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G_{M1b} and G_{M1b}-GalNAc: major gangliosides of murine-derived macrophage-like WEHI-3 cells

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WEHI-3 cells, derived from a BALB/c mouse, are a myelomonocytic leukemic cell line with macrophage-like properties. We have isolated, purified and characterized the monosialogangliosides from WEHI-3 cells by 1D-HPTLC, 2D-HPTLC, enzymatic degradation, HPTLC-immunostaining, gas liquid chromatography and fast atom bombardment-mass spectrometry (FAB-MS). Quantitative 2D-HPTLC shows two monosialogangliosides are the major components, constituting 77% of the total, with a third monosialoganglioside being 3%. The two major components were identified as (NeuAc)G_{M1b} and (NeuAc)G_{M1b}-GalNAc and the minor component as (NeuAc)G_{M1b}-GalNAc-Gal. The presence of G_{M1b} in this myelomonocytic cell line is consistent with its presence in other murine immune cells and tissues. G_{M1b}-GalNAc and GM_{1b}-GalNAc-Gal have been reported in T-lineage cells but not in resident or stimulated murine macrophages. Each of these monosialogangliosides belongs to the asialoG_{M1} synthetic pathway. Preliminary results indicate a disialo member of this pathway, G_{D1c}, may also be present as a minor component. This ganglioside pathway, containing species which are not sialylated on the internal galactose, appears to be dominant in and may be characteristic of murine immune cells.

Introduction

WEHI-3 cells are a myelomonocytic macrophage-like cell line derived from a BALB/c mouse. G_{M1}-Fuc was reported to be the major ganglioside of this cell line, constituting about 45% of the total gangliosides [1]. It has been implicated as a tumor-associated glycolipid [2], a putative receptor for the macrophage migration inhibition factor [3–5] and a human peripheral nerve antigen potentially involved in autoimmune disease [6]. Additionally, fucogangliosides have been identified as components of human peripheral blood monocytes and elicited mouse peritoneal macrophages [7]. Because of its reported high concentration in WEHI-3 cells, we intended to exploit this cell line as an endogenous source of G_{M1}-Fuc for studying murine macrophage function.

In the course of isolating and confirming the previous characterization of this ganglioside, we found that its 2D-HPTLC mobility was not that of G_{M1}-Fuc. Concomitantly, we found that the second most abundant ganglioside of this cell line, previously identified as G_{M1a} [1], was sialidase-sensitive, a finding inconsistent with a G_{M1a} structure. Therefore, we initiated a more vigorous characterization of the monosialogangliosides. Preliminary results have been reported [8].

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Abbreviations: NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid; aG_{M1}, $GgOse_4Cer$; G_{M3}, II^3NeuAc-LacCer; G_{M2}, II^3NeuAc-$GgOse_3Cer$; G_{M1a}, II^3NeuAc-$GgOse_4Cer$; G_{M1b}, IV^3NeuAc-$GgOse_4Cer$; G_{M1b}-GalNAc, IV^3NeuAc-$GgOse_5Cer$; G_{M1b}-GalNAc-Gal, IV^3NeuAc-$GgOse_6Cer$; G_{D1c}, $IV^3(NeuAc)_2$-$GgOse_4Cer$; G_{M1}-Fuc, IV^2Fuc,II^3NeuAc-$GgOse_4Cer$; FAB-MS, fast atom bombardment-mass spectrometry.

Materials and Methods

Cell culture

The WEHI-3 cell line was obtained from ATCC (Rockville, MD) and, using references supplied by ATCC, was identified as the differentiable WEHI-3B (D^+) line [9,10]. Cells were cultured in 75 cm^2 flasks in RPMI 1640 containing 5% FCS and penicillin-streptomycin solution (25 U/ml-25 μg/ml) (Whittaker Bio-

products, Walkerville, MD). Media was changed every 1 or 2 days and cells were harvested, with concomitant reculture, every 4–5 days.

Ganglioside isolation and quantitation

Gangliosides were isolated by DEAE-Sephadex A-25 (Pharmacia, Piscataway, NJ) column chromatography, base treatment, Sep-Pak (Waters Associates, Waltham, MA) desalting and Iatrobeads 6RS-8060 (Iatron Laboratories, Tokyo, Japan) column chromatography as previously described [11]. Total lipid bound sialic acid was determined by the resorcinol assay [12] and the percentage composition of each ganglioside spot on 2D-HPTLC (E. Merck, Darmstadt, Germany) was quantitated [13,14] using a Microscan 1000 densitometer (Technology Resources, Nashville, TN).

DEAE-Toyopearl column chromatography

Total gangliosides (40 μg sialic acid) from WEHI-3 cells were applied to a 2 ml bed volume DEAE-Toyopearl-650M column (TosoHaas, Philadelphia, PA) in 1 ml of methanol and the column was washed with an additional 3 ml of methanol. Monosialogangliosides were eluted with 12 ml of 0.025 M sodium acetate in methanol and a small polysialoganglioside fraction with 16 ml of 0.05 M sodium acetate in methanol. Following rotary evaporation of the solvent, each fraction was treated for 30 min at 37°C in 5 ml of 0.1 M aqueous NaOH to cleave any lactones possibly formed during the column fractionation. Each fraction was then desalted on Sep-Pak before analysis.

