Glycosphingolipids of Clonal Lines of Mouse Neuroblastoma and Neuroblastoma X L Cell Hybrids*

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SUMMARY

Glycosphingolipid analyses were performed on three clonal lines of the mouse Cl300 neuroblastoma (NZA, NA, and NB41A) and on three neuroblastoma X L cell hybrid lines (NLI-15A, NLI-7A, and NLI-11A) differing in their membrane electrical activities. Striking variations of ganglioside but not of neutral glycosphingolipid patterns were observed in the neuroblastoma lines. No differences were observed between the glycosphingolipid patterns of neuroblastoma cells (N2A) grown in suspension as “undifferentiated” neuroblasts or in monolayer as “differentiated” neurons. The neuroblastoma X L cell hybrid lines exhibited neutral glycosphingolipid patterns similar to the sum of the parental neuroblastoma and L cell patterns, whereas their ganglioside patterns differed from those of the two parents. Despite their widely differing membrane electrical activities, the glycosphingolipid profiles of these three hybrid lines were generally similar.

The physiological functions of GSLs in neural cells are difficult to study because of the variety of different cell types present in the central nervous system, and because it is difficult to alter experimentally the GSL content in attempts to correlate glycolipid composition with cellular function. For these reasons we wished to study variations in the GSL profiles of cloned neuronal cell lines during differentiation in culture. In this regard, the neuroblastoma lines derived from the mouse Cl300 tumor are of particular interest because these cells retain many of the characteristics of bona fide neurons, including the capacity to generate neurites, to synthesize known neurotransmitters, and to undergo at least some of the early steps in the formation of neuromuscular junctions (6-9). In addition, these cells offer the potential of studying neuronal function at the genetic level by the isolation of appropriate mutants, and by cellular hybridization using the techniques of somatic cell genetics (9).

In the present investigation we have examined the GSL profile of several different clonal isolates of the Cl300 neuroblastoma and of three neuroblastoma X L cell hybrids differing in their membrane electrogenic response. Basically, three questions were asked. (a) Do different clonal isolates of neuroblastoma cells, like other cultured mammalian cells previously studied (10-12), show marked differences of GSL profile? (b) Does a change of GSL pattern occur during the transition in culture of “undifferentiated” neuroblasts to “differentiated” neurons? (c) Do neuroblastoma X L cell hybrids of differing membrane electrogenic response display significant differences in ganglioside pattern, perhaps permitting a correlation to be made between complexity of ganglioside pattern and this response? Studies on the GSL composition of two of the neuroblastoma lines studied here (NB41A and N2A) have recently been reported by Dawson et al. (13, 14).

MATERIALS AND METHODS

Cell Lines and Their Properties—Table I lists some of the relevant properties of the cell lines used in these studies. N2A

The abbreviations used are: GSL, glycosphingolipid; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; TK, thymidine kinase; GM1, GM2, etc., ganglioside notation according to the system of Svennerholm (5).
N2A to check the GSL variation within one cell line. These had different GSL patterns, we selected several subclones in contrast to the monolayer-grown cells which are referred to as microscopy is shown in Fig. 1.

