Glycoconjugate Journal (1996) **13:** 433–443

Glycolipids and myelin proteins in human oligodendrogliomas

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Received 29 September 1995, revised 22 December 1995

We studied myelin proteins and glycolipids in 24 human oligodendrogliomas (16 pure, eight mixed), including two grade I, 13 grade II, five grade III, and four grade IV. Tumours with a 1b ganglioside content (GD1b, GT1b and GQ1b) over 30% of total gangliosides occur more frequently in the WHO grade I and II (47%) and grade III (40%) than in the grade IV (25%) group; there was no difference in the amounts of total ganglioside or individual gangliosides between pure and mixed oligodendrogliomas. The presence of 6′-LM1 correlated with higher grades of tumours (χ^2 $p \simeq 0.02$); however, 3′-LM1 and total neolacto-series gangliosides did not correlate with grade. Immunohistochemical studies of oligodendrocyte and myelin markers (GalCer, sulfatide, 2′,3′-cyclic nucleotide phosphodiesterase, myelin basic protein and proteolipid protein) using specific antibodies showed only a very small proportion of tumour cells staining. These data do not support the hypothesis that tumours classified as oligodendrogliomas are derived from mature oligodendrocytes.

Keywords: brain tumours, glioma, gangliosides, glycolipids, myelin proteins

Abbreviations:

Svennerholm	*IUPAC-IUB*	*Schematic structure*
GM3	II^3NeuAc-LacCer	NeuAcα2 → 3Galβ1 → 4Glcβ1 → 1Cer
GM2	II^3NeuAc-$GgOse_3$Cer	GalNAcβ1 → 4(NeuAcα2 → 3)Galβ1 → 4Glcβ1 → 1Cer
GM1	II^3NeuAc-$GgOse_4$Cer	Galβ1 → 3GalNAcβ1 → 4(NeuAcα2 → 3)Galβ1 → 4Glcβ1 → 1Cer
GD3	$II^3(NeuAc)_2$-LacCer	NeuAcα2 → 8NeuAcα2 → 3Galβ1 → 4Glcβ1 → 1Cer
GD1a	IV^3NeuAc, II^3NeuAc-$GgOse_4$Cer	NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4(NeuAcα2 → 3)Galβ1 → 4Glcβ1 → 1Cer
GD2	$II^3(NeuAc)_2$-$GgOse_3$Cer	GalNAcβ1 → 4(NeuAcα2 → 8NeuAcα2 → 3)Galβ1 → 4Glcβ1 → 1Cer
GD1b	$II^3(NeuAc)_2$-$GgOse_4$Cer	Galβ1 → 3GalNaCβ1 → 4(NeuAc2 → 8NeuAcα2 → 3)Galβ1 → 4G1cβ1 → 1Cer
GT1b	IV^3NeuAc, $II^3(NeuAc)_2$-$GgOse_4$Cer	NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4(NeuAcα2 → 8NeuAcα2 → 3)Galβ1 → 4Glcβ1 → 1Cer
GQ1b	$IV^3(NeuAc)_2$, $II^3(NeuAc)_{2''}$-$GgOse_4$Cer	NeuAcα2 → 8NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4(NeuAcα2 → 8NeuAcα2 → 3)Galβ1 → 4Glcβ1 → 1Cer
3′-LM1	IV^3NeuAc-$nLcOse_4$Cer	NeuAcα2 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer
6′-LM1	IV^6NeuAc-$nLcOse_4$Cer	NeuAcα2 → 6Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer

Glc, glucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; GlcNAc, n-acetylglucosamine; NeuAc, *N*-acetylneuraminic acid; Lac; lactose; Cer, ceramide; $GgOse_3$, gangliotriosyl; $GgOse_4$, gangliotetraosyl; $nLcOse_4$, neolactotetraosyl.

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Introduction

Oligodendrogliomas are less common than astrocytomas, accounting for only 5–20% of intracranial gliomas [1]. Oligodendrocytes, myelin-forming cells in the central nervous system (CNS), were postulated to be the progenitors of oligodendrogliomas by Bailey and Cushing [2]. Microscopically, they present a relatively uniform appearance. Tumour cells in oligodendrogliomas are small, round, and closely packed, possess a darkly staining nucleus, and are surrounded by an artifactual clear halo. In addition to these features, it is not uncommon that many tumours contain both reactive and neoplastic astrocytes of varying types in different proportions. Glial fibrillary acidic protein (GFAP), generally considered as a marker for astroglial differentiation, has also been found in developing oligodendrocytes studied *in vitro* [3], immature oligodendrocytes [4], and tumour cells in oligodendrogliomas [5–9].

Patients with oligodendrogliomas usually have better survivals than those with astrocytomas of the same grade. In that the therapy of oligodendrogliomas is also different than that of astrocytomas, it is important to distinguish the two tumours. Herein we report the results of a study that employed a panel of antibodies against glycolipids and myelin proteins to study antigenic expression by 24 oligodendrogliomas using immuno-thin layer chromatography and immunohistochemistry.

Methods

TISSUE SPECIMENS

Tumour specimens were obtained from the Ohio State University, Barrow Neurological Institute, Mayo Clinic and the National Cancer Institute Cooperative Human Tissue Network. A total of 24 human oligodendrogliomas were studied, which consisted of two grade I, 13 grade II, five grade III and four grade IV tumours according to the WHO grading system. These included 16 pure oligodendrogliomas and eight mixed lesions i.e. oligoastrocytomas. Representative H&E stained slides from paraffin embedded tissues were examined by four neuropathologists from the three participating institutions. Disagreement was resolved by simultaneous viewing through a multihead microscope.

