on the growth of cultured cells in attempts to determine the cellular basis for the diseases associated with FB₁ (reviewed in Merrill et al. (8)). It has been shown that FB₁ stimulates DNA synthesis in confluent cultures of fibroblasts (9), but inhibits the proliferation of renal epithelial cells (10) and the growth of Saccharomyces cerevisiae (11). In cultured hippocampal neurons, FB₁ disrupts axonal growth by affecting the formation or stabilization of axonal branches (12, 13) and disrupts dendrite growth in cerebellar Purkinje neurons (14). Together, these studies suggest that SL synthesis is required for cell growth and morphogenesis.

To further study the roles of SLs in cell morphogenesis, we have now analyzed the effects of FB₁ on Swiss 3T3 fibroblasts cultured at subconfluent densities. We previously demonstrated that the delivery of new membrane to the leading edge of these cells is required for pseudopodial activity and for directional migration (15), and we now show that long term incubation with FB₁ causes profound changes in a number of morphological processes associated with the actin cytoskeleton. Remarkably, all the effects of FB₁ on cell morphology could be reversed by addition of low concentrations of ganglioside GM₃.

These results suggest that SLs may be involved in the assembly of both new membrane and of the underlying cytoskeleton.

EXPERIMENTAL PROCEDURES

Materials—FB₁ was from the Division of Food Science and Technology, CSIR, Pretoria, South Africa, or from Sigma. Ganglioside GM₃, ganglioside GM₁₂, short acyl chain analogs of ceramide (N-hexanoyl-D-erythro-sphingosine, N-hexanoyl-t-threo-sphingosine, and N-hexanoyl-D-erythro-dihydro sphingosine), t-threo-sphinganine, and t-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDPMP) were from Matreya (Pleasant Gap, PA). 1-(5-Isouquinolinesulfonyl)-2-methylpipperazine (H-7), tetramethylrhodamine isothiocyanate (TRITC)-conjugated phalloidin, mitomycin C, 4,6-diamidino-2-phenylindole (DAPI), staurosporine, and t-erythro-sphingosine were from Sigma. Silica Gel 60 plates were from Merck. Lissamine-rhodamine-conjugated goat anti-rabbit antibodies were from Jackson ImmunoResearch Laboratories Inc. 6-3H[thymidine was from Amer sham International plc, Amersham, UK. Other chemicals were from Sigma, and solvents (analytical grade) were from Bio-Lab Laboratories Ltd., Jerusalem, Israel.
Cell Culture—Swiss mouse 3T3 cells were cultured in Dulbecco’s modified medium containing 10% calf serum, and maintained in a water-saturated atmosphere of 5% CO2. Cells were dissociated with trypsin/EDTA and plated in either 60-mm culture dishes for biochemical experiments, or on glass coverslips for morphological analysis, both at densities of $2 \times 10^4$ cells/ml of medium.

**Drugs and Lipids—**FB1 was added to cells from a 1 mM stock solution in 20 mM HEPES, pH 7.4; in most experiments FB1 was added to cultures immediately after plating. Mitomycin C was prepared as a 1 mg/ml stock solution in 50% ethanol. Staurosporine was prepared as a 20 µM stock solution in Me2SO. H-7 was prepared as a 30 mM stock solution in H2O. Gangliosides GM1 and GM2 were prepared as 0.5 mM stock solutions in methanol, dried under a stream of N2, dissolved in medium, sonicated, and added to cultures to give final concentrations of 25 or 100 nM.

**SL Metabolism—**[4,5-3H]Sphinganine was synthesized by reduction of $\alpha$-d-threo-sphingosine with NaB[3]H4 (10 Ci/mmol) (16–18) and used to analyze [3H]SL synthesis (16). Briefly, [4,5-3H]Sphinganine (5 × 106 cpm) was added to the culture medium of 3T3 cells. After 24 h, cells were washed with phosphate-buffered saline, removed by scraping with a rubber policeman, and centrifuged (15,000 g, 5 min). The cell pellet was resuspended in 20 mM HEPES, pH 7.4; in most experiments FB1 was added to give final concentrations of 10–100 nM FB1. Using this concentration, [3H]SL synthesis was observed (Fig. 1A), with significant inhibition obtained using 20 µM FB1. This concentration, 

