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O-Acetylation of a cell-surface carbohydrate creates discrete molecular patterns during neural development

(ganglioside/monoclonal antibody/retina/melanoma)

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ABSTRACT **The cell-surface antigen detected by the monoclonal antibody JONES is expressed in the retina and a number of other central nervous system regions of the rat during the latter part of embryonic development and the early postnatal period. In addition to the expression on certain neuroblast populations it is found on some but not all axons and is also expressed at high levels on the end feet of radial glia in regions through which axons actively grow. In the perinatal rat retina, almost all the antigenic activity was carried on a ganglioside migrating between GM1 and GM2. The epitope recognized by antibody JONES was base labile and treatment with 0.1 M sodium carbonate or ammonia vapor converted the antigen into GD3. Resistance to oxidation by sodium periodate and reformation of the epitope by chemical acetylation of base-treated gangliosides with *N*-acetylimidazole identify the antigen as 9-*O*-acetyl GD3. The acetylation of GD3 seems to be regulated independently from GD3 expression itself since acetylated and nonacetylated GD3 do not have identical immunocytochemical distributions in the developing central nervous system. In addition, five independent human melanoma cell lines varied substantially in their expression of 9-*O*-acetyl GD3, even though they all expressed high levels of GD3. Acetylation of ganglioside-linked sialic acid provides a mechanism for generating unique patterns of surface carbohydrates, which may influence cell interactions in development.**

Pattern formation during embryogenesis remains poorly understood at the molecular level. Within the developing nervous system compartments of restricted developmental potential are formed within a morphologically homogeneous neuroepithelium. The mechanisms defining these compartments and guiding subsequent cell and process migration within and between them may involve graded concentrations of cell-surface molecules. In support of this, chicken nasal retinal ganglion cell axons showed a preferential growth over tectal cell monolayers compared with retinal cell monolayers and axons from temporal retina preferred anterior rather than posterior monolayers (1). Other work has shown that dorsal and ventral chicken retinal cell suspensions differ reciprocally in their adhesion to monolayers of ventral and dorsal tectal cells (2). Based on the sensitivity of these adhesive interactions to enzyme treatments, a double-gradient model involving a ganglioside and a β-*N*-acetylgalactosamine-binding protein was postulated (3).

Positional specification during neural development can be analyzed by deriving specific antibodies that can detect molecules whose distribution correlates with defined developmental events. Characterization of such molecules will provide insights into regulatory mechanisms operating during early neural development and may also lead to a better understanding of the developmental processes themselves. We have described an antibody, JONES, that labels a ganglioside antigen in embryonic and early postnatal rat retina in a dorsoventral gradient (4). This gradient could be detected both immunocytochemically on fixed specimens and by binding to living tissue. The antigen is also found in a number of other neural regions. In cerebellum, for example, expression of the antigen on the external granule cell layer correlates better with the onset of migration of the cells than with the proliferation of their neuroblast precursors (5).

In this paper, we provide further evidence of the precise temporal and spatial regulation of the epitope recognized by the JONES antibody. We show that the major epitope recognized in rat retina is 9-*O*-acetyl GD3. Comparison of the patterns of expression of GD3 and 9-*O*-acetyl GD3 suggests that they can be regulated separately. Selective acetylation of sialic acid residues on specific gangliosides may provide an additional biochemical mechanism for extending the molecular specificity of cell-surface carbohydrate groups.

MATERIALS AND METHODS

Antibodies and Cell Lines. The JONES antibody is an IgM subclass mouse monoclonal antibody and was used at a 1:100 dilution of an ascites fluid (4). R24 (a gift of K. Lloyd, Memorial Sloan–Kettering Cancer Center) is an IgG3 mouse monoclonal antibody that recognizes GD3 and was used at a dilution of 1:200 of an ascites fluid (6, 7). Its specificity was confirmed by TLC/blot analysis on extracts of rat neural tissue. A rabbit anti-BSP-2/N-CAM serum was the gift of C. Goridis (Centre d'Immunologie, Marseille).