ANTIBODIES

The antibodies for detection of MBP, PLP and CNPase, all rabbit polyclonal antibodies, were generous gifts from Dr Denise Wood (Hospital for Sick Children, Toronto), Dr Wendy B. Macklin (Mental Retardation Research Center, UCLA) and Dr Peter Braun (Department of Biochemistry, McGill University), respectively. Monoclonal antibodies O1 and O4, ones recognizing galactosylceramide and sulfatide, respectively, were obtained from Dr S.E. Pfeiffer (University of Connecticut, Farmington, CT), F1H11 (directed against neolactotetraosyl-containing structures) was obtained from Dainabot Corp. (Tokyo, Japan). The specificities for these antibodies were previously described [10, 11].

GANGLIOSIDE EXTRACTION, PURIFICATION AND IDENTIFICATION

The methods for ganglioside isolation and identification were as described by Ledeen and Yu [12] and Sung *et al.* [13]. In brief, tumour samples weighing 0.3–1.0 g were lyophilized overnight and extracted twice in 20 volumes of chloroform:methanol:water (1:2:20%, by vol) and (1:1:5%, by vol) with respect to their fresh weights. Total lipid extracts were then subjected to either a modified Folch partition [14] or DEAE-Sephadex chromatography [15] to separate gangliosides from neutral glycolipids. Ganglioside portions were desalted by Bakerbond SPE*C-18 reverse phase column (J.T. Baker, Phillipsburg, NJ). Total gangliosides were quantitated by the colorimetric resorcinol-HCl method based on the sialic acid content [16, 17]. For each tumour sample, aliquots of 1.5 μg total ganglioside sialic acid were spotted on HPTLC plates (Kieselgel 60, E. Merck, Darmstadt, W. Germany) and developed in chloroform:-methanol:0.2% $CaCl_2$ in water (55:45:10). The HPTLC plates were sprayed with resorcinol reagent and heated to visualize separated gangliosides and densitometrically scanned in the reflection mode (Shimadzu CS-9000, Kyoto, Japan) at 580 nm.

Neolacto- and lactotetraosyl-containing gangliosides were identified by HPTLC immunostaining using the monoclonal antibody F1H11 after *Clostridium perfringens* neurominidase (Type V, Sigma) treatment with the Avidin-Biotin alkaline phosphatase system [18]. These gangliosides were quantitated by external calibration curves using known amounts of sialylparagloboside and lactotetraosylceramide (Eric Holms, Pacific Northwest Research Foundation, Seattle, WA and Bruce Macher, San Francisco State University, San Francisco, CA) as standards and quantitated by scanning densitometry.

IMMUNOHISTOCHEMISTRY

Immunoperoxidase staining

The methods for immunohistochemical staining of frozen tissues were previously described [19]. Frozen sections were cut 6 μm thick on a cryostat, air-dried and fixed in 4% paraformaldehyde in phosphate buffered saline for 5 min. To block endogenous peroxidase activities and non-specific binding, tissue sections were treated with 2.25% H_2O_2 in methanol and 5% normal goat serum for 15, and 30 min, respectively. For F1H11 staining, sections

were treated with neuraminidase isolated from *Arthrobacter ureafaciens* (Calbiochem, La Jolla, CA) at the concentration of 40 mU ml^{-1} in 0.05 M acetate buffer, pH = 5.0 prior to H_2O_2 application. Tissue sections were then incubated with primary antibodies individually (anti-MBP, PLP, CNPase, F1H11, O1 and O4) for 1.5 h at 37 °C as described above with subsequent exposure to secondary antibodies for 1 h at 37 °C. Peroxidase conjugated goat anti-rabbit IgG (Boehringer Mannheim, Indianapolis, IN) and peroxidase conjugated goat anti-mouse IgM (Sigma, St. Louis, MO) were used as secondary antibodies against myelin proteins (MBP, PLP and CNPase) and F1H11, O1 and O4, respectively. Colour development was with the 3-amino-9-ethyl carbazole (AEC) chromogen system (Lipshaw, Pittsburg, PA). Slides were counterstained for 1 min in Gill's Hemotoxylin. Normal rabbit serum and mouse IgM (DAKO) were used as negative controls for primary antibodies.

Staining frequence and intensity

Estimations of staining intensity were estimated as faint, intermediate, and strong. The immunopositive proportion of cells was scored as follows: 0 = completely undetectable; rare = those with immunopositive cells less than 10% of the total cell population; + = those with immunopositive cells between 10% and 20%; ++ = those with immunopositive cells between 20% and 50%, +++ = those with immunopositive cells over 50% of the total cell population. Normal human brain provided an external positive control and positive staining of residual myelin provided internal positive controls.

Results

Ganglioside patterns

Total ganglioside sialic acid contents as well as percent distributions of individual gangliosides for different grades and subtypes of tumours are shown in Tables 1a, 2a,b. Differences in the patterns of major gangliosides between pure oligodendrogliomas and mixed gliomas were not statistically significant. Neither did the compositions of major gangliosides correlate with WHO grade. Tumours with a 1b ganglioside content (GD1b, GT1b and GQ1b) over 30% of total gangliosides occurred more frequently in WHO grade I and II tumours (47%) and Grade III tumours (40%) than in the grade IV (25%) group. Although this pattern was not statistically significant, it followed that seen in our previous studies of astrocytomas [13].

Minor gangliosides with the neolacto-series backbones were studied by immunostaining HPTLC using monoclonal antibody F1H11 after neuraminidase treatment. Major neolacto-series gangliosides in these tumours were

Table 1. a. Total and individual ganglioside content in 24 oligodendrogliomas by WHO histological grade

	Grade 1 and 2[a] *(n^b = 15)*	*Grade 3 (n^b = 5)*	*Grade 4 (n^b = 4)*
Total NeuAc[c]	*990 ± 140*[d]	*1020 ± 120*	*900 ± 290*
GM3	84 ± 28	99 ± 30	98 ± 42
GM2	63 ± 17	56 ± 39	63 ± 38
GM1	127 ± 35	130 ± 23	85 ± 49
GD3	205 ± 47	210 ± 40	196 ± 59
GD1a	123 ± 38	166 ± 26	122 ± 76
GD2	91 ± 19	71 ± 4	97 ± 12
GD1b	144 ± 34	145 ± 22	105 ± 41
GT1b	125 ± 34	101 ± 12	104 ± 36
GQ1b	27 ± 7	25 ± 7	23 ± 6