High-performance thin-layer chromatography of gangliosides

Both analytical and preparative 1D-HPTLC were done using 10 × 20 cm plates (E. Merck) chromatographed in the 10 cm dimension using chloroform/methanol/0.25% aqueous KCl (50:45:10, v/v) as the developing solvent. The preparative ganglioside sample (45 μg sialic acid) was applied via a mechanical plate streaker (Alltech Associates, Deerfield, IL). The 2D-HPTLC system, which uses chloroform/methanol/0.25% aqueous KCl (50:45:10, v/v) in the first dimension and chloroform/methanol/0.25% KCl in 2.5 M aqueous NH_3 (50:40:10, v/v) in the second dimension, was previously described [11]. The mobility and composition of bands 5 and 6 were reexamined by spotting aliquots of these isolated bands on 2D-HPTLC. Resorcinol-positive moieties were distinguished between 1D-HPTLC and 2D-HPTLC as numbered bands and numbered spots, respectively.

Enzymatic treatment of the monosialoganglioside fraction

For sialidase treatment, the monosialoganglioside fraction (3 μg sialic acid) was dried under nitrogen then lyophilized for 30 min. *Clostridium perfringens* sialidase (0.5 U; Sigma, St. Louis, MO) in 250 μl of 0.05 M sodium citrate/sodium phosphate buffer (pH, 5.5) was added and the mixture was incubated at 37°C for 1.5 h. The reaction was stopped by adding 2 ml of 0.1 M aqueous NaOH. This mixture was further incubated for 30 min at 37°C to cleave any lactones that may have formed. The sample was desalted on a Sep-Pak column and examined by 2D-HPTLC using the system described above.

For β-galactosidase treatment [15], the monosialoganglioside fraction (6 μg sialic acid) was dried under nitrogen, 100 μl of 50 mM citrate/phosphate buffer (pH 4.5) containing 50 μg Triton X-100 was added and the sample lyophilized. Bovine testes β-galactosidase (0.06 U in 200 μl of 3.2 M ammonium sulfate (pH 5), as shipped (Sigma) was added and the mixture incubated at 37°C for 20 h. The reaction was stopped by adding 1 ml of 0.1 M aqueous NaOH and the mixture was made slightly acidic by adding 0.1 M aqueous HCl and then immediately desalted via a Sep-Pak column. Human brain G_{M1a} was used as a control and in all experiments was converted to G_{M2}.

HPTLC-immunostaining of gangliosides

HPTLC-immunostaining of the total ganglioside mixture (3 μg sialic acid) was previously described [14] using *C. perfringens* sialidase (1 U/ml) and an anti-asialoG_{M1} monoclonal antibody derived from the SH34 cell line [16]. BALB/c thioglycollate elicited macrophage gangliosides, which have been previously described [14], were used as a positive control.

Gas liquid chromatography of the monosialoganglioside fraction

The monosaccharides of the monosialoganglioside fraction were analyzed as the trifluoroacetyl derivatives of their methyl glycosides [14], using a Perkin-Elmer 8500 gas chromatograph (Perkin-Elmer, Norwalk, CT), fitted with a 30 m DB-5 capillary column (J&W Scientific, Folsom, CA).

Negative ion fast atom bombardment-mass spectrometry

Mass spectrometry (VG ZAB-SE double focusing mass spectrometer, VG Analytical, U.K.) of bands 4, 5 and 6 (0.4 μg sialic acid each) and the total monosialoganglioside fraction (1–2 μg sialic acid) was done as previously described [14] with the exception of hexamethylphosphoramide(HMPA)-glycerol being used as the matrix [17].

Results

Isolation and quantitation of gangliosides from WEHI-3 cells

The 2D-HPTLC of the total gangliosides from WEHI-3 cells is shown in Fig. 1. From the resorcinol-

TABLE I
Ganglioside silic acid distribution in WEHI-3 cells

Ganglioside [a] number	% Distribution ($n = 3$, ± S.D.)
1	10.9 ± 2.2
2	2.3 ± 0.9
3	0.3 ± 0.4
4	5.0 ± 2.4
5	0.9 ± 0.7
6	0.2 ± 0.3
7	2.9 ± 0.8
8	0.2 ± 0.1
9	0.1 ± 0.1
10	26.4 ± 3.0
11	7.1 ± 1.9
12	0.3 ± 0.6
13	32.9 ± 3.2
14	10.5 ± 3.4
15	0.1 ± 0.1
Total sialic acid (ng/10^6 cells)	65 ± 18

[a] Ganglioside number corresponds to Fig. 1, bottom.

sprayed chromatogram (top) and the accompanying schematic derived from a densitometric scan (bottom), 15 resorcinol-positive spots are apparent, representing five components which migrate as groups of triplets. The schematic also indicates which gangliosides were eluted from the DEAE-Toyopearl column in the mono- and polysialoganglioside fractions as identified by 1D- and 2D-HPTLC of the column fractions. The percentage distribution of these 15 gangliosides and the total lipid bound sialic acid is shown in Table I. Several minor unidentified resorcinol-positive spots which were less than 1% of the total and migrated faster than these 15 spots (in the regions of G_{M2} and G_{M3}) were also detected.