Human melanoma cell lines (a gift of K. Lloyd) have been described (7, 8) and were grown in RPMI 1640 medium containing 10% fetal bovine serum.

Immunocytochemistry. Freshly dissected tissue from Long–Evans rats was fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) by immersion at 4°C. Sections were prepared and labeled as described elsewhere (9). A fluorescein isothiocyanate-conjugated goat anti-mouse IgG and IgM (Boehringer Mannheim) was used as secondary antibody. Two-color fluorescence double-labeling studies were carried out as described (10). Controls showed neither inappropriate reactivity of secondary antibodies nor nonspecific labeling with other nonreactive monoclonal antibodies. Melanoma cell lines were harvested, spun onto polylysine-coated coverslips, fixed in 1% paraformaldehyde, and labeled as described (9). Approximately 100 cells were counted for each experiment.

Rat Retinal Ganglioside Preparation. Freshly dissected postnatal day 3 (PN3) Long–Evans rat retinas were homogenized in choloroform/methanol (2:1, vol/vol) (11), and extracted with 0.1 M KCl following Suzuki's method (12). Alkali treatment was omitted from the preparation. Gangli-

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Abbreviations: PN3, postnatal day 3; E20, etc., embryonic day 20, etc.

oside yield was determined by measuring sialic acid content using the resorcinol assay (13).

Radioimmunoassay. PN3 retinal gangliosides in chloroform/methanol (2:1, vol/vol) were dried onto polyvinyl chloride microtiter wells (0.025 μg of sialic acid in 50 μl per well). After rinsing three times with PBS containing 0.1% bovine serum albumin, radioactive binding assays were carried out using appropriate dilutions of primary antibody and a ^{125}I-labeled $(Fab')_2$ fragment of an affinity-purified rabbit anti-mouse IgG (14). Specific binding was defined as the average binding above that of subclass-matched control antibodies.

TLC/Blot Analysis. Retinal gangliosides (≈1 μg of sialic acid) were spotted onto high-performance TLC plates (E. Merck, silica gel 60, 0.2 mm thick), which were developed in chloroform/methanol/0.2% aqueous $CaCl_2$ (60:40:10, vol/vol/vol) (15). After drying, the plate was wetted with isopropanol/water (3:1, vol/vol), and the gangliosides were transferred to nitrocellulose (16) and processed for JONES binding as described elsewhere using an overnight incubation at 4°C with primary antibody and a 6-hr incubation at 4°C with secondary antibody (4). Ganglioside standards were run in parallel and visualized with resorcinol. Gangliosides are termed according to the nomenclature of Svennerholm (17).

Chemical O-Acetylation. PN3 retinal gangliosides were dried under nitrogen and redissolved in 25 μl of pyridine (Sigma) containing *N*-acetylimidazole (Sigma). Reactions were carried out at 50°C for 16 hr. Under these conditions *N*-acetylimidazole selectively acetylates the methyl ester of sialic acid methyl glycoside at the 9-O position (18). After incubation, the reaction mixture was evaporated under nitrogen, dissolved in methanol and dried under nitrogen (three times), and finally redissolved in chloroform/methanol (2:1, vol/vol).

RESULTS

JONES Immunoreactivity Shows a Discrete Temporal and Spatial Distribution During Neural Development. The determinant recognized by the JONES antibody is found primarily during the embryonic development of the nervous system. Immunocytochemical labeling of sections of E20 (embryonic day 20) rat cerebral vesicles showed that the determinant was present on cells in the ventricular zone, the glial limiting membrane, and some faint radial fibers across the tissue (Fig. 1*A*, see also Fig. 6*A*). In the adult rat cerebral cortex, no JONES immunoreactivity was observed (data not shown). In the E20 retina, JONES immunoreactivity was found across the whole thickness of the retina in regions undergoing active migration and differentiation but not in the peripheral regions, which consisted of a simple proliferative epithelium (Fig. 1*B*). In the adult retina, labeling was confined to the outer plexiform layer (Fig. 1*C*).