The N2A line is able to grow in suspension culture as well as from the Cl300 tumor (Jackson Laboratories, Bar Harbor, Me.) by Klebe and Ruddle (15) and Augusti-Tocco and Sato (6). NA is an 8-azaguanine-resistant hypoxyaniline-guanine phosphoribosyltransferase (EC 2.4.2.8)-deficient (HGPTmut) mutant derived from N2A. These lines have been grown in cell culture for periods of 2 to 4 years. LM(TK-) is a 5-bromodeoxyuridine-resistant thymidine kinase (EC 2.7.1.21)-deficient (TK-) L cell (16). The NLI lines are neuroblastoma X L cell hybrids derived from Sendai virus-mediated fusion between NA and LM(TK-) cultures. These are independent clones isolated on the basis of their ability to grow in the HAT selection medium (17) and have been characterized as hybrids by the detection of both parental and hybrid bands of phosphohexose isomerase by Klebe and Ruddle (15) and Augusti-Tocco and Sato (6). The patterns of gangliosides isolated and analyzed according to the general method described previously (11, 21). The patterns of gangliosides and neutral GSLs of at least three separate cultures of each cell type studied were examined qualitatively by thin layer chromatographic analyses. Quantitative analyses of gangliosides and neutral GSLs were performed on two of these cultures.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Chromosome number</th>
<th>Acetylcholine esterase</th>
<th>14-3-3 antigen</th>
<th>Membrane electrogentic response</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2A</td>
<td>84 ± 2</td>
<td>n.d.</td>
<td>n.d.</td>
<td>++</td>
<td>Klebe and Ruddle (15)</td>
</tr>
<tr>
<td>NA</td>
<td>92 ± 3</td>
<td>107</td>
<td>0.84</td>
<td>++</td>
<td>Klebe²</td>
</tr>
<tr>
<td>LM(TK-)</td>
<td>47 ± 3</td>
<td>0</td>
<td>&lt;0.10</td>
<td>-</td>
<td>Kit et al. (16)</td>
</tr>
<tr>
<td>NLI-7A</td>
<td>151 ± 10</td>
<td>90</td>
<td>0.19</td>
<td>++</td>
<td>McMorris³</td>
</tr>
<tr>
<td>NLI-11A</td>
<td>102 ± 14</td>
<td>50</td>
<td>&lt;0.10</td>
<td>+</td>
<td>McMorris³</td>
</tr>
<tr>
<td>NLI-15A</td>
<td>152 ± 13</td>
<td>30</td>
<td>0.12</td>
<td>++</td>
<td>McMorris³</td>
</tr>
</tbody>
</table>

*Mean chromosome numbers and standard deviations are quoted.

Activity is expressed in nanomoles of acetyl released per min per mg of protein at 37°C.³

Neuron-specific protein, micrograms of protein per mg of soluble protein.⁴

Membrane electrogentic response expressed on a graded scale of − to ++, where − indicates only passive changes in the transmembrane potential following current injection, and ++ indicates repetitive action potential generation.⁵

*Not determined.

and N841A are independent cloned cell lines isolated directly from the C1300 tumor (Jackson Laboratories, Bar Harbor, Me.) by Klebe and Ruddle (15) and Augusti-Tocco and Sato (6). NA is an 8-azaguanine-resistant hypoxyaniline-guanine phosphoribosyltransferase (EC 2.4.2.8)-deficient (HGPTmut) mutant derived from N2A. These lines have been grown in cell culture for periods of 2 to 4 years. LM(TK-) is a 5-bromodeoxyuridine-resistant thymidine kinase (EC 2.7.1.21)-deficient (TK-) L cell (16). The NLI lines are neuroblastoma X L cell hybrids derived from Sendai virus-mediated fusion between NA and LM(TK-) cultures. These are independent clones isolated on the basis of their ability to grow in the HAT selection medium (17) and have been characterized as hybrids by the detection of both parental and hybrid bands of phosphohexose isomerase by starch gel electrophoresis, and by the presence of marker chromosomes from both parents. A more detailed description of the production and characterization of the NLI hybrids will be presented elsewhere.³

Isolation and Characterization of Glycosphingolipids—The frozen cell pellets were extracted and the neutral GSLs and gangliosides isolated and analyzed according to the general methodology described previously (11, 21). The isolations of gangliosides and neutral GSLs of at least three separate cultures of each cell type studied were examined qualitatively by thin layer chromatographic analyses. Quantitative analyses of gangliosides and neutral GSLs were performed on two of these cultures.

Radioautography—Radioactive preparations of cell gangliosides and neutral GSLs (containing approximately 2 × 10⁴ dpm) were separated as described below. The thin layer plates were covered with Kodak No-Screen x-ray film, kept in the dark for approximately 1 week and the films were then developed. The plates were sprayed with appropriate reagents to detect gangliosides and neutral GSLs and the positions of the radioactive GSLs were compared with standard GSLs.

