A Role for Gangliosides in Astroglial Cell Differentiation
In Vitro

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Abstract. Rat cerebral astroglial cells in culture display specific morphological and biochemical behaviors in response to exogenously added gangliosides. To examine a potential function for endogenous gangliosides in the processes of astroglial cell differentiation, we have used the B subunit of cholera toxin as a ganglioside-specific probe. The B subunit, which is multivalent and binds specifically to GM1 ganglioside on the cell surface, induced a classical star-shaped (stellate) morphology in the astroglial cells and inhibited DNA synthesis in a dose-dependent manner. The morphological response was massive and complete within 2 h, with an ED50 of 0.8 nM, and appeared to depend on the direct interaction of the B subunit with GM1 on the cell surface. A B subunit-evoked inhibition of DNA synthesis and cell division (ED50 = 0.2 nM) was observed when the cells were stimulated with defined mitogens, such as epidermal growth factor and basic fibroblast growth factor. Maximal inhibition approached 80% within 24 h. The effects of the B subunit were unrelated to increases in cAMP. These observations, taken together with previous studies, demonstrate that both endogenously occurring plasma membrane gangliosides and exogenously supplied gangliosides can influence the differentiative state (as judged by morphological and growth behaviors) of astroglial cells in vitro.

Gangliosides, sialic acid-containing glycosphingolipids (44), are normal membrane constituents, largely localized to the outer leaflet of the plasma membrane and thus presenting their carbohydrate moieties on the surface of the cells (49). They are abundant in neural tissues, and the ganglioside compositions of neuronal and glial cell membranes appear to be different (25). Numerous studies have identified effects of exogenously supplied gangliosides on neuronal cells, both in vitro (11, 26, 27, 38) and in vivo (8, 34, 39, 45). There have been, however, few investigations addressing possible interactions of gangliosides with glial cells.

Rat astroglial cells in vitro have been reported to display two responses to GM1 and other gangliosides. One ganglioside effect is prevention or reversal of the assumption by flat astroglial cells of a stellate (star shaped) morphology under the influence of certain agents (12, 37), which include neuron-released molecules such as neurotransmitters and neuropeptides (29). The other ganglioside effect is that of growth modulation in these cells (23, 36). A possible role for endogenously derived gangliosides in these astroglial cell behaviors was never examined.

Several studies have indicated that the cross-linking of cell surface gangliosides can influence some (41, 42), but not other (9, 10) cellular behaviors. For example, the B subunit of cholera toxin, which is multivalent and binds exclusively to GM1 ganglioside on the cell surface (15), has been reported to induce the proliferation of lymphocytes (42) and growth-arrested, nontransformed 3T3 fibroblasts (41). The B subunit, however, inhibited the growth of rapidly dividing normal 3T3 cells and virally transformed 3T3 cells (41). The molecular basis for these opposing effects of B subunit could conceivably reflect differences in cell metabolic properties, or the amount or distribution of surface GM1 being occupied by B subunit, the latter suggested by Spiegel and co-workers (41). It has been reported that the levels of cell surface gangliosides vary as a function of 3T3 cell growth state (20), and that transformed 3T3 cells have less GM1 than normal 3T3 cells (20).

It is tempting to speculate that the previously observed astroglial cell morphology and growth responses to exogenous gangliosides also depend on the amount or distribution of ganglioside that may be incorporated. At the same time, nothing is known concerning the significance of these findings with respect to the function of endogenous gangliosides in the same astroglial cellular behaviors. Until now, there has been no assessment of the manipulation of endogenous ganglioside in cells displaying well-defined responses to the same exogenous ganglioside molecules. To investigate this question, we have used the B subunit of cholera toxin as a ganglioside-specific probe in order to examine a role for membrane GM1 ganglioside in the regulation of astroglial cell morphology and growth. The results presented here provide the first evidence that manipulation of endogenously oc-
curving plasma membrane gangliosides can influence specific behaviors of astroglial cells in vitro.

