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GANGLIOSIDE COMPOSITION OF SUBSTRATE-ADHESION SITES OF NORMAL AND VIRALLY-TRANSFORMED BALB/C 3T3 CELLS

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ABSTRACT. The ganglioside composition of the so-called substrate-attached material (SAM), which remains tightly bound to the tissue culture dish after cells are detached by chelating agents, was compared with the ganglioside composition of released cell bodies in the cultures of normal and various virally-transformed Balb/c 3T3 cells. Regardless of whether the cells were untransformed or transformed, the SAM of their cultures shows a ganglioside structure characterized by a prevalence of the higher homologs, mainly GD1a, over the simpler gangliosides, even when the level of higher homologs was reduced in the cell bodies of transformed cells. This result cannot be ascribed to the presence of plasmamembranes in the SAM as shown by ganglioside analysis of the plasmamembranes of some of the cells under study. Only in a highly metastatic transformed cell line did the SAM contain the same low GD1a level as found in the cell bodies.

Cell adhesion to an extracellular matrix is a biological property involved in various physiological and pathological conditions (1). Although cell adhesion implies specific interactions between molecules located at the cell periphery, our understanding of the mechanism of the adhesion process is still incomplete. However, knowledge of the molecular basis of cell adhesion to an extracellular matrix has been greatly advanced by the identification of the so-called substrate-attached material (SAM), which remains tightly bound to the tissue culture substrate when cells are detached by the use of the Ca^{2+}-specific chelator, ethylenebis(oxyethylenenitrilo) tetraacetic acid (EGTA) (2). Rosen and Culp (3) have demonstrated that the SAM consists, at least in part, of specialized adhesion sites on the cell periphery through which cells are anchored to the substrate. Several cell surface components, such as phospholipids (4), cytoskeletal proteins (5), fibronectin (5,6) and glycosaminoglycans

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(7) were found to contribute to the molecular make-up of the SAM. The gangliosides of the SAM, however, have not yet been systematically analyzed. Only recently has a study of the glycosphingolipids of the SAM of BHK cells been reported (8).

In the present investigation,we compared the ganglioside structure of the SAM with that of the EGTA-released cells in cultures of normal Balb/c 3T3 cells (3T3 cells), Rous sarcoma virus (B77 strain)-transformed 3T3 cells (B77-3T3 cells) and SV40-transformed 3T3 cells (SV3T3 cells).

This in vitro cell system was used to determine whether the gangliosides of the SAM show structural characteristics different from those of the cell bodies and, if so, whether such characteristics undergo modifications in transformed cells which might be related to their reduced adhesiveness (9). Concanavalin A-selected SV3T3 revertant cells (SV3T3 rev cells), which have a normal phenotype (10), were also studied to see whether any transformation-associated changes of the SAM gangliosides revert to a normal pattern.Moreover, in view of the involvement of cell adhesion in the metastatic process (11), the gangliosides of the SAM and EGTA-released cells of a highly metastatic variant of B77-3T3 cells selected by growth in hard agar (AA6 cells) (12) were also examined.

MATERIALS AND METHODS

Cells: The Balb/c 3T3 (clone A31), the SV3T3 (clone SVT2) and the SV3T3 rev cells were kindly donated by Dr.P.H.Black (Boston University School of Medicine, Dept. of Microbiology); the B77-3T3 and the AA6 cells were supplied by Dr.P.M.Comoglio (Dept. of Anatomy, University of Turin, Italy). All the cell lines were cultivated in our laboratory under the conditions described in a previous paper (13).

Labeling of gangliosides and fractionation of cell cultures: Cells were seeded in 150 mm glass petri dishes at $3x10^6$ cells per dish and grown for 24 hours in minimal essential medium (containing a fourfold concentration of essential amino acids and vitamins) supplemented with 10% foetal calf serum. Cell cultures were then changed to a fresh medium containing 1.0 μCi/ml of [^{3}H]galactose (specific activity 10 Ci/mmol) and incubated for an additional 48-hour period, when cells became 80-90% confluent. At this time, medium was decanted and the cell layer was washed three times with phosphate-buffered saline (PBS). 10 ml of an EGTA solution (0.5 mM in PBS containing 0.1 mM phenylmethylsulfonyl fluoride) were added and the

culture was incubated at 37°C on a rotary shaker for 30 min to release cells from the dish. The suspension of released cells (cell bodies) was gently pipetted on the surface of the dish to obtain the detachment of all the cells.