Characterization of WEHI-3 cell gangliosides

(a) Column chromatography and HPTLC. To characterize the monosialogangliosides, the total mixture was separated into individual bands by preparative 1D-HPTLC and into mono- and polysialoganglioside fractions by DEAE-Toyopearl column chromatography. Fig. 2 shows the total mixture with the corresponding isolated bands and indicates which components were eluted from the DEAE-Toyopearl column in the polysialoganglioside (bands 1 and 2) and the monosialoganglioside (bands 3–6) fractions.

2D-HPTLC of isolated bands (plates not shown) revealed that band 6 contained spot 13 as the major component and spot 15 as a trace component and band 5 contained predominantly spot 10, and spots 12 (as a trace component) and 14 (Fig. 1). Both cases indicate the heterogeneity of single bands on 1D-HPTLC.

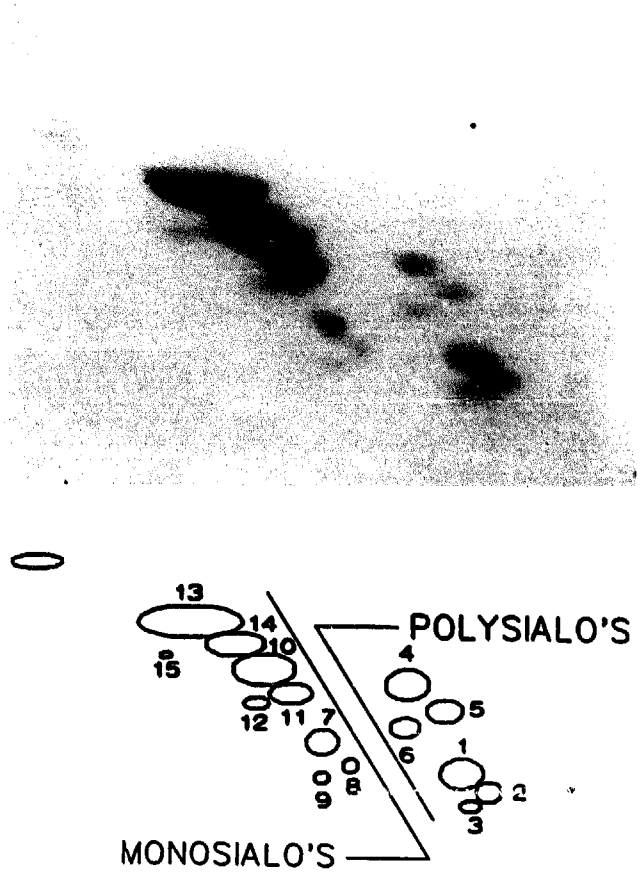

Fig. 1. 2D-HPTLC of the total ganglioside mixture from WEHI-3 cells. Resorcinol-HCl visualized chromatogram (top); and schematic derived from the densitometric scan showing ganglioside numbering and which components eluted in the mono- and polysialoganglioside fractions from the DEAE-Toyopearl column (bottom). The amount spotted is equivalent to $8 \cdot 10^7$ cells. The origin is at the lower right and chromatographic development was with chloroform/methanol/0.25% aqueous KCl (50:45:10, v/v) in the first dimension (right to left) and chloroform/methanol/0.25% KCl in 2.5 M aqueous NH_3 (50:40:10, v/v) in the second dimension (bottom to top).

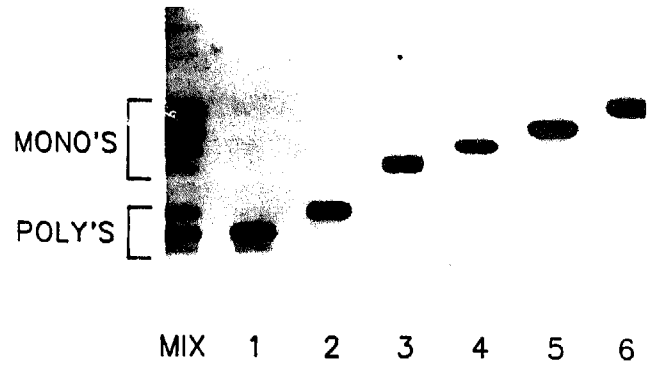

Fig. 2. 1D-HPTLC of the total ganglioside mixture (MIX) and isolated bands (1–6) from WEHI-3 cells. Bands 1 and 2 were eluted in the polysialoganglioside fraction (POLY'S) and bands 3–6 in the monosialoganglioside fraction (MONO'S) from the DEAE-Toyopearl column. Chromatographic development was with chloroform/methanol/0.25% aqueous KCl (50:45:10, v/v) and visualization was by resorcinol-HCl spray.