Data are shown as the mean ± SEM.
[a]Only two cases were grade I.
[b]*n* is the number of cases studied.
[c]Total and individual gangliosides are expressed as μg sialic acid per g dry weight.
[d]One specimen was not large enough to perform total sialic acid estimation.

b. Percentage distribution of sialic acid among gangliosides in 24 oligodendrogliomas by WHO histological grade

	Grade 1 and 2[a] *(n^b = 15)*	*Grade 3 (n^b = 5)*	*Grade 4 (n^b = 4)*
GM3	9.5 ± 3.3	8.9 ± 1.9	11.0 ± 2.1
GM2	6.9 ± 1.9	4.7 ± 2.2	5.3 ± 1.7
GM1	11.8 ± 0.6	13.1 ± 2.3	7.5 ± 3.2
GD3	20.7 ± 3.4	20.2 ± 2.5	25.2 ± 7.7
GD1a	11.1 ± 2.1	16.0 ± 1.1	10.1 ± 4.6
GD2	10.0 ± 1.7	7.4 ± 1.2	14.8 ± 4.4
GD1b	13.6 ± 2.3	14.6 ± 2.3	10.2 ± 2.7
GT1b	11.8 ± 1.8	10.0 ± 1.0	11.1 ± 1.8
GQ1b	3.2 ± 0.2	2.7 ± 0.8	3.0 ± 0.8

Data are shown as the mean ± SEM.
[a]Only two cases were grade I.
[b]*n* is the number of cases studied.

3′-LM1 and 6′-LM1. The presence of 6′-LM1 correlated significantly with higher grades (III and IV) of tumours ($p \simeq 0.02$ in χ^2 test) (Table 3). However, ganglioside 3′-LM1 and total neolacto gangliosides did not correlate with higher grade tumours. The presence of 3′-LM1 correlated with the low 1b ganglioside content (less than 30% of total gangliosides), χ^2 $p \simeq 0.0078$. Ganglioside 6′-LM1 did not have the same relationship.

Immunohistochemical studies of galactocerebroside, sulfatide, and neolacto-series glycoconjugates

Galactocerebroside (GalCer), one of the major components of myelin and a differentiation marker of oligodendrocytes, can be detected immunohistochemically using O1 antibody. Among 22 tumours studied, 16 were immunonegative; only two (one a mixed tumour, the

Table 2. a. Total and individual ganglioside contents in pure and mixed oligodendrogliomas

	Pure ($n^{a,b} = 16$)	*Mixed* ($n^{a} = 8$)	*Grey matter* ($n = 2$)
Total NeuAc[c]	*1000 ± 100*	*800 ± 200*	*2874*
GM3	94 ± 25	79 ± 29	18
GM2	57 ± 14	71 ± 29	198
GM1	118 ± 28	125 ± 45	415
GD3	223 ± 42	168 ± 41	484
GD1a	143 ± 35	109 ± 42	757
GD2	93 ± 17	77 ± 12	219
GD1b	152 ± 30	108 ± 28	607
GT1b	136 ± 30	77 ± 19	466
GQ1b	28 ± 7	21 ± 5	51

Data are shown as the mean ± SEM.
[a]*n* is the number of cases studied.
[b]One specimen was not large enough to perform total sialic acid estimation.
[c]Total and individual gangliosides are expressed as μg sialic acid per g dry weight.

b. Percentage distribution of sialic acid among gangliosides in pure and mixed oligodendrogliomas

	Pure ($n^{a,b} = 16$)	*Mixed* ($n^{a} = 8$)	*Grey matter* ($n = 2$)
GM3	10.0 ± 3.0	8.8 ± 1.9	1.0
GM2	6.3 ± 1.6	6.0 ± 2.1	3.4
GM1	10.6 ± 1.8	12.8 ± 3.5	14.5
GD3	20.9 ± 3.1	22.7 ± 4.2	8.5
GD1a	12.6 ± 2.0	10.5 ± 2.2	26.6
GD2	9.8 ± 1.6	11.2 ± 2.5	7.7
GD1b	13.4 ± 1.2	12.9 ± 2.1	21.0
GT1b	12.1 ± 1.6	9.9 ± 1.3	16.1
GQ1b	2.7 ± 0.5	3.8 ± 1.1	1.7

Data are shown as the mean ± SEM.
[a]*n* is the number of cases studied.
[b]One specimen was not large enough to perform total sialic acid estimation.

Table 3. Relationship of neolacto-series gangliosides and WHO histological grade

WHO Grade	*3′-LM1*	*6′-LM1*	*Total neolacto gangliosides*
Grade 1 and 2 ($n^{a} = 15$)	presence (5/15) 33%	presence (1/15) 6.7%	presence (6/15)[b] 40%
Grade 3 ($n^{a} = 5$)	presence (2/5) 40%	presence (3/5) 60%	presence (3/5) 60%
Grade 4 ($n^{a} = 5$)	presence (3/4) 75%	presence (2/4) 50%	presence (4/4) 100%
χ^2 test, *p* value	0.32	0.02	0.09

[a]*n* is the number of cases studied.
[b]Neolacto-series ganglioside other than 3′-LM1 and 6′-LM1 was detected in one case.