**Materials and Methods**

**Materials**

Eagle's basal medium (EBM) and penicillin G sodium were purchased from Gibco (Grand Island, NY); fetal calf serum from Seromed (Berlin, Federal Republic of Germany); l-glutamine, poly-l-ornithine HBr (44,000 mol wt), bovine pancreatic insulin, ovalbumin, cholera toxin, and forskolin from Sigma Chemical Co. (St. Louis, MO); bovine plasma fibronectin, bovine pituitary basic fibroblast growth factor (bFGF), and mouse salivary gland epidermal growth factor (EGF) from Bethesda Research Laboratories (Bethesda, MD); B subunit of cholera toxin from List Biological Labs (Campbell, CA); [methyl-3H]Tdr (2.0 Ci/m mole) from Amersham International (Buckinghamshire, England); Instagel II from Packard Instrument Co., Inc. (Downers Grove, IL).

**Cell Cultures**

Primary and secondary cultures of astroglial cells were prepared from cerebres of 1-2-d-old Sprague-Dawley rats as described previously (33). Dissociated cells were cultured in EBM modified to contain 33.3 mM d-glucose, 2 mM l-glutamine, 26.4 mM NaHCO3, 100 U/ml penicillin, and 10% (vol/vol) heat-inactivated FCS. These primary cultures were harvested 10 d later in serum-free modified EBM containing 1% (vol/vol) ovalbumin and diluted to 3.2 x 105 cells/ml in the modified EBM. The secondary cultures to be used experimentally were set up by seeding 50 µl of the final cell suspension into 6-mm plastic culture wells (No. 3070; Falcon Labware, Becton, Dickinson & Co., Oxnard, CA) already containing 50 µl of medium, and which had been precoated with polyornithine and fibronectin (33). Thus, each well started with 16,000 cells, 12,000 of which attached. All cultures received one medium change at 2 h and another at 24 h. Most cultures were treated and studied beginning 1 d after seeding (DI cultures) with the new medium containing the agent to be tested. Cultures to be used for morphological evaluation or cell counting were fixed with 2% (vol/vol) glutaraldehyde in EBM, stained with 0.1% toluidine blue, and observed under phase contrast microscopy.

**DNA Synthesis**

To evaluate DNA synthesis, cultures were pulsed at various times after pretreatment of B subunit or mitogen with 2 µCi/ml [methyl-3H]Tdr (2 Ci/m mole). After a 2-h pulse, cultures were washed twice with 100 µl of ice-cold PBS, 100 µl of cold 5% TCA was added per well, and the cells were kept at 4°C for 20 min. The TCA extract was discarded and each well washed three times with 100 µl of 5% TCA. The cells were dissolved in 100 µl/well of 1 N NaOH, and the NaOH extract transferred to a scintillation vial. Each well was washed with 100 µl of 1 N HCl, and this added to the counting vial. 5 ml of Instagel II were added to each vial, and radioactivity counted in a liquid scintillation spectrometer (Tricarb 460 C; Packard Instrument Co.).

**cAMP Measurements**

Levels of intracellular cAMP were determined by radioimmunoassay with the use of protocols and kit (No. RPA 509; Amersham International) as described previously for astroglial cells (12). The larger amounts of cellular material needed for cAMP analysis were prepared by seeding 1.2 x 106 primary astroglial cells into 16-mm culture wells (Costar 3524; Data Packaging Corp., Cambridge, MA), coated with polyornithine and fibronectin (12).

**Results**

**B Subunit of Cholera Toxin Induces Morphological Differentiation in Astroglial Cells**

Astroglial cells prepared from neonatal rat cerebrum and cultured in serum-free medium maintain a flat, polygonal shape (33). Exposure of these cells to a variety of agents thought to activate cAMP-synthesizing systems (e.g., cAMP analogues, forskolin, cholera toxin, norepinephrine, vasoactive intestinal polypeptide) promotes a rapid (1-2 h) and near complete conversion to a process-bearing (stellate) morphology (12, 29, 30, 37, 48). When the astroglial cells are treated with N6, O2'-dibutyryl cyclic AMP, forskolin, or cholera toxin, the concurrent or delayed administration of GM1 inhibits or reverses the morphological response (37). GM1 does not block the elevation of cAMP that occurs in response to these agents (12).

To further explore this morphological modulation of astroglial cells by gangliosides, the following experiments were carried out. The B subunit of cholera toxin, which is pentavalent and recognizes exclusively ganglioside GM1 on the cell surface (15), was used as a probe to assess a role for endogenous gangliosides in the determination of astroglial cell morphology. When secondary astroglial cells at DI were treated with 1 µg/ml of B subunit, a massive conversion to the stellate morphology occurred and was complete within 2 h (Fig. 1). The morphology induced by B subunit is indistinguishable from that elicited by cAMP analogues (30, 37) or other cAMP-elevating drugs (12, 29, 48) in these cells. Fig. 2 illustrates the concentration dependence of the B subunit-evoked flat-to-stellate conversion in the astroglial cells. A maximal effect was observed at a B subunit concentration of 250 ng/ml (4.3 nM) or greater, with an ED50 of 45 ng/ml (0.8 nM).