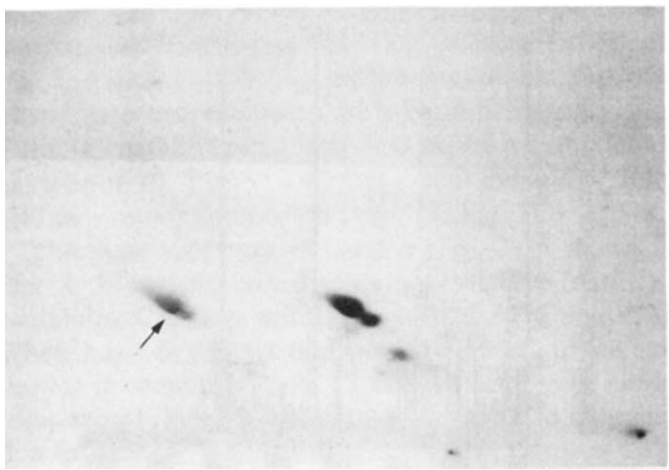

Fig. 3. 2D-HPTLC of the monosialoganglioside fraction (3 μg sialic acid) from WEHI-3 cells following in vitro treatment with 0.5 U of *Clostridium perfringens* sialidase for 1.5 h at 37°C. The origin is at the lower right and chromatographic development was with chloroform/methanol/0.25% aqueous KCl (50:45:10, v/v) in the first dimension (right to left) and chloroform/methanol/0.25% KCl in 2.5 M aqueous NH_3 (50:40:10, v/v) in the second dimension (bottom to top). Visualization was by resorcinol-HCl spray followed by orcinol-H_2SO_4. The reaction product (asialoG_{M1}) is indicated by the arrow.

(b) Enzymatic treatment of monosialogangliosides. Fig. 3 shows the 2D-HPTLC of the monosialoganglioside fraction following in vitro enzymatic treatment with *Clostridium perfringens* sialidase. The remaining resorcinol-positive spots correspond to triplets 7–9 and 10–12 (Fig. 1) and show no change in 2D-HPTLC mobility, indicating that they are not susceptible to *C. perfringens* sialidase, whereas triplet 13–15 completely disappeared. Spots resulting from the sialidase treatment were also produced which were resorcinol-negative, orcinol-positive and migrated like asialoG_{M1} (based on the 2D-HPTLC mobility of standard asialoG_{M1}). These results suggest that spots 13–15 have the G_{M1b} structure

Fig. 4 shows the 2D-HPTLC of the monosialoganglioside fraction following in vitro enzymatic treatment with bovine testis β-galactosidase. Triplet 7–9 completely disappeared, indicating that these spots have a terminal galactose, whereas triplets 10–12 and 13–15 were resistant. No new resorcinol- or orcinol-positive spots appeared, suggesting that the degradative products of spots 7–9 had structures which comigrated with spots 10–15.

(c) HPTLC-immunostaining of total gangliosides. Following in situ treatment of the total ganglioside mixture with *C. perfringens* sialidase 3 spots (4, 13 and 14; Fig. 1) reacted with the anti-asialoG_{M1} monoclonal antibody (Fig. 5), indicating that they are terminally sialylated and also have the asialoG_{M1} core carbohydrate structure. This reactivity of spots 13 and 14 is consistent with their identification as G_{M1b} (containing different fatty acids as determined by FAB-MS, below).

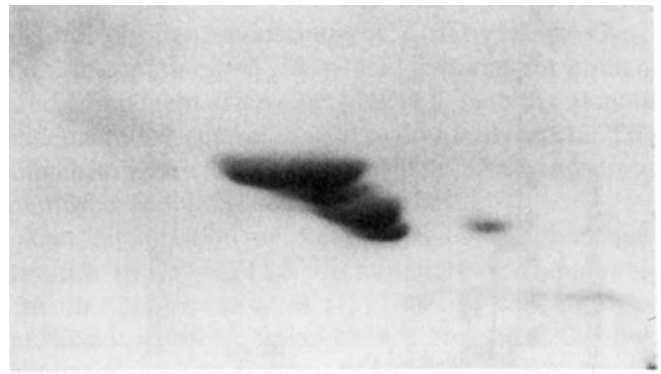

Fig. 4. 2D-HPTLC of the monosialoganglioside fraction (6 μg sialic acid) from WEHI-3 cells following in vitro treatment with 0.06 U of bovine testis β-galactosidase for 20 h at 37°C. The origin is at the lower right and chromatographic development was with chloroform/methanol/0.25% aqueous KCl (50:45:10, v/v) in the first dimension (right to left) and chloroform/methanol/0.25% KCl in 2.5 M aqueous NH_3 (50:40:10, v/v) in the second dimension (bottom to top). Visualization was by resorcinol-HCl spray.