Table 4. Immunohistochemistry with different antibodies against myelin glycolipids and proteins in oligodendrogliomas

Degree of positivity	*O1*	*O4*	*MBP*	*PLP*	*CNPase*	*F1H11*
O[a]	16[b]	23	23	20	19	10
rare	2[c]	0	0	1[d]	0	7
+	0	0	0	0	0	2
++	1[e]	1[e]	0	0	0	4
+++	2[f]	0	0	0	0	1
Uncertain	1	0	1	0	1	0
Total cases studied	22	24	24	21	20	24

[a]The percentage of neoplastic positive cells was scored as: 0, completely undetectable; rare, those with immunopositive cells less than 10% of the total cell population; +, those with immunopositive cells between 10% and 20%; ++, those with immunopositive cells between 20% and 50%; +++, those with immunopositive cells over 50% of the total cell population; uncertain, not certain whether the stained material is myelin debris, tumour cell membranes or trapped oligodendrocytes.
[b]Values represent the number of cases in each subgroup.
[c]Both cases were pure oligodendrogliomas.
[d]Some cells were positive, but it is not certain whether the stained cells were tumour cells or vascular elements.
[e]This was a mixed oligodendroglioma; the cells positively staining with O1 and O4 were astrocytic elements.
[f]One of these cases is a mixed oligodendroglioma containing immunopositive astrocytic elements.

other a pure oligodendroglioma) contained over 50% positive tumour (Table 4). O1 stained most of the fibrillary cells, gemistocytes and minigemistocytes, polar cells and some small tumour cells (Fig. 1A,B). The mixed tumours (oligoastrocytomas) consisted of negatively stained small cells and positively stained fibrillated cells. In contrast; O1-immunopositive tumour cells occurred very frequently in astrocyomas [19].

Sulfatide is also a marker for oligodendrocytes and myelin, one detectable using O4 antibody. The O4 immunopositive cells types were cytologically similar to O1 immunopositive cell types (Fig. 1C,D), but were less frequent (Table 4). One tumour with an immunopositivity score of '++' consisted of <1% O4 positive small cells and 50% O4 positive fibrillary cells.

We also immunohistochemically examined sialylneolactotetraosyl-containing backbone structures using F1H11 after neuraminidase treatment of tissue sections. F1H11 with neuraminidase treatment resulted in staining of small cells, some fibrillary cells, macrophages, and endothelial cells (Fig. 2A,B). In contrast to O1 and O4, 14 of 24 tumours demonstrated varying degrees of immunopositivity. No significant difference in terms of the percentage of tumour cells stained by O1, O4 and F1H11 was seen between pure oligodendrogliomas and mixed tumours.

Immunohistochemical studies of myelin proteins

Three major myelin proteins, myelin basic protein (MBP), proteolipid protein (PLP), and 2′,3′-cyclic nucleotide phosphodiesterase (CNPase) were selected as markers for the further immunohistochemical study of these 24 tumours. Frozen sections of normal human brain obtained at autopsy were processed as positive controls. In addition, residual myelinated fibres within tumour tissues always stained positively, serving as internal positive controls. The polyclonal antibodies for MBP, PLP, and CNPase generally stained myelin and myelin debris, but did not label neoplastic oligodendrocytes (Fig. 2C,D). The observed morphological patterns of myelin staining are summarized in Fig. 3. These data indicate that tumours classified as oligodendrogliomas based upon histopathological diagnoses rarely stained with antibodies that recognize myelin markers.

Discussion

Immunohistochemical studies of potential markers of oligodendrogliomas have previously been reported by several groups [5–9, 20]. To date, however, there has been no detailed study of the ganglioside composition of oligodendrogliomas. In our present study we combined immunoTLC and immunohistochemical techniques to study the expression of glycolipids and myelin proteins in a series of 24 oligodendrogliomas.

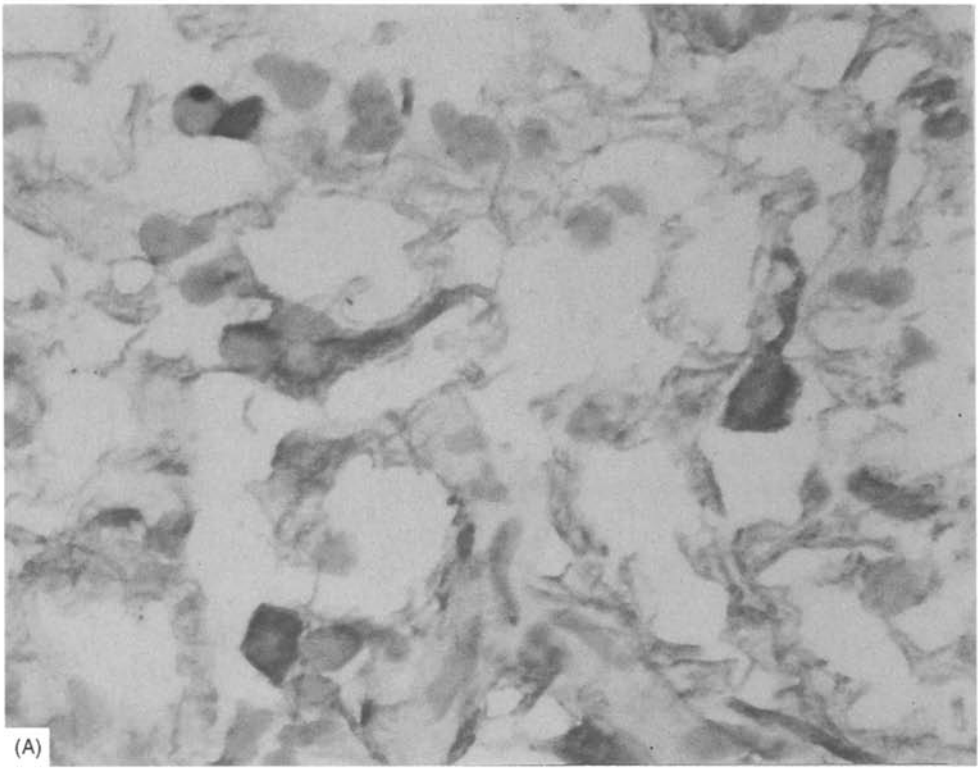
In a previous study of astrocytomas and primitive neuroectodermal tumours [13], we found that a decrease in the proportion of gangliosides of the 1b pathway correlated with an increase in histological tumour grade. Patients with tumours in which 1b gangliosides accounted for less than 30% of total ganglioside content had poorer survival rates [21]. In this study, grade III and IV oligodendrogliomas more often had a lower proportion of 1b gangliosides than did tumours of grades I or II. The ganglioside compositions of our 24 oligodendrogliomas were somewhat similar to those of anaplastic astrocytomas (AA), the proportions of 1b ganglioside being higher than either glioblastoma multiforme or primitive neuroectodermal tumour [13]. Singh *et al.* [22] reported that the neutral glycolipid composition of oligodendrogliomas was also similar to that of AA, having higher levels of ceramide monohexoside and infrequently containing ceramide trihexoside or globoside.