**Thymidine Incorporation—**Cells were plated in 24 multiwell dishes at a density of 5 × 104 cells/well, and incubated for 2 h with 0.5 µCi of [6-3H]thymidine (50 Ci/mmol). Cells were washed in phosphate-buffered saline, incubated with trichloroacetic acid (5%, 4°C, 20 min), washed with ethanol, and dissolved in 0.1 N NaOH. Radioactivity was determined by liquid scintillation counting in a Packard Tri-Carb 1500 scintillation counter using Ultima gold scintillation fluid (Packard): water (8:1, v/v).

**RESULTS**

**FB1 Inhibits SL Synthesis in 3T3 Fibroblasts—**To determine the extent of inhibition of SL synthesis by FB1 in Swiss 3T3 fibroblasts, various concentrations of FB1 were added to the medium at the time of plating. A dose-dependent inhibition of [3H]SL synthesis was observed (Fig. 1A), with significant inhibition obtained using 20 µM FB1. Using this concentration, [3H]SL synthesis was inhibited to a similar extent when analyzed on each of the first 5–6 days in culture, and even short incubations with FB1 resulted in similar levels of inhibition. When expressed as a function of the amount of protein per culture dish on day 5, the incorporation of [4,5-3H]Sphinganine into total [3H]SLs was inhibited by >75% at 20 µM FB1 and above (Fig. 1B). The extent of inhibition appeared less when expressed as incorporation of [4,5-3H]Sphinganine per µg protein (compare Fig. 1, A and B), since FB1-treated cells did not proliferate (see below), and the amount of protein per dish was much lower in FB1-treated cells, particularly at high concentrations of FB1 (20 or 50 µM).

Analysis of the [3H]SL profile demonstrated that levels of individual [3H]SLs remaining after FB1-treatment varied (Fig. 1C). Whereas [3H]glucosylceramide (3H)GlcCer, [3H]sphingomyelin ([3H]SM), and [3H]GM2 were reduced by 73–77% (see also Fig. 1A), [3H]lactosylceramide ([3H]LacCer) was reduced by only 37%, and [3H]ceramide trihexoside ([3H]Gb3) increased by 2.3-fold (Fig. 1C). This resulted in different profiles of [3H]SLs in control compared to FB1-treated cells. [3H]Gb3 comprised 1.9% of the total [3H]SLs in control cells, but 16.5% in FB1-treated cells. [3H]GlcCer comprised 8.7 and 7.7%, [3H]LacCer 4.5 and 8.9%, [3H]SM 58.7 and 48.1%, and [3H]GM2 com-
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Projected cell area was measured on day 5 after addition of sphingoid bases and SLs on day 4. For cells treated with FB1 (20 μM), FB1 was added at the time of plating, SLs or long chain bases added on day 4, and cell area was analyzed on day 5. 100 cells were analyzed for each treatment, and data are shown as means ± S.E. with the number of experiments shown in parentheses.

Table I
The effects of sphingoid long chain bases and SLs on cell morphology

| Treatmenta | Projected cell area | Percent change versus untreated cells |
|------------|---------------------|---------------------------------------|
| None       | 1666 ± 52 (8)       | %                                     |
| D-sphinganine (10 μM)b | 1640 ± 102 (4) | 98.4                                 |
| n-erythro-Sphingosine (10 μM)b | 1569 ± 40 (2) | 94.2                                 |
| C6-α-erythro-Cer (5 μM) | 1821 ± 46 (2) | 108.4                               |
| C6-α-erythro-dihydroCer (5 μM) | 1784 ± 7 (2) | 106.2                               |
| C6-α-threo-Cer (5 μM) | 1800 ± 4 (2) | 107.1                               |
| C6-α-threo-dihydroCer (5 μM) | 805 ± 33 (7) | 54.3                                 |
| FB1 (25 nM) | 1517 ± 44 (6) | 91.1                                 |
| FB1 + GM2 (100 nM) | 1634 ± 53 (4) | 98.1                                 |
| FB1 + GM1 (25 nM) | 1100 ± 39 (5) | 66.0                                 |
| FB1 + GM1 (100 nM) | 1206 ± 112 (4) | 72.4                                 |
| FB1 + C6-α-erythro-Cer (5 μM)c | 889 ± 56 (5) | 52.1                                 |
| FB1 + C6-α-threo-Cer (5 μM)c | 793 ± 21 (2) | 47.6                                 |
| FB1 + C6-α-erythro-dihydroCer (5 μM) | 841 ± 87 (2) | 56.1                                 |