Spot 4, which was eluted from the DEAE-Toyopearl column in the polysialoganglioside fraction and has a lower mobility on HPTLC than the monosialogangliosides, may be G_{D1c}. A fourth very minor spot (upper left-hand corner of the figure) also reacted with the SH34 antibody. Its mobility is similar to (NeuAc)G_{M3} in murine macrophages, which also reacted with this antibody following sialidase treatment [14]. This cross-reactivity is consistent with the original description of the specificity of this antibody [16].

(d) Gas–liquid chromatography of monosialogangliosides. Gas–liquid chromatography of the monosaccha-

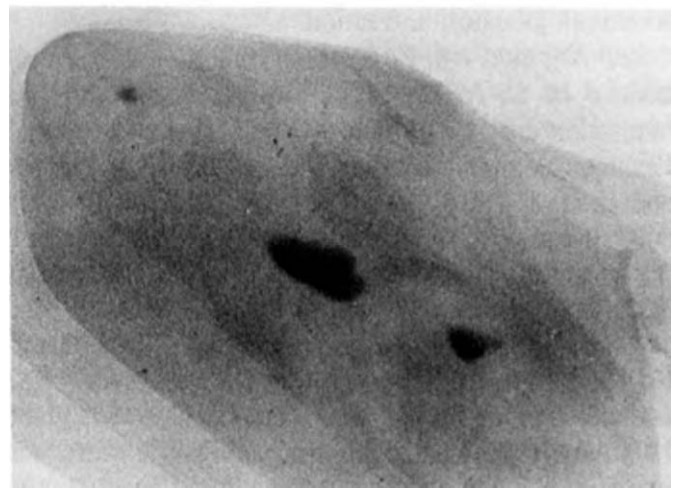

Fig. 5. HPTLC-immunostaining of the total ganglioside mixture (3 μg sialic acid) from WEHI-3 cells with anti-asialoG_{M1} monoclonal antibody, following in situ treatment with *Clostridium perfringens* sialidase. The origin is at the lower right and chromatographic development was with chloroform/methanol/0.25% aqueous KCl (50:45:10, v/v) in the first dimension (right to left) and chloroform/methanol/0.25% KCl in 2.5 M aqueous NH_3 (50:40:10, v/v) in the second dimension (bottom to top).

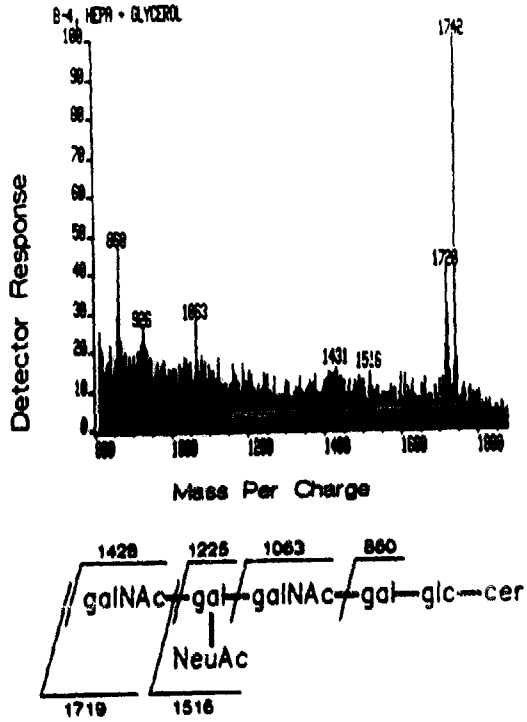

Fig. 6. FAB-MS and fragmentation diagram of band 4 from preparative 1D-HPTLC. The peak at m/z of 1742 is the sodium adduct (Na^+ minus H) of m/z peak 1720.

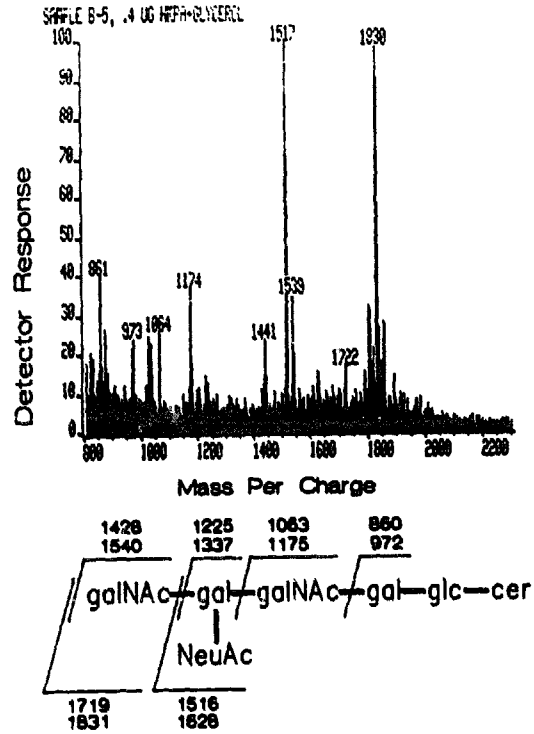

Fig. 7. FAB-MS and fragmentation diagram of band 5 from preparative 1D-HPTLC. Superior numbers of each couple on the fragmentation diagram correspond to C_{16} fatty acid-containing ceramide and inferior numbers to C_{24} fatty acid-containing ceramide.