Lipid extraction and ganglioside analysis: The released cells were washed twice and resuspended at $20X10^6$/ml in PBS, sonicated and extracted according to the method of Folch et al. (14). Total lipids of the SAM were extracted by soaking the dish in 20 ml of a chloroform:methanol 2:1 mixture followed by two rinses with 5 ml of the same mixture. Gangliosides were isolated from the other lipid fractions by chromatography of the lipid extracts on Sephadex columns following the procedure of Siakotos and Rouser (15). The total ganglioside fraction was chromatographed on HPTLC plates (Merck) with chloroform:methanol:0.25% $CaCl_2$ (55:40:9). The labeled gangliosides were revealed by fluorography and quantitatively transferred into scintillation vials for counting.

Plasmamembrane preparation: Plasmamembranes were prepared from EGTA-detached cells following the procedure of Brunette and Till (16). The sonicated plasmamembranes were extracted according to Folch et al. (14) and gangliosides were analyzed as described above.

RESULTS

Significant amounts of gangliosides, i.e., 7.1% of the whole cell culture gangliosides, were found in the SAM of Balb/c 3T3 cells. Compared to the untransformed 3T3 cells, all the transformed cell lines showed lower percentages of the whole cell culture gangliosides in their SAM: 1.4%, 3.8%, 1.6%, and 3.6% in B77-3T3, SV3T3, SV3T3 rev and AA6 cells, respectively.

As shown in Table 1, the ganglioside structure of the SAM of 3T3 cell cultures was characterized by a prevalence of the higher gangliosides, mainly GD1a, over the simpler homologs, a pattern identical to that found in the released cell bodies. The profile of the SAM gangliosides of B77-3T3 cultures resembled that found in 3T3 cells while their cell bodies showed a reduction of GD1a accompanied by an increase of GM2, a typical transformation-associated ganglioside alteration (17). A similar observation holds also for the SV3T3 cells where a reduction of GD1a was accompanied by an increase of GM3 in their cell bodies, while in the SAM there was a higher level of GD1a and a smaller increase of GM3. This tendency to maintain a ganglioside structure similar to that of normal 3T3 cells was still more marked in the SAM of SV3T3 rev cell cultures whose cell bodies showed the same ganglioside behaviour as found in SV3T3 cells.

Table 1 Ganglioside composition of EGTA-released cells (Cells) and substrate-attached material (SAM) in cultures of the 3T3, B77-3T3, SV3T3, SV3T3 rev and AA6 cells [a]

	3T3		B77-3T3		SV3T3		SV3T3 rev		AA6	
Ganglioside[b]	Cells	SAM	Cells	SAM	Cells	SAM	Cells	SAM	Cells	SAM
GM3	7.8	10.4	7.8	8.1	53.8	38.5	67.3	23.4	4.1	4.3
GM2	27.2	21.7	50.7	27.5	13.0	13.5	6.2	19.1	80.5	76.2
GM1	15.0	17.0	18.1	13.1	12.3	12.3	9.6	17.9	7.6	9.5
GD1a	50.9	51.1	23.7	51.2	21.8	34.2	17.0	39.6	7.8	10.0

[a] Values are percentage of total ganglioside-associated radioactivity. "Total" refers to the sum of the radioactivities of individual gangliosides fractionated by TLC.

[b] Gangliosides are indicated according to the nomenclature of Svennerholm (22). GM3: (sialyl) galactosyl-glucosyl-ceramide; GM2: N-acetylgalactosaminyl-(sialyl)galactosyl-glucosyl-ceramide; GM1: galactosyl-N-acetylgalactosaminyl-(sialyl)galactosyl-glucosyl-ceramide; GD1a: (sialyl)galactosyl-N-acetylgalactosaminyl-(sialyl)galactosyl-glucosyl-ceramide.

Thus, whereas the cell bodies exhibit the loss of the higher ganglioside homologs typical of transformation (17), the SAM of transformed cells tends to maintain the characteristic ganglioside structure observed in 3T3 cell cultures. In contrast, in the highly metastatic AA6 cell line there was a marked decrease of GD1a both in the cell bodies and in the SAM.