In addition to the temporal regulation of the JONES immunoreactive determinant, there is a striking spatial regulation in many areas of the developing rat nervous system. Examples of this are shown in Fig. 2. A complete map of the labeling will be presented elsewhere (5). In the E15 rat lamina terminalis, the junction between the telencephalon and the diencephalon, JONES labeling is confined to a narrow band at the glial limiting membrane (Fig. 2*A*). The presence of many processes in this region unlabeled by JONES is shown when the same section is labeled with anti-BSP-2/N-CAM serum (Fig. 2*B*). Similarly, some but not all axon bundles in the head region are labeled by JONES in the E15 rat (Fig. 2 *C* and *D*).

Biochemical Characterization of the JONES Antigen. We have shown previously that the JONES antibody recognizes a minor ganglioside migrating between GM1 and GM2 on TLC plates. Other work using two-dimensional TLC analysis has shown the existence of base-labile modifications of GD3 that give species with similar migratory behavior (19) and O-acetylated derivatives of GD3 have been isolated from human melanoma tumor cell lines (20). PN3 retinal gangliosides were separated by TLC and blotted onto nitrocellulose. Blots were treated with ammonia vapor or water vapor and then reacted with JONES antibody or R24, an antibody specific for GD3. The JONES reactive band (Fig. 3, lane 1) disappeared after ammonia treatment (lane 3). The native GD3 band was unaffected by this treatment (lanes 2 and 4). In addition, base treatment led to another GD3 band in the same position as the JONES immunoreactive band in the untreated sample (compare lanes 1 and 4), suggesting that JONES recognizes a form of GD3 that contains a base-labile modification.

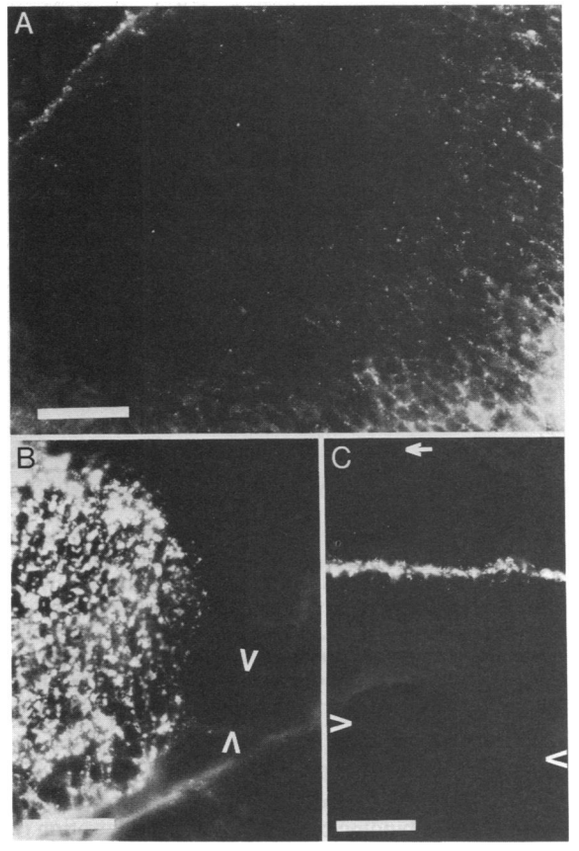

FIG. 1. Immunofluorescent labeling of rat central nervous system by antibody JONES. (*A*) Coronal section of E20 cerebral cortex showing labeling of cells in the ventricular zone (lower right) and the glial limiting membrane (upper left). (*B*) Transverse section of E20 retina showing bright labeling to the edge of the differentiating tissue but no labeling of the peripheral retinal epithelium (between arrowheads), which is morphologically continuous with it. (*C*) Transverse section of adult retina showing labeling of only the outer plexiform layer. No other labeling is apparent from the optic nerve fiber layer (arrowheads) to the photoreceptor outer segments (arrow). (Bars = 50 μm.)

This modification protects the molecule from periodate oxidation. Treatment of retinal gangliosides with 6 mM periodate decreases R24 reactivity (Fig. 4, bar 2) but does not alter JONES immunoreactivity. Base treatment after periodate oxidation shows that the modified GD3 was protected and could react with antibody R24 after removal of the substituent. Only substitutions at the 8-O and 9-O positions of the terminal sialic acid will prevent its oxidation by periodate.