Since agents that elicit cAMP elevations in astroglial cells also induce the stellate morphology, a trivial explanation for the effect of the B subunit of cholera toxin is that the preparation used here is contaminated by the A subunit (adenylate cyclase activating). Several lines of evidence, however, show this not to be the case. In the first, the B subunit dose-response obtained with the commercial material was identical to that found using a B subunit preparation (46) kindly provided by Dr. M. Tomasi (Laboratorio di Biologia cellulare e Immunologia, Istituto Superiore di Sanita, Roma). This latter preparation has been biologically characterized as devoid of any adenylate cyclase–activating A subunit (2). Secondly, measurements of intracellular cAMP levels at different times failed to show any increase of cAMP after treatment by B subunit. As Table I illustrates, concentrations of the B subunit up to 20 µg/ml were unable to stimulate cAMP production at any time tested. Cholera toxin at 1 µg/ml, however, caused a 200-fold elevation of cAMP at 1 h in the astroglial cells, tapering off to about an 18-fold stimulation by 16 h. Previous studies have shown this concentration of cholera toxin to elicit a maximal rise of cAMP at about 1 h in astroglial cells (12). Significant, though smaller, increases of cAMP were observed using as little as 5 ng/ml of cholera toxin at 1 h (data not shown). Similar results were obtained in independent experiments using HPLC analysis instead of radio-immunoassay. Thus, the B subunit used in our studies is devoid of any A subunit; the effects of B subunit on astroglial cells are unrelated to increases of cAMP.

To confirm that the observed morphological activity was due to the binding of B subunit to the cells, we measured the ability of cholera toxin to elevate cAMP and its inhibition by the B subunit. The increase in cAMP at 1 h with 5 ng/ml of cholera toxin (59 pmol/10⁶ cells) vs. 2 pmol/10⁶ cells for con-
Figure 1. Photomicrographs of secondary rat cerebral astroglial cells treated 2 h with no agent (top) or 1 μg/ml B subunit of cholera toxin (bottom). Bar, 25 μm.

Control) was inhibited by the addition of B subunit in a dose-dependent manner: 0.5 μg/ml of B subunit reduced the cholera toxin-induced rise to 39 pmol cAMP/10⁶ cells, while 1.5 μg/ml B subunit totally inhibited the effect of cholera toxin. Furthermore, presentation of B subunit (20 μg/ml) 20 s be-

Figure 2. Dose responsiveness of the B subunit–induced conversion of astroglial cells to the stellate morphology. DI cultures (16,000 cells seeded/6 mm well) were treated with varying concentrations of the B subunit of cholera toxin for 2 h, fixed, stained, and the percent of stellate cells determined. Values are averaged from three replicate experiments (n = 6).
Astroglial cells were seeded as secondary cultures (1.2 x 10^5 per well in 24-well plates), and treated at D1 with cholera toxin or B subunit for 1, 4, and 16 h. The cells were then extracted with 5% TCA and cAMP measured. Each value is given as the mean ± SD (n = 6).

**Table I. Effect of Cholera Toxin and the B Subunit on cAMP Levels in Astroglial Cells**

<table>
<thead>
<tr>
<th>Addition</th>
<th>cAMP (pmol/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>B subunit (20 µg/ml)</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Cholera toxin (1 µg/ml)</td>
<td>400 ± 50</td>
</tr>
</tbody>
</table>

The inhibitory effect of the B subunit was also observed when astroglial cells were stimulated with either EGF or bFGF, at high or low cell densities. Table II illustrates the results. The B subunit (1 µg/ml) reduced DNA synthesis at 24 h by 80–85% without mitogen addition, irrespective of initial cell seeding density. In the presence of 10 ng/ml EGF (and 5 µg/ml insulin), proliferation was stimulated ∼4.5-fold at 24 h. Polypeptide growth factors active on astroglial cells generally require insulin (6, 28, 31, 36). The growth-stimulating effect of EGF was essentially abolished in the concurrent presence of 1 µg/ml B subunit, although not to the level seen in control cultures with the B subunit. Similar results were obtained with bFGF; however, this latter mitogen was less effective than EGF. Insulin alone is reported to have no effect on DNA synthesis in cultured astroglial cells (6, 31, 36).