Gangliosides have a variety of effects that have been demonstrated in whole animals, cultured cells and cell-free biochemical reactions. The most consistent biological responses to gangliosides are an inhibition of cell division and stimulation of cellular differentiation [23–26]. Evidence is accumulating that at least some of these effects are mediated by gangliosides modulating the activation of specific cell surface receptors. Some, such as the platelet-derived growth factor receptor are inhibited [27], while TrkA is activated [28]. Because gangliosides affect such a variety of receptor mediated cell functions, it has been suggested that they may be involved in coordinating diverse receptor mediated events by altering functional protein-protein interactions, such as receptor dimerization [29]. This requires specific oligosaccharide binding domains on critical regions of such regulated proteins. Gangliosides are biosynthesized by adding one sugar at a time to the nascent oligosaccharide chain (Fig. 4). This provides an efficient mechanism for regulating their oligosaccharide structures and, thus, activities of these regulatory molecules. By this model the loss or activation of one glycosyltransferase could have a profound effect on the biology of a cell due to the change in its ganglioside composition.

There is considerable evidence that the ganglioside compositions of cells change as a consequence of malignant transformation [30, 31], but this is the first report on the ganglioside composition of a sizable series of human oligodendrogliomas. The differences in gangliosides between the tumours and brain could be due to a change in the ganglioside metabolism of the tumour cells or reflective of the nontumour cellular elements within the tumour mass. It is possible that coincident with the events involved in malignant transformation that there is a shift away from the ganglio series gangliosides that predominate in normal brain (GM3 and its derivatives shown in Fig. 4) towards the neolacto series gangliosides such as 6′-LM1. Alternatively (or additionally) 6′-LM1 could be present on non-tumour cells such as infiltrating hematogenous cells. However, this is not a usual histological feature of oligodendrogliomas, so it seems even less likely of an explanation for the presence of these minor gangliosides than for astrocytomas in which they also occur. Resolution of this issue must await results of immunohistochemical studies.

Whereas the presence of 6′-LM1, as detected by immunostaining on HPTLC, also correlated with higher tumour grade, 3′-LM1 did not have this association. Here again we demonstrated that 6′-LM1 is associated with higher grade gliomas. Interestingly, 6′-LM1 correlated with higher grades [13] and poorer survival in astrocytic tumours [21]. Determination of the prognostic value of 6′-LM1 and 1b gangliosides in oligodendrogliomas will require a larger series of cases.