rides of the WEHI-3 monosialoganglioside fraction showed the presence of glucose, galactose, *N*-acetylgalactosamine and sialic acid. Neither fucose nor *N*-acetylglucosamine was detected, ruling out fucose-containing gangliosides or sialoglycolipids of neolacto structure as major components; either of these gangliosides, if present, would constitute less than 2% of the monosialoganglioside fraction.

(e) Negative ion FAB-MS of monosialogangliosides isolated by 1D-HPTLC. The mass spectrum and fragmentation diagram of band 4 (Fig. 2) is shown in Fig. 6. Fragmentation masses are present for G_{M1b}-GalNAc containing C_{16} fatty acid (m/z = 1720, 1516, 1063 and 860). Because band 4 was a very small percentage of the total ganglioside mixture it was not spotted on 2D-HPTLC.

The mass spectrum of band 5 (Fig. 2) is shown in Fig. 7. Fragmentation masses are present for G_{M1b}-GalNAc containing C_{24} fatty acid (m/z = 1830, 1539, 1174 and 973) and G_{M1b} containing C_{16} fatty acid (m/z = 1517, 1064 and 861). When band 5 was spotted on 2D-HPTLC (plate not shown) it contained predominantly spot 10 and spots 12 (as a trace component) and 14 (Fig. 1). Spot 10 was resistant to degradation in vitro by β-galactosidase and sialidase (Figs. 4 and 3, respectively) and did not react with anti-asialoG_{M1} antibody following in situ-HPTLC sialidase treatment (Fig. 5). Spot 14 was resistant to degradation in vitro by β-

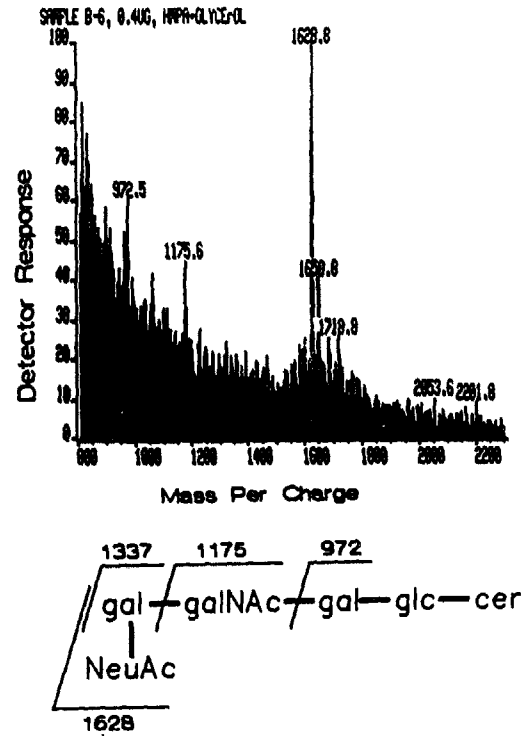

Fig. 8. FAB-MS and fragmentation diagram of band 6 from preparative 1D-HPTLC. The peak at m/z of 1650.8 is the sodium adduct (Na^+ minus H) of m/z peak 1628.8.

galactosidase (Fig. 4), but was sialidase susceptible (by in vitro, Fig. 3, and in situ-HPTLC sialidase treatment, Fig. 5), yielding asialoG_{M1} as the degradative product. These data are consistent with band 5 having G_{M1b}-GalNAc containing C_{24} fatty acid as the major component (spot 10, Fig. 1) and G_{M1b} containing C_{16} fatty acid as a minor component (spot 14, Fig. 1).

The mass spectrum of band 6 (Fig. 2) is shown in Fig. 8. Fragmentation masses are present for G_{M1b} containing C_{24} fatty acid (m/z = 1628, 1175 and 972). When band 6 was spotted on 2D-HPTLC (plate not shown) it contained spots 13 and 15 (as a very minor component) (Fig. 1). Spot 13 was resistant to degradation in vitro by β-galactosidase (Fig. 4), but was sialidase susceptible (by in vitro, Fig. 3, and in situ HPTLC sialidase treatment, Fig. 5), yielding asialoG_{M1} as the degradative product. These data are consistent with band 6 having G_{M1b} containing C_{24} fatty acid as the major component (spot 13, Fig. 1).