Acetylation of base-treated retinal gangliosides, under conditions that favor reaction at the 9-O position of the terminal sialic acid, was sufficient to recreate JONES immu-

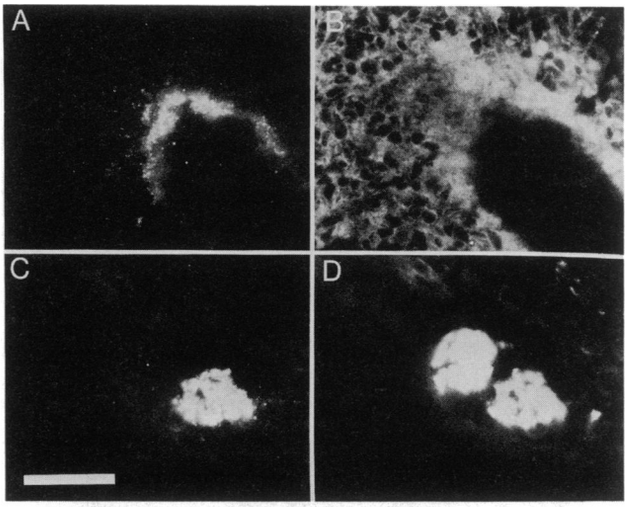

FIG. 2. Selective labeling within axonal pathways by antibody JONES. (*A* and *B*) Lamina terminalis of E15 rat. A narrow band of label at the glial limiting membrane is seen with antibody JONES (*A*). A much more extensive area of processes is revealed by labeling the same section with anti-N-CAM/BSP-2 (*B*). (*C* and *D*) Labeling of axon bundles innervating facial structures in the E15 embryo. JONES labels one of two adjacent bundles (*C*), both of which can be labeled by anti-N-CAM/BSP-2 (*D*). (Bar = 50 μm.)

noreactivity (Fig. 5*A*). Unmodified GD3, as detected by antibody R24 binding, decreased reciprocally with the increase in JONES binding over the same concentration range of *N*-acetylimidazole. The material acetylated was shown to have the same TLC migration as the native molecule (Fig. 5*B*). An additional minor band that migrated faster, probably representing diacetylated GD3, was found after chemical acetylation but not in tissue extracts. The identity of migration, together with the other results, suggests that JONES recognizes 9-*O*-acetyl GD3.

GD3 and 9-*O*-Acetyl GD3 Are Regulated Independently. Immunocytochemical labeling of adjacent sections of developing rat nervous system with antibodies R24 and JONES showed clear differences in the localization of GD3 and 9-*O*-acetyl GD3. In the E18 cerebral vesicles, JONES labeling was confined to the proliferating ventricular zone and to some radial fibers extending to the pial surface (Fig. 6*A*). R24 labeled the entire thickness of the tissue including both the ventricular zone and the postmitotic cells of the cortical plate (Fig. 6*B*).

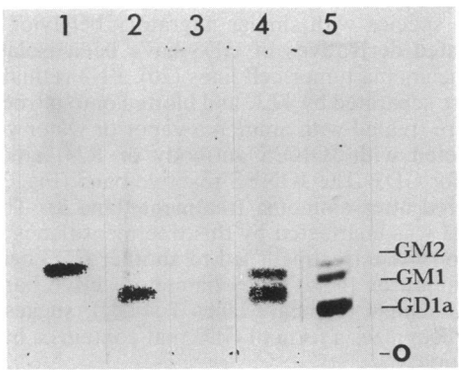

FIG. 3. TLC/blot analysis of retinal gangliosides and the effect of base treatment on JONES and R24 binding. PN3 retinal gangliosides (0.18 μg of sialic acid per lane) were subjected to TLC/blot analysis. After transfer, the blot was dried and the lanes were cut. Lanes 1 and 2 were incubated in a humidified chamber for 5 hr at 22°C. Lanes 3 and 4 were similarly incubated with ammonia vapor. Following a 15-min incubation in PBS/5% bovine serum albumin at 22°C, lanes 1 and 3 were labeled with JONES and lanes 2 and 4 were labeled with R24. The bands recognized by JONES and R24 were clearly resolved. Base treatment removed JONES binding and revealed a second GD3 band at that position. O, Origin.