Thin Layer and Gas-Liquid Chromatographic Analyses of Glycosphingolipids—The gangliosides were separated and detected by thin layer chromatography on glass plates coated with Silica Gel G in a solvent system of chloroform-methanol-ammonia-water (60:35:1.7, v/v/v) as described by Wherrett and Cumings (22).

The neutral GSL fraction was separated by thin layer chromatography on glass plates coated with Silica Gel G in a solvent system of chloroform-methanol-water (65:25:4, v/v/v) and detected by spraying with aniline-diphenylamine reagent (23).
For quantitative analyses and determination of the carbohydrate composition of the GSLs by gas-liquid chromatography, the gangliosides and neutral GSLs were separated as described above and detected by their opaque appearance after spraying with methanol-water (1:2, v/v). Appropriate areas of the chromatograms were scraped into screw-capped tubes, a measured aliquot of mannitol added as an internal standard, and the contents subjected to methanolysis (methanolic hydrochloride

Fig. 1. Morphology of the various cell lines used in these studies. A, neuroblastoma clones N2A, NA, and NB41A. The bar represents 100 μm. B, Neuroblastoma X L cell hybrids and their parents. The hybrids NLI-18A, NLI-7A, and NLI-11A are arranged in order of decreasing membrane electrical activity. The NA 8-azaguanine-resistant neuroblastoma and the LM(TK−) bromodeoxyuridine-resistant L cell parents are shown below the hybrids. The bar represents 100 μm.
nitol standard and the concentration of individual neutral GSLs.

The amount of glucose present in the various GSLs was determined by comparison with the internal mannitol standard and expressed relative to cell protein (nanomoles of GSL per mg of total cell protein). The amounts of the other carbohydrates present in the various GSLs were also determined, yielding the stoichiometry of each sugar relative to glucose.

**Standards of GSLs**—Gangliosides and neutral GSL standards were prepared from human brain and kidney obtained at autopsy. Standards of monogalactosyl diglyceride and digalactosyl diglyceride were purchased from Applied Sciences Laboratories, Inc. (State College, Pa).

**RESULTS**

Ganglioside Content of Various Cells Studied—Qualitative and quantitative data on the gangliosides in the various cell lines studied are shown in Fig. 2 and Table II, respectively. Relatively complex patterns of gangliosides were found in all the neuroblastoma and NLI hybrid cells excepting NB41A (Fig. 2A, Channel 1). For example, N2A cells grown in suspension as small round cells lacking neurites ("undifferentiated") (Fig. 2A, Channel 1) exhibited ganglioside bands corresponding in position in subclonal lines over this time span. Contrasting with this latter finding was the marked difference in ganglioside patterns between N2A (Fig. 2A, Channels 1 to 4) and NB41A (Fig. 2B, Channel 1), two neuroblastoma lines isolated independently from the C1300 tumor. Similarly, the ganglioside pattern of NA cells (the HGPRT-deficient mutant of N2A) differed markedly from that of the parent N2A cells (comparison of Fig. 2C, Channel 1 with Fig. 2A, Channels 1 to 4).

The quantitative analyses shown in Table II confirmed the differences in ganglioside pattern just described. GM1 was found to be the principal ganglioside of N2A cells (comprising approximately 75% of the total ganglioside on a molar basis), whereas it was almost absent in the NB41A and NA cells. On the other hand, GM3 was a relatively minor component in the NA cells (approximately 10% of the total), whereas it was almost absent in the NB41A and NA cells. These results indicate that no change in ganglioside profile accompanied the process of neurite extension and also provided a measure of the constancy of ganglioside composition in subclonal lines over this time span. Contrasting with this latter finding was the marked difference in ganglioside patterns between N2A (Fig. 2A, Channels 1 to 4) and NB41A (Fig. 2B, Channel 1), two neuroblastoma lines isolated independently from the C1300 tumor.