When a larger amount of the total monosialoganglioside fraction (1–2 μg sialic acid) was analyzed by mass-spectrometry, molecular ions for G_{M1b}-GalNAc-Gal having C_{16} and C_{24} fatty acid-containing ceramides (m/z = 1882 and 1992, respectively) were detected as minor components of the mixture (data not shown). This is consistent with spots 7–9 being susceptible in vitro to β-galactosidase (Fig. 4), resistant in vitro to sialidase (Fig. 3), and not reactive with anti-asialoG_{M1} antibody following in situ-HPTLC sialidase treatment (Fig. 5). Fragments characteristic of G_{M1a} (i.e., G_{M2} and G_{M3}) were not detected in any of the spectra for the monosialogangliosides.

The collective data indicate that spots 7–9 are the G_{M1b}-GalNAc-Gal structure, spots 10–12 are the G_{M1b}-GalNAc structure, and spots 13–15 are the G_{M1b} structure.

Discussion

The two major gangliosides of WEHI-3 cells, constituting 77% of the total, are the monosialogangliosides G_{M1b} and G_{M1b}-GalNAc. A third monosialoganglioside, G_{M1b}-GalNAc-Gal, was 3% of the total. Their identity is based upon combined ion exchange column chromatography, 1D-HPTLC, 2D-HPTLC, enzymatic degradation, HPTLC-immunostaining, GLC and FAB-MS data. Previously, these two major monosialogangliosides were identified as G_{M1a} and G_{M1}-Fuc [1] solely by 1D-HPTLC comigration with ganglioside standards. There are many reports, however, of G_{M1b} species comigrating with G_{M1a} species on 1D-HPTLC [14,18–20] and G_{M1}-Fuc has a 1D-HPTLC mobility similar to G_{M1b}-GalNAc [21]. This heterogeneity is also evident from our 2D-HPTLC which revealed that band 6 (Fig. 2) consists of G_{M1b} containing C_{24} fatty acid and a minor unidentified component and band 5 (Fig. 2), which comigrates with G_{M1}-Fuc [1] is actually a mixture of G_{M1b} containing C_{16} fatty acid, G_{M1b}-GalNAc containing C_{24} fatty acid and a minor unidentified component. Therefore, HPTLC mobility alone is inadequate for determining ganglioside structure. This point has been clearly demonstrated with neutral glycolipids as well [22].

Our quantitation of the two major monosialogangliosides by 2D-HPTLC densitometry is comparable with that of Pessina et al. [1] (77 vs. 73% of the total ganglioside content, respectively). However, the percentage of each of the two monosialogangliosides differs considerably. G_{M1b} (spots 13–15, Fig. 1) is 44% compared with their faster migrating monosialoganglioside band which is 28% and G_{M1b}-GalNAc (spots 10–12, Fig. 1) is 34% compared to their slower migrating monosialoganglioside which is 45%. This difference is again attributable to inadequate resolution on 1D-HPTLC, where a single band can be a mixture of ganglioside species with different ceramide as well as carbohydrate compositions and a single ganglioside carbohydrate structure can produce multiple bands due to different ceramide structures, as described above. Therefore, the greater resolving power of 2D-HPTLC is necessary for the purification, characterization and also the quantitation of gangliosides.

A reexamination of the 2D-HPTLC ganglioside patterns for *E. coli* activated and thioglycollate elicited murine macrophages [14] revealed that they also have the triplet pattern seen for WEHI-3 cell gangliosides, but it was not evident on every HPTLC plate. This is partly due to the pattern within the triplet itself. For both WEHI-3 cells and murine macrophages, the intensity of the spots is greatest for the uppermost one and decreases for each succeeding spot in the triplet, in a clockwise orientation. Consequently, depending on the quantity of sample spotted, the least intense spot of the triplet may not appear for some or all triplets. Also, some of the spots in a triplet may overlap with those of another triplet. Thus even 2D-HPTLC alone is inadequate for characterization of gangliosides.

Proliferation in both the WEHI-3 cell line and a B lymphocytic cell line (L1210) was shown to be inhibited by both cholera toxin and its B subunit [1]. Because cholera toxin is known to bind to G_{M1a}, a major monosialoganglioside isolated from both cell lines was identified as G_{M1a} based solely on 1D-HPTLC comigration with standard G_{M1a}. On a protein basis, however, the L1210 cell line was shown to contain almost 4-times as much G_{M1a} as the WEHI-3 line yet was 5000-times less responsive to the holotoxin. Our finding that the faster migrating monosialoganglioside in the WEHI-3 cell line is G_{M1b} (and not G_{M1a}) makes this difference even greater but also suggests that the major monosialoganglioside in the L1210 line may not be G_{M1a}, either. As suggested [1], this paradoxical

responsiveness of the WEHI-3 cell line may be due to the presence of other choleragenoid gangliosides and our detection of G_{M1b}-GalNAc-Gal, a choleragenoid ganglioside first described in mouse spleen [23], supports this. This underscores the necessity of rigorously characterizing, by biochemical and/or chemical means, the presence and structure of gangliosides when carrying out and interpreting data from functional studies.