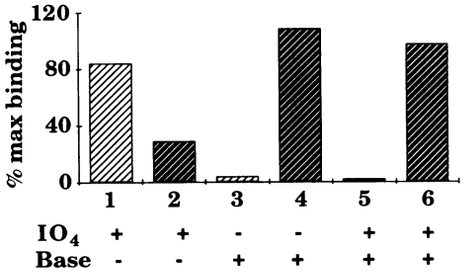

FIG. 4. Effect of base and periodate treatments on antibody binding to retinal gangliosides. PN3 retinal gangliosides (in triplicate) were incubated with PBS or 6 mM periodate in PBS. After rinsing, wells were treated with H_2O or 0.1 M Na_2CO_3 at 22°C overnight. Wells were then processed for JONES (bars 1, 3, and 5) and R24 (bars 2, 4, and 6) binding. Counts bound were normalized to untreated samples. Periodate treatment destroys most R24 binding (bar 2) but leaves JONES binding intact (bar 1). Base treatment destroys JONES binding (bar 3) but leaves R24 binding (bar 4). Periodate treatment followed by base treatment destroys JONES binding (bar 5) but leaves R24 binding to the GD3 molecules revealed by base treatment (bar 6).

Labeling of five human melanoma cell lines provided further evidence for the differential control of GD3 and 9-*O*-acetyl GD3. As shown in Fig. 7, all five lines were largely positive for GD3 expression. JONES expression varied significantly, however, from a low of 4% to a high of 68%.

DISCUSSION

We had previously identified the molecule reacting with the JONES monoclonal antibody as a ganglioside migrating between GM2 and GM1 on TLC plates (4). The removal of JONES reactivity by base treatment and the consequent

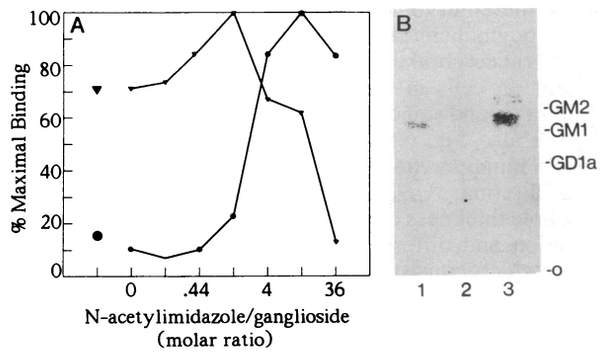

FIG. 5. Effect of chemical O-acetylation on JONES and R24 binding. (*A*) Concentration dependence of the acetylation reaction. PN3 retinal gangliosides were base treated by incubation in 0.1 M Na_2CO_3. Samples (5 μg of sialic acid) were dried under N_2 and chemically O-acetylated by *N*-acetylimidazole in pyridine. After the reaction, treated gangliosides were applied to polyvinyl chloride microtiter wells in duplicate and analyzed by radioimmunoassay as described in *Materials and Methods*. Untreated samples, represented by large symbols, were also assayed in parallel for JONES (●) and R24 (▼) binding. Specific antibody binding was normalized to samples exhibiting maximal binding. (*B*) TLC/blot analysis of acetylated PN3 rat retinal gangliosides. Lanes: 1, untreated gangliosides (0.5 μg of sialic acid); 2, base-treated (3 μg of sialic acid); 3, gangliosides (3 μg of sialic acid) O-acetylated using a ×10 molar excess of *N*-acetylimidazole. JONES labeling reveals a major band in lane 3 that migrates at the same position as the authentic band in lane 1. A minor faster migrating band is also seen. O, Origin.