Immunohistochemical staining for GalCer and sulfatide in our 24 oligodendrogliomas utilizing O1 and O4 demonstrated that the tumour cells do not express these glycolipids nearly as frequently as do astrocytic tumours [19]. Furthermore, immunohistochemical studies showed that the tumour cells in oligodendrogliomas do not express significant amounts of myelin proteins (MBP, PLP, and CNPase). Others have similarly reported that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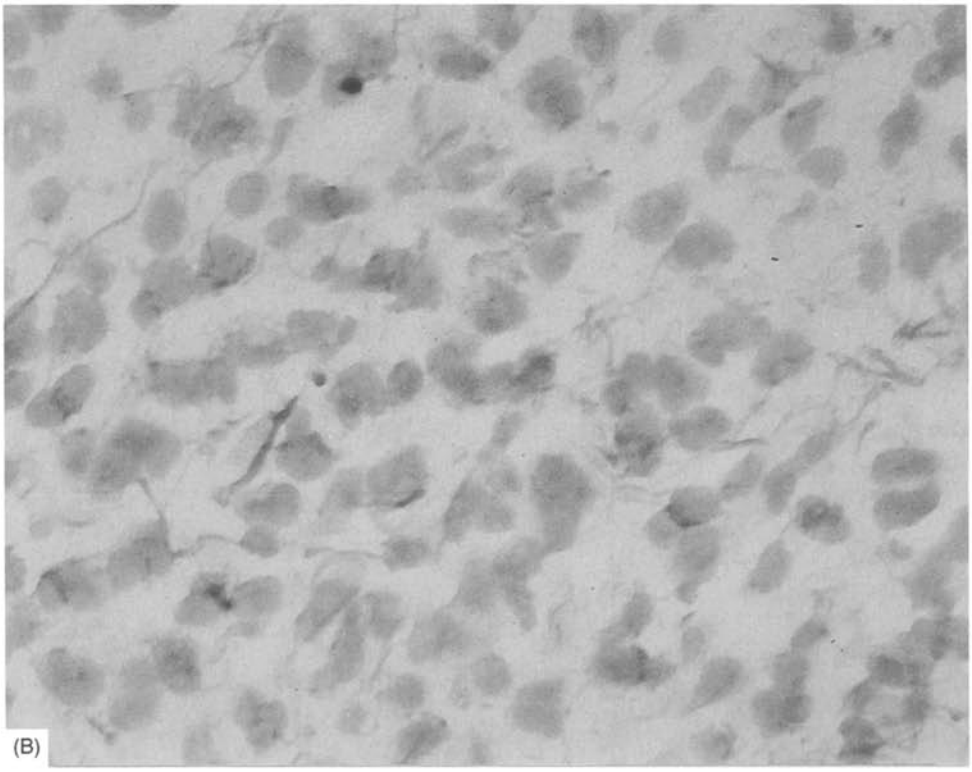
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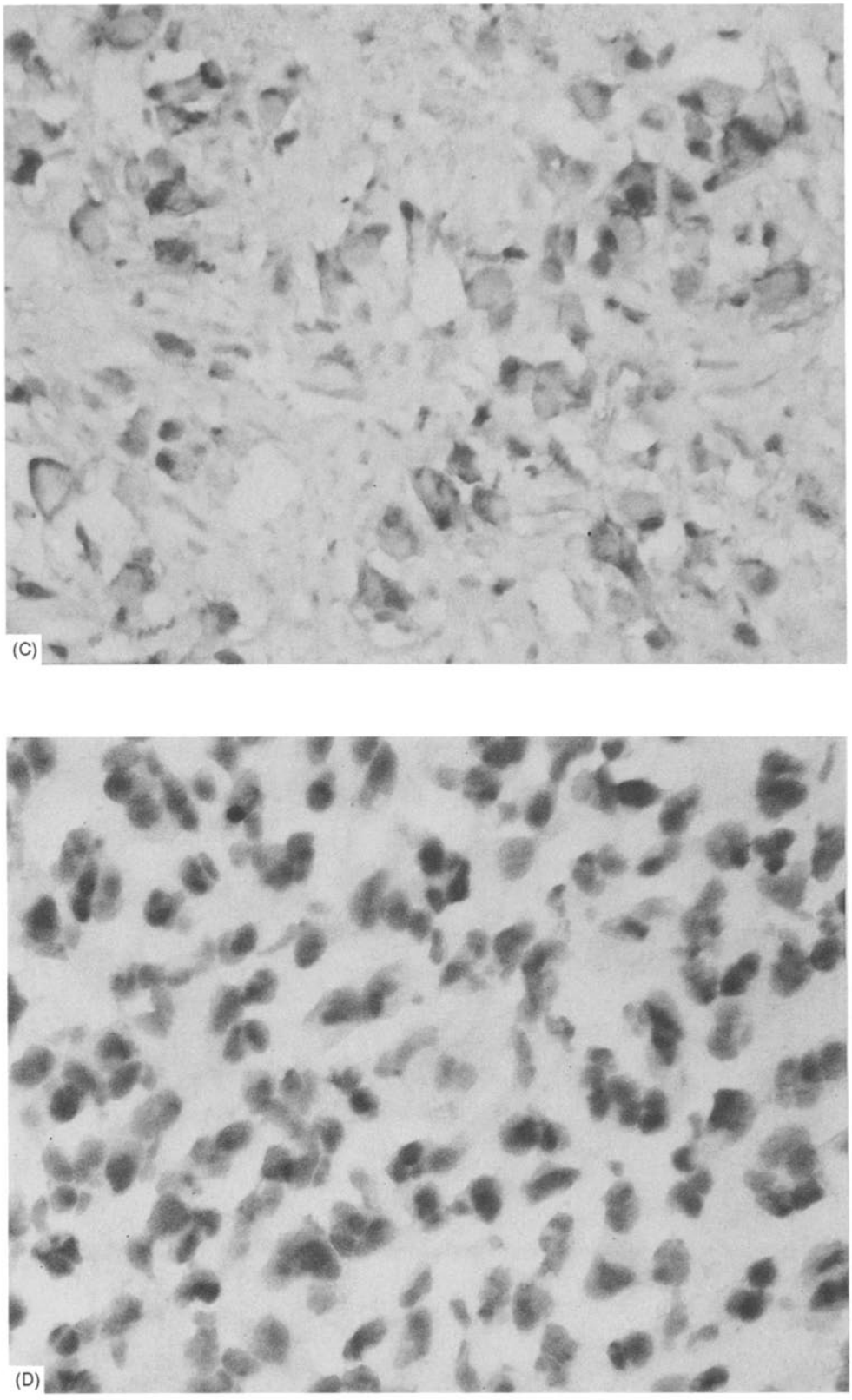
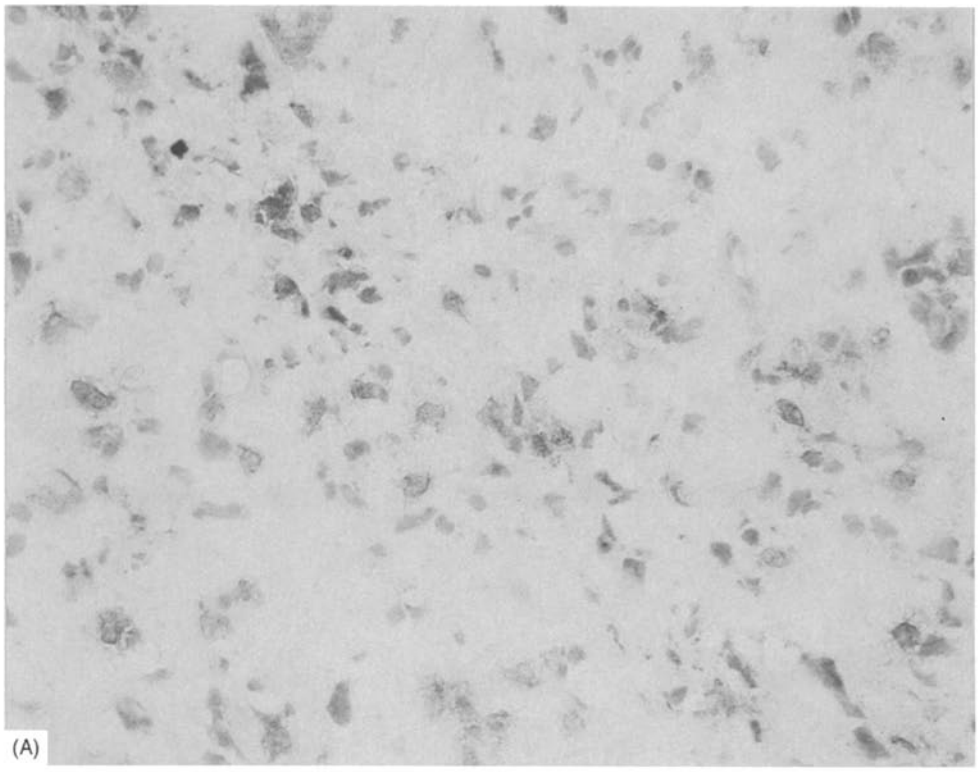
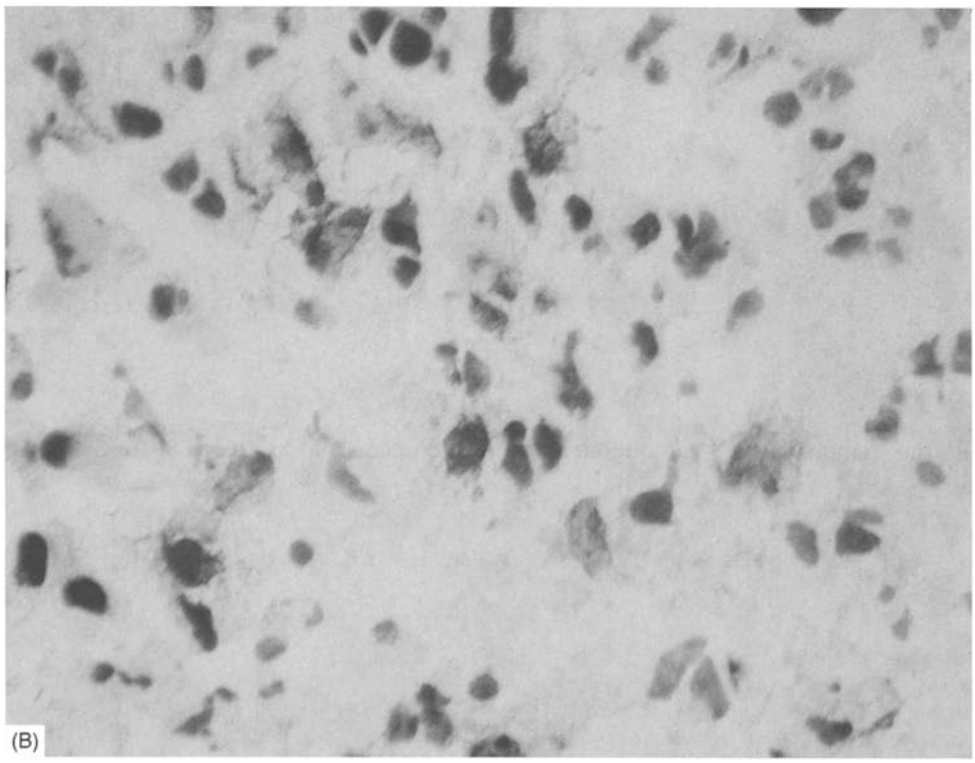