The hybrids differed from each other in their membrane electrophoretic response (see Table I) and were selected for study in part to examine a possible role of gangliosides in membrane excitability. As shown in Fig. 2C (Channels 2 to 4) and Table II, the three different NLI hybrid cell lines showed relatively minor differences in ganglioside profile. NLI-15A contained con-
**Table II**

**Glycosphingolipid content of various cells**

The results presented are the averages of duplicate analyses on two cultures of each cell type. The amounts of individual GSLs in the cells studied were determined from quantitative gas-liquid chromatographic analyses of their sugar content, using mannitol as an internal standard. The values were calculated from their glucose content; all of the neutral GSLs and gangliosides contained 1 mole of glucose per mole (see Table III).

<table>
<thead>
<tr>
<th>GSL</th>
<th>N2A</th>
<th>NB41A</th>
<th>NA</th>
<th>L Cells Lm(TK-)</th>
<th>NLI hybrids</th>
<th>Neuroblastoma Lines Cl300</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gangliosides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4.0, 4.2</td>
<td>1.9, 2.3</td>
<td>1.2, 1.4</td>
<td>0.4, 0.6</td>
<td>2.4, 2.9</td>
<td>1.9, 2.1</td>
</tr>
<tr>
<td>GM2</td>
<td>2.8, 3.2</td>
<td>0.1, 0.1</td>
<td>0.1, 0.1</td>
<td>n.d.</td>
<td>0.6, 0.9</td>
<td>0.2, 0.2</td>
</tr>
<tr>
<td>GM1</td>
<td>0.4, 0.4</td>
<td>1.7, 1.9</td>
<td>0.3, 0.3</td>
<td>n.d.</td>
<td>1.4, 1.4</td>
<td>1.3, 1.5</td>
</tr>
<tr>
<td>GM0</td>
<td>0.3, 0.3</td>
<td>0.1, 0.3</td>
<td>0.3, 0.5</td>
<td>n.d.</td>
<td>0.3, 0.5</td>
<td>0.3, 0.3</td>
</tr>
<tr>
<td>GD1a</td>
<td>0.5, 0.3</td>
<td>e</td>
<td>0.4, 0.4</td>
<td>n.d.</td>
<td>0.1, 0.1</td>
<td>0.1, 0.1</td>
</tr>
<tr>
<td>GPd</td>
<td>e</td>
<td>e</td>
<td>0.1, 0.1</td>
<td>n.d.</td>
<td>e</td>
<td>e</td>
</tr>
<tr>
<td><strong>Neutral GSLs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.0, 6.1</td>
<td>6.9, 6.9</td>
<td>7.0, 7.1</td>
<td>9.7, 9.6</td>
<td>15.2, 15.8</td>
<td>5.9, 5.5</td>
</tr>
<tr>
<td>Monoglycosphingolipid</td>
<td>6.0, 6.9</td>
<td>0.6, 0.9</td>
<td>1.6, 1.3</td>
<td>2.2, 2.2</td>
<td>2.3, 2.7</td>
<td>2.6, 2.9</td>
</tr>
<tr>
<td>Diglycosphingolipid</td>
<td>0.9, 0.9</td>
<td>0.6, 0.6</td>
<td>0.8, 0.8</td>
<td>6.7, 6.7</td>
<td>2.6, 2.1</td>
<td>1.0, 1.1</td>
</tr>
<tr>
<td>Triglycosphingolipid</td>
<td>0.1, 0.1</td>
<td>0.7, 0.7</td>
<td>0.2, 0.2</td>
<td>5.8, 5.0</td>
<td>2.0, 2.4</td>
<td>0.5, 0.7</td>
</tr>
<tr>
<td>Tetroglycosphingolipid</td>
<td>e</td>
<td>e</td>
<td>e</td>
<td>e</td>
<td>0.3, 0.3</td>
<td>e</td>
</tr>
</tbody>
</table>

* Not determined.
* The pattern of L cell gangliosides was similar to that reported previously (21), consisting of approximately equimolar amounts of GM1, GM2, and GM3.
* Less than 0.05 nmoles of GSL per mg of protein.