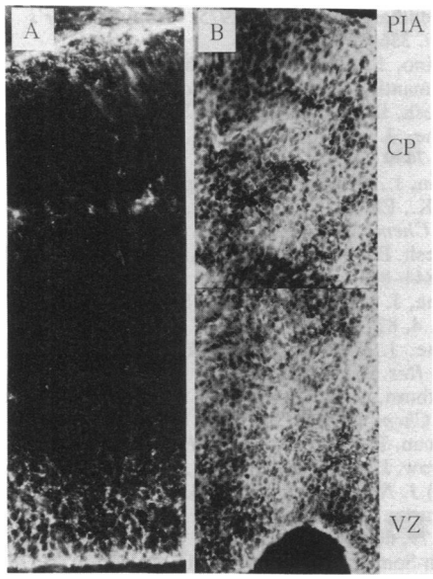

FIG. 6. Comparison of JONES and R24 labeling of adjacent coronal sections of E18 rat brain. Labeling with JONES (*A*) reveals only the ventricular zone, the glial limiting membrane, and faint radial fibers. Labeling with R24 (*B*) reveals cells in the ventricular zone as well as throughout the cortical plate. CP, cortical plate; VZ, ventricular zone.

production of GD3 (Fig. 3) showed that the molecule recognized is a modified form of GD3. The ability of the modification to protect the molecule from periodate oxidation (Fig. 4) and the reconstitution of JONES immunoreactivity that migrates to the same position as the authentic molecule (Fig. 5) all indicate that the antigen recognized is 9-*O*-acetyl GD3. A slower migrating immunoreactive band has been found on TLC/immunoblot analysis of gangliosides from several postnatal (postmitotic) central nervous system regions, particularly cerebellum. This band is also base labile (data not shown). This suggests that JONES may recognize another acetylated ganglioside or possibly a ganglioside modified into a lactone.

9-*O*-Acetyl GD3 has been detected previously in several tumor cell lines. A monoclonal antibody, ME 311, generated against a human metastatic melanoma cell line was used to monitor the purification of the molecule whose structure was determined by fast atom bombardment mass spectrometry

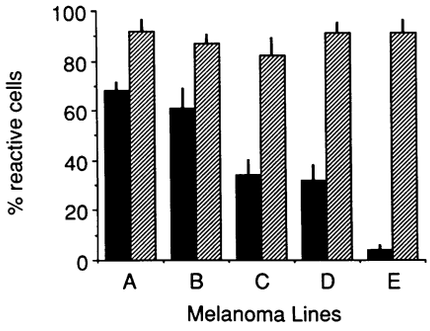

FIG. 7. Immunofluorescent labeling of human melanoma cell lines with JONES and R24 antibodies. JONES (solid) and R24 (hatched) labelings were performed as described in *Materials and Methods* and independently repeated twice for melanoma lines A and C and four times for lines B, D, and E. The results are expressed as the mean ± SEM of the measurements. Melanoma lines have been designated as follows: A, SK-MEL-193-1; B, SK-MEL-23-22; C, SK-MEL-93-2; D, SK-MEL-216; E, SK-MEL-217, as described in refs. 7 and 8.

and ^{1}H NMR (21). This antibody also reacted with sections of a pheochromocytoma. Another monoclonal antibody, D1.1, also detected 9-*O*-acetyl GD3 in human melanoma cells (20, 22). This antibody was generated against a rat tumor cell line expressing both glial and neuronal properties (23). From the patterns of reactivity of D1.1 in the developing nervous system, it has been suggested that it detects a marker of the proliferating neuroepithelium that gives rise to differentiated neurons and glia (24). The JONES antibody does react during proliferative phases of neural development but clearly does not detect all proliferating neuroepithelia (Fig. 1*B* and unpublished results). It is also expressed on both some axon bundles of postmitotic neurons and possibly the glial end feet over which some new axons develop (Figs. 1 and 2). From its pattern of expression in retina (4) and cerebellum it has been suggested that 9-*O*-acetyl GD3 expression is a better marker of cell migration and process outgrowth (5).