Figure 1. Immunohistochemical stains of a mixed glioma with areas of oligodendroglioma and astrocytoma. (A) O1 antibody against galactocerebroside staining astrocytoma cells positively X640. (B) O1 antibody; small cell (oligodendroglioma) area staining negatively X640. (C) O4 antibody against sulfatide staining minigemistocytes and fibrillary astrocytoma cells ×400. (D) O4 antibody; small cell (oligodendroglioma) area staining negatively ×640.


(A)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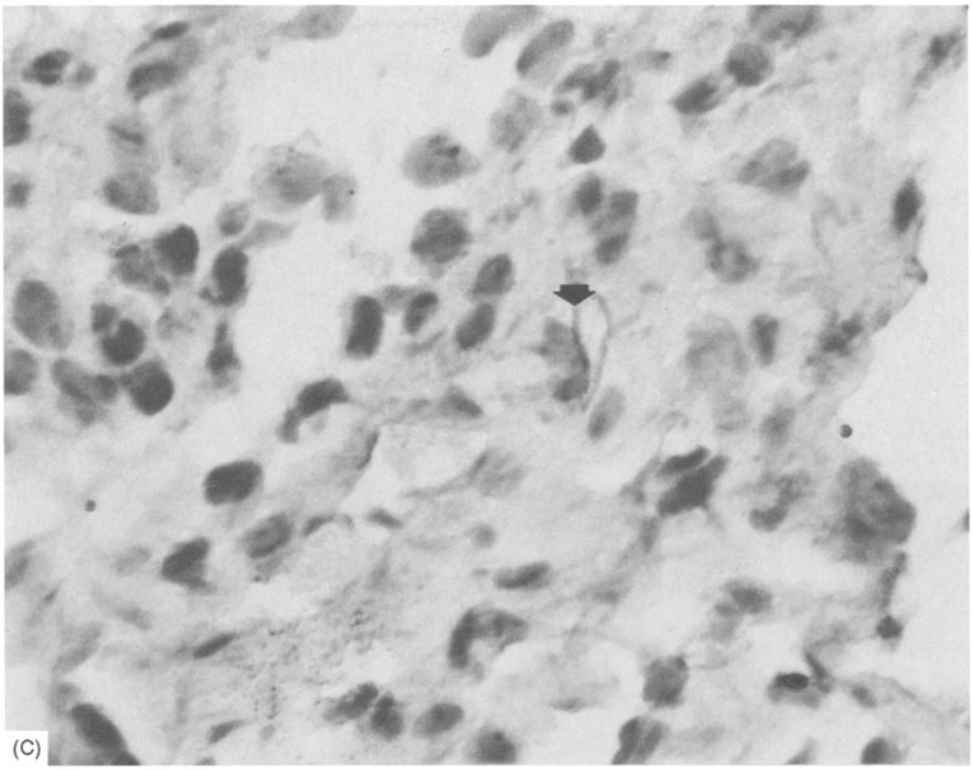

Figure 2. Immunohistochemical stains of oligodendroglioma. (A) F1H11 antibody against lactoneotetraosyl (Type 2 Lacto) oligosaccharide ×250; (B) Same specimen as Fig. 2A at a magnification of ×640. (C) Example of myelin staining with O4 that might be confused with tumour cell staining. Arrow head points to one of two myelinated structures similar to that shown in Fig. 3 where myelin staining adjacent to the membrane continues away from the cell ×640.

oligodendroglioma cells were immunonegative for MBP [8], but reactive for GFAP [5–8, 20]. Kennedy *et al.* [20] studied the expression of several antigens related to oligodendroglial development by cells cultured from human gliomas (including oligodendrogliomas). Their results indicated no correlation with morphological classification. Collectively, these findings suggest either that the expression of glycolipids and proteins characteristic of oligodendrocytes is lost by oligodendroglioma tumour cells or present at levels undetectable by the methods we used. Alternatively, the tumours we refer to as oligodendrogliomas are not oligodendrocyte derived.