The antiproliferative effect of the B subunit was confirmed by direct cell counting and autoradiography. The increase in cell numbers elicited by EGF or bFGF at 48 h was reduced in the presence of B subunit by 85 and 45%, respectively, of the mitogen-stimulated values relative to control (Table III). In addition, autoradiographic analysis revealed fewer [3H]Tdr-labeled nuclei after a 24-h exposure to B subunit. As Table IV illustrates, the percent of labeled nuclei in cultures without mitogen decreased from 10 to 6% with B subunit, while the value of 28% in EGF-treated cultures was reduced to 10%. These results substantiate the data on direct cell number measurements in Table III. The inhibitory action of the B subunit on astroglial cells stimulated to grow with EGF or bFGF most likely is not a consequence of an interference by B subunit of mitogen binding to the cells. When cells were first exposed to the mitogen for 24 h and then to B subunit, the same inhibition was observed. For example, 48-h control cultures incorporated [3H]Tdr at 554 dpm/h, with EGF (plus insulin) yielding 2,445 dpm/h. Addition of 1 µg/ml B subunit after 24 h of EGF/insulin treatment, in the continued presence of the mitogen, decreased [3H]Tdr labeling to 642 dpm/h 24 h later. Similar results were obtained with bFGF. Thus, B subunit is still effective when added after a prior mitogen activation.

**Figure 3.** Concentration dependence of B subunit inhibition of DNA synthesis in astroglial cells. Cultures of secondary astroglial cells at D1 (16,000 cells seeded/6 mm well) were exposed to various concentrations of the B subunit of cholera toxin for 24 h, and assayed for [3H]Tdr incorporation into DNA during the last 2 h. Each value is the mean ± SD (n = 6, two experiments).
Table II. Inhibition of DNA Synthesis in Astroglial Cells by the B Subunit of Cholera Toxin

<table>
<thead>
<tr>
<th>Additions</th>
<th>16,000 cells</th>
<th>4,000 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- B Subunit</td>
<td>+ B subunit</td>
</tr>
<tr>
<td></td>
<td>dpm/h/well</td>
<td>dpm/h/well</td>
</tr>
<tr>
<td>None</td>
<td>1,050 ± 140</td>
<td>171 ± 27</td>
</tr>
<tr>
<td>EGF</td>
<td>5,077 ± 423</td>
<td>1,305 ± 220</td>
</tr>
<tr>
<td>bFGF</td>
<td>3,448 ± 435</td>
<td>1,557 ± 325</td>
</tr>
</tbody>
</table>

Astroglial cells were seeded as secondary cultures in 96-well tissue culture plates in 0.1 ml EBM at 4 x 10^3 or 16 x 10^3 cells per well. After 1 d, 0.1 ml of EBM was added containing 10 ng/ml EGF or bFGF (and 5 μg/ml insulin), and 1 μg/ml of B subunit where indicated. Incorporation of [3H]TdR was determined during the last 2 h of the 24-h incubation. Values are the mean ± SD (n = 8, two experiments).

Table III. The B Subunit of Cholera Toxin Reduces Mitogen-induced Cell Number Increases

<table>
<thead>
<tr>
<th>Treatment (48 h)</th>
<th>Cells/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>446 ± 63</td>
</tr>
<tr>
<td>EGF</td>
<td>905 ± 100</td>
</tr>
<tr>
<td>EGF + B subunit</td>
<td>604 ± 90*</td>
</tr>
<tr>
<td>bFGF</td>
<td>862 ± 101</td>
</tr>
<tr>
<td>bFGF + B subunit</td>
<td>603 ± 98*</td>
</tr>
</tbody>
</table>

Secondary astroglial cells at D1 (16,000 cells seeded/6 mm well) were treated with the indicated agents for 48 h: 1 μg/ml B subunit; 10 ng/ml EGF or bFGF (plus 5 μg/ml insulin). Cells were fixed, stained, and counted under phase microscopy. Values are the mean ± SD (n = 8, two experiments).

* P < 0.05 compared with mitogen without B subunit (Student's t test).