a C6-α-erythro-Cer, N-hexanoyl-α-erythro-sphingosine; C6-α-threo-Cer, N-hexanoyl-α-threo-sphingosine; C6-α-erythro-dihydroCer, N-hexanoyl-α-erythro-dihydrosphingosine.

b Neither D-sphinganine nor n-erythro-sphingosine (10 μM) had any effect when added every day during the first 5 days in culture (projected cell areas of 1751 ± 34 and 1739 ± 41 (n = 2), respectively). In addition, neither 0.1 or 1 μM concentrations had any effect.

c On day 5 the projected cell area after removal of FB1 on day 4 was 1673 ± 188, n = 2.

d In one experiment, C6-α-erythro-Cer was added on day 4, and cell area measured on days 5 through 8; no increase in cell area versus FB1-treated cells was observed (cell area was 931 ± 3 μm on day 5; 997 ± 63 on day 6; 827 ± 50 on day 7; 966 ± 41 on day 8).

Cell Spreading Is Reduced by FB1 Treatment—Inhibition of SL synthesis by FB1 (20 μM) for 5 days caused profound changes in cell morphology. Whereas control cells displayed typical fibroblast morphology, with a leading edge and a trailing edge (Fig. 2A), FB1-treated cells were less well spread on the substrate and displayed reduced pseudopodial activity (Fig. 2B). After incubation with FB1 for 5 days, the projected cell area was reduced by ~45% (Table I), and there was a significant decrease in the morphometric index (21) of dispersion (dispersion index of control cells was 0.91 ± 0.09, and of FB1-treated cells was 0.55 ± 0.09, n = 40), but not of elongation (elongation index of control cells was 1.23 ± 0.1 and of FB1-treated cells was 1.10 ± 0.08, n = 40). Elongation is a measure of the extent to which shape must be compressed along its longitudinal axis in order to minimize its difference from a circle, while dispersion is invariant to stretching, compressing, or shearing the shape in any direction; both of these indices are equal to zero for a circle. Elongation is considered as a measure of cell bipolarity, whereas dispersion is a measure of multipolarity (21). Thus, the formation of protrusions is reduced in FB1-treated cells, while cells remain elongated to a similar extent to control cells.

The effects of FB1 on morphology were completely reversed 24 h after its removal from the medium, or by addition of exogenous ganglioside GM2 to the medium for 24 h, even in the presence of FB1 (Table I); GM2 itself had no effect on projected cell area. Neither of the bioactive intermediates, sphinganine or sphingosine, which both accumulate upon long term treatment with FB1 (8, 23), had any effect on morphology even for up to 5 days incubation, and even after multiple additions (Table I). Short acyl chain analogues of ceramide, whose level is depleted upon FB1 treatment (8), and a short acyl chain analogue of dihydroceramide were also unable to reverse the effects of FB1 (Table I).

Since in fibroblasts, short acyl chain analogues of ceramide are metabolized mainly to GlcCer and SM (not shown; see also Meivar-Levy et al. (24)), and since sphinganine, sphingosine, and ceramides do not significantly affect cell morphology, these data together demonstrate that the ability of GM2 to restore cell morphology is due to depletion of an essential higher order glycosphingolipid, probably GM4, and not due to accumulation or depletion of bioactive intermediates. This is supported by observations that exogenously added GM2 was metabolized to only a limited extent by fibroblasts during a 24-h incubation (see also Chigorno et al. (25)). Upon incubation of control and FB1-treated cells with 100 pmol [3H]GM2 on day 4, 68–72% of the [3H] radioactivity remained in [3H]GM2 on day 5, with most of the remainder in [3H]GlCer (5–6%), [3H]SM (5%), [3H]LacCer (6%), and [3H]Gb3 (3–4%). A related ganglioside, GM1, was able to partially restore the effects of FB1 (Table I). After incubation of cells with [3H]GM2, ~10% was degraded to [3H]GM3; the small amount of GM4 formed from GM2 may be responsible for the partial ability of GM2 (Table I) to restore the effects of FB1 on cell morphology.