The functions of gangliosides in the nervous system are poorly understood. Whether the correlation of 9-*O*-acetyl GD3 expression with cell and process motility is related to the function of the molecule is still unclear but gangliosides have been implicated in both cellular adhesion and process outgrowth. For example, gangliosides specifically support chicken neural retinal cell adhesion (25), and may participate in cellular binding and organization of matrix molecules like fibronectin (26). Antibodies to gangliosides have been shown to inhibit goldfish optic nerve regeneration (27) and nerve growth factor-induced neurite outgrowth from dorsal root ganglion (28), while exogenous gangliosides appear to augment neurite outgrowth *in vitro* (29). The mechanisms of these effects are unknown. Some may involve the modulation of other protein receptors or enzymes. For example, recent work has suggested that gangliosides can modulate protein kinase C activity *in vitro* (30). Other effects may be due to direct interaction with gangliosides. The best example of this is the ganglioside GM1, which acts as a receptor for cholera toxin binding (31).

Although the antibody JONES is selective for the acetylated derivative of GD3, it was possible that the patterns of reactivity we had observed were due to changes in GD3 expression that were secondarily mirrored in the patterns of 9-*O*-acetyl GD3 expression. Previous reports have shown that GD3 expression declines in postnatal rat development (32), although there is still some debate as to the cellular localization of the remaining antigen (33, 34). In E20 developing cerebral cortex, the only time point examined in this study (Fig. 6), GD3 expression is clearly maintained after the cells have migrated from the ventricular zone into the cortical plate, whereas 9-*O*-acetyl GD3 expression decreases to undetectable levels. Although masking of ganglioside determinants has been reported, this is unlikely to account for the results because the decrease seen immunocytochemically is matched by a decrease in 9-*O*-acetyl GD3 as measured biochemically (35). Generalized masking is also unlikely since GD3 itself was readily detected. It is not yet clear whether this is due to changes in the biosynthesis or the degradation of the molecule. Acetylation of free sialic acid and intact gangliosides has been described in several tissues of different species but little is yet known about the enzymes involved (22, 36). Various esterases can cleave the acetyl group from acetylsialic acid but whether this can occur on molecules on the cell surface is unknown (37).

9-*O*-Acetyl GD3 is one of a growing list of cell-surface carbohydrate determinants that show specific patterns of expression in the nervous system. Others include the HNK-1 determinant (38) SSEA-1, which reacts with subpopulations of dorsal root ganglion cells (39), and other fucosylated glycolipids (40). Since these carbohydrate groups are so precisely regulated in both time and space, it remains a reasonable hypothesis that many cell–cell and cell–substrate

interactions are regulated through these moieties. Thus, many aspects of neural development may be controlled by changes in enzymatic activities at several points along complex metabolic pathways.