Siderably more GM3 than the other two hybrids, but otherwise the patterns were similar. The hybrid cells did, however, differ in their ganglioside patterns from those of both parents. For example, they contained more GM3 than the parent NA cells and lesser amounts of the more complex gangliosides. The ganglioside pattern of the parent L cells is not shown in Fig. 2C. It resembles each other much more than did the ganglioside patterns. The three subclones of N2A exhibited essentially identical neutral GSL patterns, consisting of double zones with the chromatographic migration of standards of mono- and di-glycosphingolipids. The "undifferentiated" N2A cells grown in suspension also contained these components (Fig. 3A, Channel 1). The glycolipid pattern of the batch of N2A cells shown in this figure exhibited bands corresponding in chromatographic migration to standards of mono- and digalactosyl diglyceride. This particular batch of cells was found to be contaminated with mycoplasma. Glucosyl- and galactosyl-containing mono- and digalactosyl diglycerides are known to be the principal glycolipids of certain mycoplasma (26). Other batches of N2A cells grown in suspension and shown to be free of mycoplasma did not exhibit these components and had a pattern essentially identical with that of the N2A subclones shown in Fig. 3A (Channels 2 to 4).

The neuroblastoma line NB41A (Fig. 3B, Channel 1) exhibited mono- and di-glycosphingolipids but also contained triglycosphingolipids, which was not an appreciable component of the other neuroblastoma lines.

The analyses of the neutral GSLs of the three hybrid cell lines were of interest. The parent NA cell (chemically detected GSL pattern not shown here, but the pattern as studied by radioautography is shown in Fig. 4, Channel 3) revealed a pattern similar to that of the N2A subclones shown in Fig. 3A (Channels 2 to 4), containing mono- and di-GSLs. The parent L-cell (Fig. 3B, Channel 2) contained mono- and di-GSLs and triglycosphingolipids but no tetroglycosphingolipids. The three hybrid cells (Fig. 3B, Channels 3 to 5) were found to contain all four neutral GSLs, including both tri- and tetroglycosphingolipids. In addition, line NLI-11A (Fig. 3B, Channel 4) contained a neutral GSL with similar chromatographic properties to asialo-GM3.
FIG. 3. Thin layer chromatograms of the neutral glycolipids of the various cells studied. A: neutral glycolipids of: 1, N2A cells in suspension culture ("undifferentiated"), mycoplasma contaminated; 2, N2A-A5 subclone in monolayer culture ("differentiated"); 3, N2A-A4 subclone in monolayer culture ("differentiated"); 4, N2A-D4 subclone in monolayer culture ("differentiated"); 5, a mixture of monoglycosylceramide and sulfatide (migrating just behind triglycosylceramide) from human brain and neutral GSL standards (principally di-, tri-, and tetraglycosylceramides) from human kidney; 6, a mixture of various glycolipid standards including mono- and digalactosyl diglyceride, asialo-GM₂, and asialo-GM₁.  B: neutral glycolipids of: 1, NB41A cells; 2, LM(TK−) parent L cells; 3, NLI-11A hybrid cells; 4, NLI-17A hybrid cells; 5, NLI-15A hybrid cells. C: neutral glycolipids of: 1, a mixture of monoglycosylceramide from human brain and the neutral GSLs of human kidney; 2, C1300 neuroblastoma solid tumor. Aliquots of neutral glycolipid fractions prepared by silicic acid column chromatography corresponding in amount to approximately 500 μg w/w were separated by thin layer chromatography on glass plates coated with Silica Gel G using chloroform-methanol-water (65:25:4, v/v) as the developing solvent. The glycolipids were detected as blue zones by spraying with aniline-diphenylamine reagent.