Table IV. Autoradiographic Analysis of the Antiproliferative Effect of the B Subunit of Cholera Toxin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent labeled nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>B subunit</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>EGF</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>EGF + B subunit</td>
<td>10 ± 3</td>
</tr>
</tbody>
</table>

Secondary astroglial cell cultures at D1 (1.2 x 10^5 cells seeded/16 mm well) were treated for 24 h with 1 μg/ml B subunit, 10 ng/ml EGF (plus 5 μg/ml insulin), or both. Cultures were pulsed with 2 μCi/ml [3H]TdR for the entire 24-h period, and then processed for autoradiography as described elsewhere (1). Each value is taken from analysis of three cultures (300–400 cells scored in each condition), and is the mean ± SD.

Discussion

The results presented here demonstrate that the B subunit of cholera toxin can induce a pronounced morphological change and inhibit DNA synthesis in rat cerebral astroglial cells in vitro. This latter response to the B subunit was also seen when astroglial cells not rapidly dividing were stimulated with defined mitogens, such as EGF or bFGF. The only identified receptor for the B subunit is the ganglioside GM1 (7, 15, 42). Studies from other laboratories have shown that the B subunit is able to stimulate lymphocytes by directly binding to GM1 on the cell surface (42). The molecules of B subunit bind only to the oligosaccharide chains of GM1 exposed on the cell surface (15, 17, 22). In this study, the observed effects of B subunit also appear to be due to the binding of B subunit to the cells, as the ability of cholera toxin to stimulate cAMP levels was completely inhibited by the addition of B subunit. The B subunit, however, was equally effective in promoting the morphological conversion of the astroglial cells.

Astroglial cells cultured in the absence of serum display a flat, polygonal morphology (33). Administration of cAMP analogues or cholera toxin (among other agents) causes the cells to convert to a stellate (star shaped) morphology (12, 29, 30, 37, 48), suggesting a possible mediation through intracellular cAMP (12, 48). Treatment of these cells with the B subunit of cholera toxin elicited a morphological change that was indistinguishable from that observed with activation of cAMP-synthesizing systems (12, 37). The B subunit used here is devoid of any cAMP-elevating A subunit, in accord with other studies (41, 42). These results raise the possibility that the effects of cholera toxin on astroglial cell morphology may be due, at least in part, to the binding of its B subunit...
to cell surface GM1 and not only to its ability to activate ade-
nylate cyclase. Since the maintenance of the stellate mor-
phology in astroglial cells is critically dependent on a stable
microtubular network (5), the action of B subunit implies
that cross-linking of surface GM1 molecules is translated to
events at the level of the cytoskeleton.

In this study, we also found that the B subunit can inhibit
DNA synthesis in cultured astroglial cells that are not rapidly
dividing. Maximal inhibition of DNA synthesis was observed
24 h after B subunit introduction, reaching $\sim 20\%$ of control
levels. Furthermore, the stimulation of DNA synthesis and
cell number increases induced by EGF or bFGF was in-
hibited to an equal extent, and was found whether the mito-
gen was presented concurrently or 24 h before B subunit.
The B subunit–evoked inhibition of DNA synthesis at 24 h
was reflected in a decreased percentage of labeled nuclei at
24 h, and a smaller increase in actual cell numbers in the
presence of mitogen at 48 h. These anti–proliferative effects
of the B subunit on astroglial cells, as with the morphological
effects, are unrelated to increases of cAMP.

In several other studies, the B subunit of cholera toxin has
been reported to stimulate DNA synthesis in resting lympho-
cytes (42) and quiescent 3T3 cells (41), but to inhibit the
growth of rapidly dividing normal 3T3 cells and transformed
3T3 cells (41). From these observations, the above authors
concluded that the ability of the B subunit to stimulate the
division of resting cells is a general phenomenon, with en-
dogenous gangliosides playing a role in the regulation of both
positive and negative signals for cell growth. In contrast the
present studies demonstrate that, at least for astroglial cells
and independent of growth state, B subunit inhibits DNA
synthesis. While most experiments were performed by seed-
ing the astroglial cells directly in a serum-free medium,
other experiments were done in which cells were seeded at
low density in 24-well culture plates in serum-containing
medium to allow continued growth, and B subunit subse-
quently added in the absence of serum after different times.
In this latter case inhibition was also observed, irrespective
of whether the cells were still actively dividing or had ceased
to show further increases in number (Skaper, S. D., L. Facci,
and M. Favaron, unpublished observations). The suggestion
that the opposing responses of 3T3 fibroblasts to B subunit
may be related to the amount or distribution of surface GM1
being occupied (41) is based upon the observations that trans-
formed 3T3 cells have less GM1 than normal 3T3 cells (16,
20), and that the levels of cell surface gangliosides increase
as normal 3T3 cells reach confluency (20). The failure of as-
troglial cells to display similar opposing growth responses to
the B subunit may result for a variety of reasons. For exam-
ple, astroglial cells may possess differences in their mem-
brane ganglioside composition in comparison with normal
3T3 cells. Treatment of the former cells with neuraminidase
to convert polysialogangliosides to GM1 produces a several-
fold increase in membrane GM1 content, as measured by
binding of a B subunit–horseradish peroxidase complex
using ELISA (Favaron, M., S. D. Skaper, and L. Facci,
unpublished observations). The inhibitory response of DNA
synthesis to B subunit, however, remains after neuroami-
dase treatment. Differences between astroglia and 3T3 cells
may also result from variations in the metabolic state of the
two cell types when used or different mechanisms whereby
B subunit acts.