FB1-Treatment Affects the Organization of the Actin Cytoskeleton—The effects of FB1 were further analyzed by exami-
In addition to its roles in the organization of the leading edge in interphase cells, the actin cytoskeleton also plays an important role in cell division, particularly in the formation of the contractile ring during cytokinesis. Incubation with FB1 interfered with cytokinesis as demonstrated by the appearance of binuclear cells (Fig. 3, B, D, and F). The percent of binuclear cells was 3–4% in control cells, but 11–12% in FB1-treated cells (Fig. 4). Addition of GM3 on day 4 in culture partially reversed the effects of FB1 on cytokinesis (Fig. 4), but neither sphingosine or sphinganine had any effect (not shown).

FB1 Interferes with Protrusional Activity and Microvilli Formation—A number of protein kinase inhibitors, such as staurosporine and H-7, induce changes in fibroblast morphology (26, 27). Upon incubation with H-7, fibroblasts acquire numerous long, thin processes (Fig. 5A) that result from the inability of cells to retract their trailing edge (26); at the leading edge, lamellipodial activity is unaffected and may even be enhanced (Fig. 5A). Pretreatment with FB1 (20 µM) for 5 days significantly decreased the number of long processes formed upon H-7 or staurosporine-treatment (Fig. 5), and GM3 almost completely restored the ability of staurosporine to induce long processes (Fig. 5).

Fibroblasts were also incubated with mitomycin C, a drug that inhibits DNA replication and arrests cells in the S phase of
the cell cycle. Since other biosynthetic processes are not affected, including synthesis of new membrane and new cytoskeletal components, cells continue to grow and acquire much larger sizes than untreated cells. The projected area of cells treated with 0.1 μg/ml mitomycin C for 5 days was 4.3-fold greater than that of untreated cells (Table II), and mitomycin C-treated cells displayed a large number of small microvilli at the cell surface that were generally located near the center of the cell (Fig. 6A). The projected area of FB1-treated cells was only 2.4-fold greater after incubation with mitomycin C (Table II), and FB1-treated cells displayed far fewer microvilli (Fig. 6B). The addition of GM3 on day 4 to cells that had been treated with both mitomycin C and FB1 resulted in a significant increase in projected cell area (Table II), demonstrating that GM3 is able to partially restore the inhibitory effects of FB1 on cell spreading after mitomycin C treatment.

Cell Proliferation and DNA Synthesis Is Blocked by FB1—Little cell proliferation was observed for up to 10 days in the presence of 20 μM FB1, although cells remained viable throughout this period. The number of cells in untreated cultures increased by ~23-fold during the first 7 days in culture, but only increased by 2.6-fold in FB1-treated cultures (Fig. 7A). The block in cell proliferation was reversible, since cell number increased after removal of FB1 on day 4, and attained values similar to untreated cells (Fig. 7A). The block of cell proliferation could not be explained solely by inhibition of cytokinesis, since only 11–12% of the cells were binuclear after FB1-treatment (see Fig. 4). Indeed, analysis of [3H]thymidine incorporation demonstrated that DNA synthesis was also significantly inhibited by FB1-treatment (Fig. 7B). The effects of FB1 on DNA synthesis (Fig. 7B) could be reversed by removing FB1 from the medium, but in contrast to its effects on cell morphology, addition of GM3 had no effect on either cell proliferation (not shown) or on [3H]thymidine incorporation (Fig. 7B). Addition of either sphingosine or sphinganine directly to the culture medium had no effect on cell proliferation, either in the absence or presence of FB1 (not shown).

DISCUSSION

Inhibition of ceramide synthesis by FB1 causes a number of responses in various cells (8). We now demonstrate that the reduction in complex SL synthesis that occurs upon incubation of Swiss 3T3 cells with FB1 results in major changes in the actin cytoskeleton and in processes related to, or dependent on, the actin cytoskeleton and that these effects can be reversed by addition of ganglioside GM3.