The neutral GSL content of the C1300 tumor was also studied (Fig. 3C, Channel 2). The total amount of neutral GSL in the solid tumor was lower than that of the cultured cells (see Table II). It contained all four neutral GSLs and diglycosylceramide was the most prominent component.

Use of D-[1-14C]Galactose to Study Glycosphingolipid Patterns—A radioautogram of the neutral GSLs extracted from a variety of neuroblastoma cells incubated with D-[1-14C]galactose is shown in Fig. 4. It is evident that all of the cells studied possessed the capacity to synthesize their complement of neutral GSLs using this isotope as a precursor. Double zones corresponding in migration to standards of mono-, di-, tri-, and tetraglycosylceramides are clearly visible among the various samples applied. With regard to the hybrid cells, the almost complete absence of triglycosylceramide in the parent NA cell (Channel 3) and tetraglycosylceramide in the parent L cell (Channel 4) are evident.

All three hybrid lines are seen to possess the four neutral GSLs, and in particular contain both tri- and tetraglycosylceramides. The sensitivity of the method is shown by the detection (Channel 5) of the zones corresponding in migration to asialo-GM₂. The radioactive zones between tetraglycosylceramide and the origin and Methods.” Aliquots of the neutral GSL fractions prepared by silicic acid column chromatography as described in “Materials and Methods.” Aliquots of the neutral GSL fractions prepared by silicic acid column chromatography and containing approximately 2 × 10⁴ dpm were separated by thin layer chromatography on a glass plate coated with Silica Gel G in a solvent system of chloroform-methanol-water (65:25:4, v/v). The x-ray plate was in contact with the chromatogram for 1 week. CM, monoglycosylceramide; CD, diglycosylceramide; CT, triglycosylceramide; CTT, tetraglycosylceramide.
FIG. 5. Gas-liquid chromatographic analyses of the sugar moieties of several gangliosides and neutral GSLs of cultured neuroblastoma cells as trimethylsilyl ethers of their O-methyl glycosides. The chromatographic conditions were as described in "Materials and Methods." A, the analysis of the ganglioside of clone N2A corresponding in chromatographic migration to GM2; B, analysis of the ganglioside of clone NB41A corresponding to GD1a; C, analysis of the ganglioside of clone N2A corresponding to GD1a; D, analysis of the neutral GSL of clone NB41A corresponding to triglycosylceramide; E, analysis of the neutral GSL of clone NLI-7A corresponding to asialo-GM1; F, analysis of the neutral GSL of clone NB41A corresponding to tetraglycosylceramide (globoside). The peaks are identified from left to right as the derivatives of: mixture of α- and β-galactofuranoside (α); α-galactopyranoside (α Gal); β-galactopyranoside (β); α-glucopyranoside (α Glu); β-glucopyranoside (β); mannos (MAN); β-N-acetylgalactosaminopyranoside (GAL NAC); α-N-acetyl-galactosaminopyranoside (GAL NAC); and N-acetylgalactosamine acid (NANA). D-Mannitol was the internal standard. The scale on the abscissae is in degrees.
represent small amounts of labeled ganglioside and nucleotide sugars (21). This method was also found to be applicable to qualitative analysis of the gangliosides of similar quantities of cells; an excellent correlation between the pattern of gangliosides as detected by this method and that revealed by chemical detection was observed.