The mechanism(s) by which the B subunit is able to regulate
the morphology and growth of astroglial cells is unknown.
Interaction of membrane proteins with specific ligands can
alter biochemical events in some cells. For example, B lym-
phocytes are activated by antibodies that cross-link mem-
brane immunoglobulins. This cross-linking leads to enhanced
inositol phospholipid metabolism and rapid increases in di-
acylglycerol (3), elevation of intracellular free calcium con-
centration, and phosphorylation of proteins associated with
the plasma membrane and cytoskeleton (21). The growth-
stimulatory action of the B subunit for resting 3T3 cells is
reported to be associated with a rise of intracellular calcium
that depends upon external calcium and increased membrane
potential, but no enhancement of phosphoinositide turnover
(40). It is also possible that gangliosides may modulate pro-
duction phosphorylation (4, 18, 24, 47), which in turn can serve
as a signal to modulate both the cytoskeleton and cell growth.
These parameters remain to be examined in the present sys-
tem. Since, however, the only known action of the B subunit
is to bind to GM1 ganglioside on the cell surface, the ex-
pected cross-linking and subsequent aggregation of GM1 by
the multivalent B subunit appears to be relevant to elicit these
cellular behaviors.

It is tempting to speculate that the same series of events
are involved in the B subunit–induced change in astroglial
cell morphology and inhibition of proliferation. Preliminary
observations suggest that both the morphological and bio-
chemical responses to the B subunit are linked to cytoskeletal
stability at the level of the microtubules (Skaper, S. D.,
M. Favaron, and L. Facci, unpublished observations). An
ability of endogenous gangliosides to modulate cytoskeletal
organization would need to be reconciled with the fact that
these lipid components do not span the cell membrane. One
explanation for a cytoskeletal connection of gangliosides
might be found in the interaction of gangliosides with one or
more integral membrane proteins, which in turn interact
with cytoskeletal proteins directly, i.e., a bridging membrane
protein that binds in a cis fashion to GM1 at the outer cell
surface and in a trans fashion to a cytoskeletal-organizing
protein at the inner plasma membrane. Recent observations
concerning cooperativity of GM1-dependent with protein-
dependent adhesion and neuritogenesis of neuroblastoma
cells lend support to this idea (32). In addition, there are sev-
eral reports of possible association of gangliosides with
transmembrane proteins and cytoskeletal elements, based on
their resistance to mild detergent extraction (19, 35, 43) and
on capping in lymphocytes (22).
surface GMI with a specific ligand one may tip the balance in one direction, and by adding exogenous gangliosides one may tip the balance in the opposite direction. The actual situation may not be so straightforward, however, as the added ganglioside could also undergo membrane incorporation, perhaps working against the effects of free ganglioside. This astroglial culture system may still provide a useful model for investigating a role for gangliosides in gliosis; such studies are presently in progress.

Taken together, these and past (12, 23, 36, 37) observations demonstrate that both endogenously occurring plasma membrane gangliosides and exogenously supplied gangliosides can play a role in the morphological and biochemical differentiation of astroglial cells in vitro. Thus, these astroglial cells may provide a model system in which one can study a role for gangliosides as membrane transducers of signals for cytoskeletal-linked events. The differences in membrane concentration and distribution of gangliosides may influence membrane fluidity, with such changes in mobility being linked to cytoskeletal proteins either physically or chemically.

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References