Characterization of the Biochemical Effects of FB1 on Swiss 3T3 Fibroblasts—Of all the inhibitors tested (5), FB1 has proved particularly useful in manipulating levels of SL synthesis (7, 8, 28). As a consequence of FB1 treatment, sphingosine and sphinganine levels are elevated, and ceramide is depleted (7). All three of these molecules can themselves disrupt cell morphology and proliferation (10, 29), and it is therefore essential when using FB1 to distinguish between effects caused by changes in levels of these bioactive intermediates or effects caused by depletion of complex sphingolipids (8). The inability of sphingoid long chain bases and of ceramide, and the ability of GM3 to reverse the effects of FB1 even in the presence of FB1, strongly suggests that the effects we observed in sparse cultures of 3T3 cells are due to depletion of complex SLs, as appears to be the case for changes in cell morphology and growth upon FB1 treatment of cultured hippocampal neurons (12, 13). The major SLs in 3T3 cells are GM3 and SM, and both of these lipids are depleted to a similar extent after incubation with FB1. Surprisingly, levels of the neutral glycosphingolipid, Gb3, are elevated after FB1 treatment. In cultured cerebellar neurons (28), SM synthesis was more sensitive to FB1 than glycosphingolipid synthesis. Since the activities of SM synthase and of glycosyltransferases were not directly affected by FB1, it was suggested that different enzymes in the metabolic pathway are sensitive to a different extent to reduction in the levels of their respective substrates (28). Our data suggest that Gb3 synthase has a relatively high $K_m$ value, which renders it relatively insensitive to changes in levels of its substrate, lactosylceramide.
TABLE II
The effects of FB1 and GM3 on the increase in cell area induced by mitomycin C

Fibroblasts were treated 3–4 h after plating with mitomycin C (0.1 
µg/ml), with mitomycin C and FB1 (20 µM), or with mitomycin C and  
FB1, on day 1 and GM3, (25 nM) on day 4. Projected cell area was  
measured on days 5 and 6; the projected area of cells treated with FB1  
alone was 909 ± 39 µm. Each value represents the mean ± S.E. of 3  
independent experiments, for which at least 50 cells were analyzed.

| Days in culture | Projected cell area after treatment with: | µm² |
|-----------------|------------------------------------------|-----|
|                 | Control | Mitomycin C | Mitomycin C + FB1 | Mitomycin C + FB1 + GM3 |
| 5               | 1710 ± 59 | 7422 ± 498 | 2574 ± 111 | 4725 ± 323 |
| 6               | ND* | 9646 ± 553 | 3200 ± 283 | 7110 ± 490 |

* Not determined.

The Ability of GM3 to Reverse the Effects of FB1—Incubation  
with FB1 affects a number of cellular processes, including cell  
spreading, microvilli formation, cytokinesis, formation of long  
processes, and disruption of DNA synthesis and cell proliferation.  
GM3 restores the disruptive effects of FB1 on cell morphology,  
but not on proliferation. Four pieces of evidence suggest that  
GM3 itself is responsible for the restoration of cell  
morphology. (i) GM3 is synthesized at far higher levels than  
either lactosylceramide or Gb3, rendering it more suitable to  
mediate interactions between the plasma membrane and the  
actin cytoskeleton (see below). Moreover, Gb3 synthesis is  
not inhibited by FB1 treatment. It should however be noted that  
no role has yet been ascribed to Gb3, and the differences in the  
sensitivity of Gb3 and GM3 synthesis to FB1-treatment may  
suggest that levels of Gb3 must be maintained at a constant  
level in the cell for it to perform whatever function it may play.  
(ii) GM3 is only degraded to a limited extent by fibroblasts (see  
also Chigorno et al. (25)). (iii) The lack of effects of short acyl  
chain analogues of ceramide (which are metabolized mainly to  
GlcCer and SM, but not to lactosylceramide, GM3 and Gb3),  
suggest that GlcCer cannot be responsible for the restoration of  
cell morphology. (iv) Neither SM or ceramide can be responsible  
for restoration since incubation with PDMP (which inhibits the  
synthesis of GlcCer and of higher order glycosphingolipids,  
but enhances SM synthesis (30) and elevates ceramide levels),  
results in a similar decrease in projected cell area to that  
observed with FB1, and addition of exogenous GM3 together  
with PDMP restores normal cell morphology.  