**Carbohydrate Composition of GSLs Studied—Assignments of general structures to the GSLs studied here were based on analyses of the carbohydrate moieties by gas-liquid chromatography. Representative analyses of GSLs corresponding in chromatographic migration to GM₃, GM₂, GD₁, triglycosylceramide, asialo-GM₂ and tetraglycosylceramide are shown in Fig. 5, A, B, C, D, E, and F, respectively. The molar ratios of galactose and galactosamine and sialic acid (where present) normalized to glucose (taken as unity) are shown in Table III. In general, the GSLs of the various cell lines studied exhibited a carbohydrate stoichiometry that might have been anticipated from their chromatographic migration relative to standards of known composition. The analyses on NLI-7A confirmed the presence of a species with a chemical composition compatible with the structure of asialo-GM₂. Of interest was the observation that glucose was virtually the sole carbohydrate in the monoglycosylceramide species of all the cells, only a trace of galactose (approximately 5% of the glucose) being present. The detection of this component by radioautography (Fig. 4) thus presumably depended on epimerization of galactose to glucose. It should be noted that no distinction between N-acetyl- and N-glycolyneuraminic acids was made in this study, as the gas-liquid chromatographic system used here does not separate them.

**Table III**

**Carbohydrate stoichiometry of principal glycosphingolipids**

Analyses by gas-liquid chromatography and quantitation of the sugar peaks were performed as described in "Materials and Methods." Results are averages of duplicate analyses of two preparations of each GSL.

<table>
<thead>
<tr>
<th>Chromatographic migration of GSL studied</th>
<th>Molar ratio relative to glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td>N2A</td>
<td></td>
</tr>
<tr>
<td>GM₃</td>
<td>1.00</td>
</tr>
<tr>
<td>GM₂</td>
<td>1.00</td>
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<tr>
<td>GM₁</td>
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<td>GD₁</td>
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<td>TTC</td>
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<tr>
<td>LM(TK⁺)</td>
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</tr>
<tr>
<td>MC</td>
<td>1.00</td>
</tr>
<tr>
<td>DC</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* MC, DC, TC, TTC: mono-, di-, tri-, and tetraglycosylceramide, respectively.

**DISCUSSION**

Marked differences were observed in the GSL patterns of the clonal isolates of neuroblastoma cells studied in the present investigation, particularly in the case of the gangliosides (see Fig. 2 and Table II). These variations were unlikely to be mediated by differences in cell density of the various cultures (10, 19, 27), as care was taken to harvest all cultures at approximately the same stage of growth. In contrast to these findings were the observations that subclones of N2A (see Figs. 2 and 3) exhibited essentially similar GSL patterns, as did two of the three hybrid lines of cells. Clonal derivatives of NIL hamster cells (10), SV40 virus-transformed 3T3 cells (11) and x-ray-induced mouse adenocortical tumor cells (12) have been previously shown to exhibit variations of GSL profile. The reasons for the existence of such variations of GSL profile between certain related clones of mammalian cells are not understood. They may reflect differences in the activities of synthetases (28, 29) or hydrolases (30) controlling directly the rates of synthesis and degradation of these compounds. It is interesting to note that such variations of GSL profile are not lethal as far as cell growth in culture is concerned, a result consistent with the marked GSL imbalances that are compatible with at least a limited lifespan in the human glycosphinilipidoses. It would seem that GSLs play a more subtle role in cell function than other membrane lipids whose presence is essential for the integrity of certain cellular membranes.

While this work was in progress, Dawson et al. (13, 14) reported the GSL patterns of cultures derived from NB41A and N2A. In agreement with their results, we found that GM₃ was the principal ganglioside in NB41A, although, unlike them, we could not detect asialo-GM₂ in this line. Furthermore, we found GM₁ to be the principal ganglioside in N2A, contrary to their observations. At present the reasons for these differences are unknown.

The substantial amounts of GM₃ in N2A cells and their subclones and of tetraglycosylceramide in all the neuroblastoma cells and NLI hybrids were unexpected, particularly as these are minor components of adult brain. The observation that these compounds were present in the parent C1300 tumor was thus of particular significance, as it apparently dispels the possibility that their appearance in the cultured cells was solely a result of adaptation to cell culture conditions. Although one could speculate that the appearance of GM₃ and tetraglycosylceramide correlates with the abnormal biological behavior of the C1300 tumor, this seems unlikely since these two compounds are the principal GSLs of certain mammalian red blood cells (cf. Reference 31).