The presence of G_{M1b} in this myelomonocytic macrophage-like cell line is not surprising, since it has been found in murine myeloid leukemia cells [24], lymphoid tumor cells [18], spleen [25], thymus and splenic T cells [20,26–28], T-lymphoma cells [29,30], T-lymphoblasts [27,28], tumors of transplanted erythroleukemic cells [31], and most recently in myelogenous leukemia cells [32] and stimulated peritoneal macrophages [14].

In the murine immune system G_{M1b}-GalNAc has been reported in spleen [20,23] T lymphoblasts [27,28], thymus [20], thymocytes [27] and the lymphoid tumor cell line MDAY-D2 [18,33] and was suggested to be T cell specific [20,28]. Following our preliminary report of its presence in myeloid cells [8] it has since been reported as a component of murine myelogenous leukemic cells, although it was not rigorously characterized [32]. Therefore its presence is not sufficient to characterize murine immune cells as being of the T cell lineage. Because it has not yet been detected as a component of mouse peritoneal macrophage monosialo-gangliosides [14] it may represent a de novo synthesized tumor-associated ganglioside in this myelomonocytic cell line.

G_{M1b}-GalNAc-Gal has been reported only in murine spleen and thymus [20,23] and this constitutes its first report in a myelomonocytic cell type. Although the synthesis of gangliosides of this asialoG_{M1} pathway (i.e., G_{M1b}, G_{M1b}-GalNAc and G_{M1b}-GalNAc-Gal) has been demonstrated in murine spleen and thymus [20,23], it is the dominant ganglioside pathway in WEHI-3 cells. The paucity (or absence) of gangliosides of other pathways, therefore, suggests that there is a deficiency in the sialyltransferase-1 (SAT-1) activity [34] in this cell line.

HPTLC-immunostaining of the total ganglioside mixture with anti-asialoG_{M1} monoclonal antibody, following 2D-HPTLC and in situ treatment with *Clostridium perfringens* sialidase (Fig. 5), revealed that spot 4 (Fig. 1) was susceptible to this treatment. Because its HPTLC mobility is much lower than the monosialogangliosides and it eluted in the polysialoganglioside fraction of the DEAE-Toyopearl column it may have the G_{D1c} structure.

G_{D1c} was first characterized as a novel ganglioside of murine thymoma [35]. It was subsequently suggested that it may be present in splenic T lymphocytes [27], but its first definitive characterization in normal cells was in rat thymocytes [36]. We previously characterized the monosialogangliosides of thioglycollate elicited macrophages from the C3H/HeN mouse and observed two minor components which reacted with anti-asialoG_{M1} antibody following sialidase treatment, indicating they had the asialoG_{M1} carbohydrate core structure [14]. These two components also have a 2D-HPTLC mobility similar to this immunoreactive ganglioside in WEHI-3 cells and were determined by GLC to be disialogangliosides. The presence of two gangliosides with these characteristics in the C3H/HeN mouse macrophage is consistent with the presence of both NeuAc and NeuGc in each of the monosialoganglioside species. Thus it appears that this ganglioside (G_{D1c}) having been described thus far only in cells of the T-lineage may also be present in transformed and normal cells of the monocytic/macrophage lineage.

G_{M1b} and other gangliosides of the gangliotetraosyl core series that contain terminal sialic acids susceptible to sialidase and are not sialylated on the internal galactose moiety seem to be emerging as common components of the murine immune system [14,18–20,24–33,35–37]. Because sialylation has been implicated as a factor in cell adhesion as well as the life span of circulating cells, the conversion of these gangliosides by sialidase from sialylated to nonsialylated without degradation of the neutral carbohydrate backbone may be significant with regard to cell migration, homing and turnover. In addition, there have been two reports [14,26] of altered quantities of G_{M1b} in mice characterized by specific immune dysfunctions.

Human and bovine brain are often used as sources of exogenous gangliosides for functional studies, simply because brain is a rich source. Murine macrophage gangliosides, however, have structures distinct from those of human brain, containing *N*-glycolylneuraminic acid in addition to *N*-acetylneuraminic acid [14]. Also, the ceramide portion of human brain gangliosides contains both C_{18} and C_{20} sphingosine and predominantly stearic acid [38] whereas murine macrophage gangliosides contain only C_{18} sphingosine and primarily C_{16} and C_{24} fatty acids [14]. Critical function has been ascribed to the ceramide portion of the molecule for sialylated [39,40] as well as nonsialylated [29,39,41] forms and it was suggested that the use of brain gangliosides as an exogenous source may be inappropriate for studies in the murine immune system [42]. Subsequent studies comparing both sources have confirmed this [43,44]. Therefore cell lines such as WEHI-3 hold great potential for preliminary in vitro studies of ganglioside function in the murine immune system and as quantity sources of exogenous gangliosides having the same structures as gangliosides endogenous to murine immune cells.

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