One group of investigators interpreted their immunohistochemical results as demonstrating that oligodendroglioma tumour cells express MBP [32]. In the present study we interpreted morphologically similar findings as being due to reactivity of myelin or myelin debris and not as staining of tumour cells. Immunoreactive entrapped and altered myelin elements within the substance of gliomas yields several patterns, some of which superficially resemble staining of tumour cell membranes, encountered several such patterns that are diagrammatically represented in Fig. 3.

Recent evidence demonstrates that, depending upon the culture medium, glial progenitor cells *in vitro* may develop into astrocytes or oligodendrocytes [33, 34]. Choi *et al.* [4, 35] postulated that oligodendroglia in the developing central nervous system may arise from astroglial precursors. This suggestion was based on the finding of: (a) an increase in the mitotic activities of rat subpial astocytes prior to the onset of myelination followed by the appearance of oligodendrocytes in the same regions; and (b) the presence of transitional cells with both astocytic and oligodendrocytic characteristics. The light and electron microscopic findings of Min and Scheithauer [36] based upon the study of 30 classic oligodendrogliomas, also suggest that oligodendrogliomas are related to or derived from a unique glial cell, one with considerable variation in cytological and astocyte associated ultrastructural features.

Our results as well as the observations of others underscore the problems inherent in traditional classifications of gliomas, ones based upon morphological and cytological criteria. Such schemes designate tumours by their resemblance to normal fully differentiated glial cells which they resemble and from which they are presumed to be derived. Future progress in tumour classification awaits molecular studies of the characteristics of glial

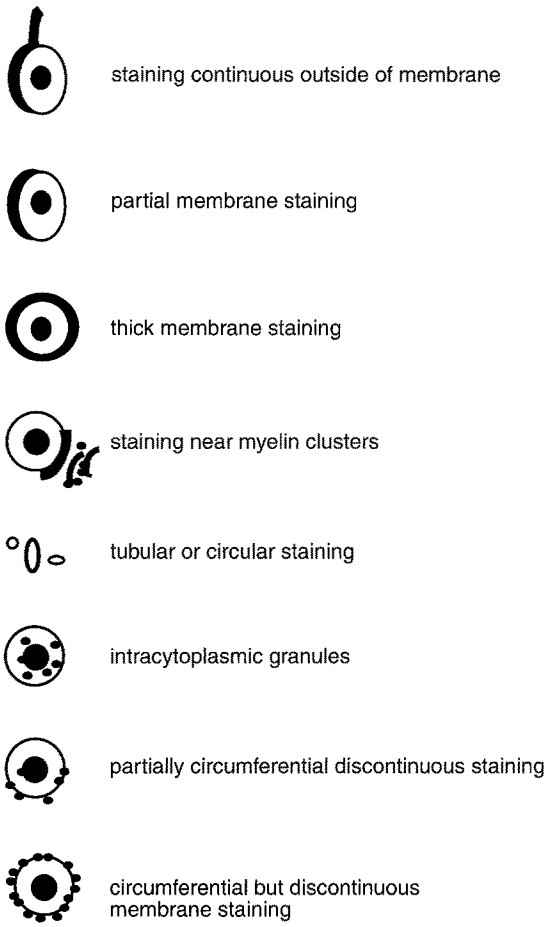

Figure 3. Diagrammatic representations of relations that immunostained myelin debris can have to tumour cells. These can give a false appearance of positive immunostaining of the tumour cells.

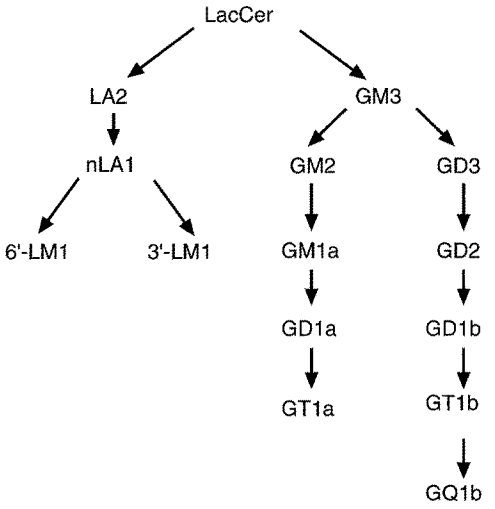

Figure 4. Schematic diagram showing the biosynthetic steps leading to the formation of the gangliosides discussed in the text. LacCer is lactosylceramide; LA2 is lactotriosylceramide; nLA1 is neolactotetraosylceramide.

progenitor cells and of gliomas. For the purpose of directing therapy and prognostication, we are confident that it will be necessary to classify brain tumours on this basis rather than upon histology alone.

Acknowledgements

The authors gratefully acknowlege the generous gifts of antibodies from the following: Dr Denise Wood, Toronto Hospital for Sick Children (MBP), Dr Wendy B. Macklin, Mental Retardation Research Center, UCLA (PLP), Dr Peter Braun, Department of Biochemistry, McGill University (CNPase), Dr S.E. Pfeiffer, University of Connecticut, Farmington, CT (O1 and O4), Dainabot Corp., Tokyo, Japan (F1H11).

This work was supported by a grant from the National Cancer Institute UO1 CA50910, NINDS 1P20 NS31087, and by the Department of Pathology and the College of Medicine, The Ohio State University. Some of the specimens were obtained from the NCI Cooperative Human Tissue Network and the Children's Cancer Group.

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