The question arises whether plasmamembrane gangliosides are responsible for the characteristic ganglioside structure of the SAM, especially in view of the morphological observation by Rosen and Culp (3) suggesting a contribution of plasmamembranes to the SAM. Therefore, we compared the ganglioside composition of the isolated plasmamembranes with that of the cell bodies and the SAM in the 3T3, SV3T3 and SV3T3 rev cell system. As seen in Table 2, the ganglioside profile of the plasmamembranes of the 3T3 cells was similar to that of their cell bodies, both showing a rather low GD1a content; yet the GD1a content of the SAM of these cultures was distinctly higher. The same trends were also observed for the SV3T3 and SV3T3 rev cells. These data bring to mind the Cathcart and Culp's observation (4) that the phospholipid composition was remarkably similar

Table 2. Ganglioside composition of EGTA-released cells (Cells), plasmamembranes (PM) and substrate-attached material (SAM) from cultures of the 3T3, SV3T3 and SV3T3 rev cells [a]

Ganglioside[b]	3T3			SV3T3			SV3T3 rev		
	Cells	PM	SAM	Cells	PM	SAM	cells	PM	SAM
GM3	28.1	25.4	17.9	49.9	60.6	25.1	85.7	89.7	74.2
GM2	36.0	35.0	21.3	15.5	12.2	9.5	3.5	2.6	3.9
GM1	21.9	31.5	20.8	11.5	9.0	14.3	7.1	5.5	5.7
GD1a	13.2	8.4	40.0	24.4	18.1	51.2	3.6	2.2	16.2

[a,b] As in Table 1.

in the cell bodies and in the plasmamembranes, while it showed a distinctly different pattern in the SAM of 3T3, SV3T3 and SV3T3 rev cells.

DISCUSSION

Although gangliosides may influence cell adhesion due to their amphipathic characteristics which favour multiple interactions with the pericellular environment, the study of the role of gangliosides in the cell adhesion is of recent date (18-20). Moreover, it is still uncertain whether the reduced adhesiveness of transformed cells may be related to the alteration of their ganglioside structure.

In this investigation, we compared the ganglioside structure of the SAM with that of the released cell bodies in an untransformed fibroblastic murine cell line, the Balb/c 3T3 cells, as well as in various transformants of these cells which differed with respect to their growth properties, their metastatic potential, and the oncogenic virus. Our major observation was that the SAM gangliosides of 3T3 cells showed the same structural characteristics as found in the cell bodies; these characteristics tend to be preserved in the SAM of B77-3T3 and SV3T3 cells, while their cell bodies showed the typical transformation-associated ganglioside changes. The tendency to reproduce the structural characteristics of 3T3 SAM gangliosides observed in

SV3T3 cell cultures was even more evident in the SV3T3 rev cells. It is worth noting that also in the normal cells used in the plasma-membrane isolation experiment there was a rather high level of GD1a in the SAM, while their cell bodies showed a low level of GD1A. All in all, these observations suggest that the gangliosides of the SAM represent a portion of the whole cell culture gangliosides distinct from those of the other cell fractions and relatively insensitive to the alterations of ganglioside synthesis which may occur in the rest of the cell. The apparent independence of the SAM's ganglioside structure from that of the cell bodies is reminiscent of the characteristic enrichment of certain glycosaminoglycans (7) and phospholipids (4) in the SAM of the 3T3, SV3T3 and SV3T3 rev cell system.

The finding that a cell surface portion which mediates anchorage of cells to substrates tends to be enriched by specific gangliosides, mainly GD1a, regardless of the levels of these gangliosides in cell bodies points to a possible role of SAM gangliosides in cell adhesion. In this regard, it is significant that cells adapted to growth in suspension cultures drastically reduce their GD1a content (21). Moreover, gangliosides were found to interact with fibronectin, a major adhesive macromolecule, in a way that is related to the complexity of their structure (18-20).

It should be noted that the reduced adhesiveness of the transformed cells (9) is accompanied by a decrease in the amount of gangliosides deposited on the substrate rather than by an alteration of the SAM ganglioside pattern. Only when metastatic behaviour appears in transformed cells, do the SAM gangliosides lose their structural characteristics, as is the case for the AA6 cells.

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