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1. Bonhoeffer, F. & Huf, J. (1982) *EMBO J.* **1,** 427–431.
2. Barbera, A. J., Marchase, R. B. & Roth, S. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2482–2486.
3. Marchase, R. B. (1977) *J. Cell Biol.* **75,** 237–257.
4. Constantine-Paton, M., Blum, A. S., Mendez-Otero, R. & Barnstable, C. J. (1986) *Nature (London)* **324,** 459–462.
5. Mendez-Otero, R., Schlossauer, B., Barnstable, C. J. & Constantine-Paton, M. (1987) *J. Neurosci.*, in press.
6. Pukel, C. S., Lloyd, K. O., Travassos, L. R., Dippold, W. G., Oettgen, H. F. & Old, L. J. (1982) *J. Exp. Med.* **155,** 1133–1147.
7. Dippold, W. G., Lloyd, K. O., Li, L. T. C., Ikeda, H., Oettgen, H. F. & Old, L. J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6114–6118.
8. Albino, A. P., Lloyd, K. O., Houghton, A. N., Oettgen, H. F. & Old, L. J. (1981) *J. Exp. Med.* **154,** 1764–1778.
9. Barnstable, C. J. & Drager, U. C. (1984) *Neuroscience* **11,** 847–855.
10. Arimatsu, Y., Naegele, J. R. & Barnstable, C. J. (1987) *J. Neurosci.* **7,** 1250–1263.
11. Folch, J., Lees, M. & Sloan-Stanley, G. H. (1957) *J. Biol. Chem.* **226,** 497–509.
12. Suzuki, K. (1965) *J. Neurochem.* **12,** 629–638.
13. Svennerholm, L. (1957) *Biochim. Biophys. Acta* **24,** 604–611.
14. Gozes, I. & Barnstable, C. J. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2579–2583.
15. Ledeen, R. W. & Yu, R. K. (1982) *Methods Enzymol.* **83,** 139–191.
16. Towbin, H., Schoenenberger, C., Ball, R., Braun, D. G. & Rosenfelder, G. (1984) *J. Immunol. Methods* **72,** 471–479.
17. Svennerholm, L. (1963) *J. Neurochem.* **10,** 613–623.
18. Haverkamp, J., Schauer, R., Wember, M., Kamerling, J. & Vliegenthart, J. F. G. (1975) *Hoppe-Seyler's Z. Physiol. Chem.* **356,** 1575–1583.
19. Sonnino, S., Ghidoni, R., Chigorno, V., Masserini, M. & Tettamanti, G. (1983) *Anal. Biochem.* **128,** 104–114.
20. Cheresh, D. A., Varki, A. P., Varki, N. M., Stallcup, W. B., Levine, J. & Reisfeld, R. A. (1984) *J. Biol. Chem.* **259,** 7453–7459.
21. Thurin, J., Herlyn, M., Hindsgaul, O., Stromberg, N., Karlsson, K., Elder, D., Steplewski, Z. & Koprowski, H. (1985) *J. Biol. Chem.* **260,** 14556–14563.
22. Cheresh, D. A., Reisfeld, R. A. & Varki, A. P. (1984) *Science* **225,** 844–846.
23. Levine, J. M., Beasley, L. & Stallcup, W. B. (1984) *J. Neurosci.* **4,** 820–831.
24. Levine, J. M., Beasley, L. & Stallcup, W. B. (1986) *Dev. Brain Res.* **27,** 211–222.
25. Blackburn, C. C., Swank-Hill, P. & Schnaar, R. L. (1986) *J. Biol. Chem.* **261,** 2873–2881.
26. Fishman, P. H. (1986) *Chem. Phys. Lipids* **42,** 137–151.
27. Sparrow, J. S., McGuinness, C., Schwartz, M. & Grafstein, B. (1984) *J. Neurosci. Res.* **12,** 233–243.
28. Schwartz, M. & Spirman, N. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6080–6083.
29. Katoh-Semba, R., Skaper, S. D. & Varon, S. (1984) *J. Neurosci. Res.* **12,** 299–310.
30. Kim, J. Y. H., Goldenring, J. R., DeLorenzo, R. J. & Yu, R. K. (1986) *J. Neurosci. Res.* **15,** 159–166.
31. Porcellati, G., Ceccarelli, B. & Tettamanti, G. (1976) *Ganglioside Function: Biochemical and Pharmacological Implications* (Plenum, New York).
32. Goldman, J. E., Hirano, M., Yu, R. K. & Seyfried, T. N. (1984) *J. Neuroimmunol.* **7,** 179–192.
33. Goldman, J. E., Geier, S. S. & Hirano, M. (1986) *J. Neurosci.* **6,** 52–60.
34. Graus, F., Cordon-Cardo, C., Houghton, A. N., Melamed, M. R. & Old, L. J. (1984) *Brain Res.* **324,** 190–194.
35. Schlossauer, B., Mendez-Otero, R. & Constantine-Paton, M. (1986) *Soc. Neurosci. Abstr.* **12,** 317.
36. Schauer, R. (1978) *Methods Enzymol.* **50,** 64–89.
37. Varki, A., Muchmore, E. & Diaz, S. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 882–886.
38. McGarry, R. C., Riopelle, R. J. & Roder, J. C. (1985) *Neurosci. Lett.* **56,** 95–100.
39. Dodd, J., Solter, D. & Jessel, T. M. (1984) *Nature (London)* **311,** 469–472.
40. Yamamoto, M., Boyer, A. M. & Schwarting, G. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3045–3049.