Although exogenous GM3 could restore all of the disruptive  
effects of FB1 on cell morphology, including cytokinesis, it was  
completely inactive in reversing the inhibition of cell proliferation  
and DNA synthesis. Other studies have also demonstrated  
inhibition of cell proliferation by FB1 (10, 11, 31). Cell  
growth is also arrested in rabbit skin fibroblasts (32) and in  
Swiss 3T3 cells (33) by PDMP, with growth arrested at the G2/S  
and G2/M transitions in 3T3 cells (33). In contrast, FB1  
stimulates thymidine incorporation in 3T3 cells (9); however,  
the conditions used in this study (9) were completely different from  
those used in the current study, inasmuch as we examined cell  
proliferation and DNA synthesis during the logarithmic phase  
of growth, whereas Schroeder et al. (9) examined stimulation of  
DNA synthesis in quiescent cultures. Although we cannot draw  
any definitive conclusions about the mechanism(s) by which  
FB1 inhibits cell proliferation in sparse cultures of 3T3 cells,  
the inability of GM3 to restore proliferation demonstrates that  

3 The projected area of PDMP-treated (100 µM) cells was 967 ± 20  
µm² (n = 3) compared to 1666 µm² in untreated cells. The projected  
area of cells treated with PDMP (100 µM) and GM3 was 1562 ± 63 (n  
= 3) and 1794 ± 7 (n = 2) µm² for 25 nM and 100 nM GM3, respectively,  
and for cells treated with PDMP and GM3 was 1081 ± 12 (n = 2) and 1165 ±  
4 (n = 2) µm² for 25 nM and 100 nM GM3.

GM3 is not the limiting factor in the regulation of cell proliferation.  
Elucidation of the possible roles of bioactive sphingolipid  
intermediates in regulating proliferation (2) requires further  
study.

The Relationship between GM3 and the Actin Cytoskeleton—The  
most interesting conclusion from the current study is that  
GM3 synthesis is required for a variety of processes that  
depend on the assembly of new membrane and of the underlying  
actin cytoskeleton. These processes are exemplified by the  
reduction in the number of microvilli in mitomycin C-treated cells  
(Fig. 6). Microvilli are surface extensions containing an actin  
core, and are found on the surface of many cells, particularly on  
cells that require a large surface area to function. Normal  
fibroblasts have few microvilli, but after mitomycin C-treat- 
ment, their levels are greatly increased, presumably since syn- 
thesis and assembly of actin and new membrane continues,  
even though cell division is arrested. The fact that FB1-treated  
cells have far fewer microvilli indicates that an intimate rela- 
tionship exists between GM3 and the actin cytoskeleton. Other  
examples of this relationship are illustrated by the reduction in  
projected cell area (Table I), pseudopodial activity (Fig. 2), the  
formation of long processes (Fig. 5), and the inhibition of cyto- 
kinesis (Figs. 3 and 4).

How might ganglioside GM3 be related to the actin cytoske- 
elon? Since gangliosides are highly enriched in the plasma  
membrane (34), the effects described above are presumably due  
to alteration in a plasma membrane function. It is not known  
whether the effects depend on alterations in a physical prop- 
erty of the membrane, or alternatively on the assembly of new  
membrane, but it should be noted that all of the processes  
described above require membrane synthesis and assembly.  
For instance, protrusional activity at the leading edge can be  
blocked by treatments that inhibit the synthesis or delivery of  
new membrane (15, 35). During the assembly of new mem- 
brane, actin-binding proteins such as ezrin, radixin, and moe-
studies showing that GM3 is functionally associated with integrin-mediated cell adhesion. However, the data reported in the current study suggest that GM3 plays a wider role since it appears necessary for mediating events associated with assembly of the actin cytoskeleton and of new membrane.

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