All of the GSLs observed in the various neuroblastoma lines studied here (with the possible exception of asialo-GM₂) have previously been detected in other cultured cells of nonneural origin (32). Thus, the GSL profiles of these cells could not be said to be unique markers for cells of neural origin. It was of interest, however, that virtually none of the GSLs that are enriched in myelin (e.g. galactocerebrosides and sulfatide (33)) were detected in the neuroblastoma cells. Galactocerebrosides constituted only some 5% of the monoglycosylceramide species. Sulfatide, which migrated just behind triglycosylceramide in the thin layer chromatographic system used (Fig. 3A, Channel 5), was not detected in any of the cells studied. A component of similar migration to sulfatide was observed in the neutral GSL fraction of NLI-7A cells (Fig. 3B, Channel 4); however, on analysis this proved to be an asialo-GM₂ species. It is possible that incubation of neuroblastoma cells with Na₂SO₄ might re-
veal the presence of trace amounts of sulfatide. All of the neutral GSLs detected in the neuroblastoma cells resolved as double bands (Fig. 3); this phenomenon has been noted in previous studies on the GSLs of cultured cells (11, 21, 34, 35) and is presumably due to the presence of either very long chain normal fatty acids (35) or of hydroxy fatty acids (36) in the slower migrating bands.

We did not detect any appreciable change in the profile of the various gangliosides and neutral GSLs of N2A cells before and after neurite outgrowth (see Fig. 2A). Our expectation at the outset of these experiments was that some shift in profile toward a preponderance of higher gangliosides in the “differentiated” state might be observed, akin to the increase of these compounds observed during neuroembryogenesis in brain (2). The present findings do not exclude GSLs from participating in neurite function, as they may have important spatial distributions along the neurite, but they do rule out gross qualitative and quantitative changes in GSLs as prerequisites for neurite extension.

The studies on the NLL hybrids were of interest, in view of their varying membrane electrogenic potentials and also because no previous investigations of the GSLs of somatic cell hybrids have been reported. The total amount of ganglioside in the hybrid cells was somewhat greater than that of the parent NA cells and much greater than that of the parent L cells. The ganglioside patterns of the hybrid cells were different from those of the parental cells, exhibiting relatively less of the more complex gangliosides than the parent NA cells and relatively less GM1 than the parent L cells. The neutral GSL pattern of the hybrid cells was noteworthy as it resembled the sum of the patterns of the two parent cells, exhibiting mono- and diglycosylceramides (present in both parentes), triglycosylceramide (present only in L cells) and tetracygosylceramide (present only in the NA cells). The GSL patterns of the hybrid cells cannot be readily interpreted in terms of dominance or recessiveness of one or other of the parental cell patterns. This finding may in part reflect the fact that both parental cells had relatively similar and complex patterns of GSLs. Fusion of parental cells with widely dissimilar patterns of GSLs might yield more information on this matter. Further studies on other hybrid cells are thus necessary to permit possible analyses of genetic factors affecting GSL composition. Application of the technique of incubation of cells with L-[14C]galactose followed by radioautographic analysis of their GSLs, as utilized here, may be useful in permitting such studies in which only limited amounts of hybrid cells are available.

The analyses on the hybrid cells did not provide support for any simple relationship between ganglioside composition and electrogenic response, as all three hybrids exhibited very similar patterns of gangliosides. As in the discussion of the studies on neurite outgrowth, the observations here cannot rigorously exclude gangliosides from participation in this response, for at least two reasons. Firstly, it is conceivable that small variations in ganglioside composition could be critical in permitting expression of this membrane response. Secondly, highly specific interactions of gangliosides with other membrane components could also be necessary for the electrogenic response; these hypothetical components could be deficient in the hybrids of low response. The fact that NB41A cells, which contain principally GM3, exhibit a full electrogenic response would argue, however, that the pattern of complex gangliosides observed in adult brain is certainly not critical for expression of this response.

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