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Glycosphingolipids of Leukocytes Are Unbranched at the Galactopyranosyl Residue and Contain Fucosylα1-3-*N*-Acetylglucosamine Structures

EWA ZDEBSKA, ANNA CHELSTOWSKA and JERZY KOŚCIELAK*

Department of Biochemistry, Institute of Hematology, ul, Chocimska 5. 00-957 Warsaw, Poland

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Glycolipids of peripheral leukocytes which had been used for the production of interferon were separated into oligoglycosylceramides, polyglycosylceramides and polyglycosylpeptides (erythroglycan). Neutral oligoglycosylceramides comprised glucosylceramide, galactosylceramide, lactosylceramide, lactotriaosylceramide, globotriaosylceramide and *neo*lactotetraosylceramide. Globotetraosylceramide was not detected. Glycolipids which were more complex than *neo*lactotetraosylceramide belonged exclusively to the *neo*lacto series of compounds and were essentially unbranched at galactopyranosyl residues. The polyglycosylceramide fraction contained a glycolipid with a probable structure Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4GlcNAcβ1-3 Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1ceramide. Polyglycosylpeptides were found only in trace amounts and were also unbranched at galactopyranosyl residues. All glycoconjugates studies did not contain significant amounts of carbohydrate structures derived from ABH immunodominant groups.

Glycosphingolipids seem to be involved in a variety of biological reactions at the membrane level including differentiation of cells [for review see ref. 1]. Thus, although all cells of human blood are derived from a common stem cell ancestor, the glycolipid composition of differentiated cells, i.e. erythrocytes and various subpopulations of leu-

*Author for correspondence

Nomenclature: Lactotrioasylcermide (LcOse$_3$Cer), GlcNAcβ1-3Galβ1-4GlcCer; globotriaosylceramide, (GbOse$_3$Cer), Galα1-4Galβ1-4GlcCer; globoside (globotetraosylceramide, GbOse$_4$Cer), GalNAcβ1-3Galα1-4 Galβ1-4GlcCer; paragloboside (lacto-*N*-*neo* tetraosylceramide, *n*LcOse$_4$Cer), Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer.

kocytes, are different [for review, see ref. 2]. This applies only to simple glycolipids because knowledge of the presence and structure of complex glycolipids is restricted to erythrocytes. Here we report the results of a structural study of the major classes of human leukocyte glycolipids, including polyglycosylceramides [3-6]. We also examined the cells for the presence of complex glycopeptides known as polyglycosylpeptides or erythroglycan [7, 8]. A preliminary report of these studies has been presented [9].

Materials and Methods

Chemicals and Reagents

Sephadex LH-20, DEAE-Sephadex A-25 and Con A-Sepharose 4B were obtained from Pharmacia, Uppsala, Sweden. Precoated Polygram Sil-G plates were from Macherey Nagel, W. Germany. Silica gel-H and precoated HP Kieselgel 60 plates were from Merck, Darmstadt, W. Germany. Iatrobeads were from Iatro Lab., Japan. Green coffee bean α-galactosidase was a gift from Dr. J. Goldstein. β-*N*-Acetylhexosaminidase and α-galactosidase from *Turbo cornutus* were purchased from Seikagaku Kogyo, Kyoto, Japan. β-*N*-Acetylhexosaminidase and β-galactosidase from *Charonia lampas* were obtained from the same manufacturer.

Solvents Used in Chromatographic Procedures

Solvent 1; chloroform/methanol/water, 65/24/8 by vol., lower phase: solvent 2; chloroform/methanol/water, 60/35/8 by vol.: solvent 3; chloroform/methanol/water, 30/60/8 by vol.: solvent 4; chloroform/methanol, 7/3 by vol.

White Cells

Packed human leukocytes (260 ml) which had been used for the production of interferon were obtained from the Central Blood Bank in Helsinki by courtesy of Prof. C. Gahmberg. The method involved collection of buffy coat, removal of residual erythrocytes by osmotic shock and subsequent infection of white cells with Sendai virus. The whole procedure takes about 1.5 days including a 17.5 h long incubation with the virus. Prior to isolation of glycoconjugates, the cells were additionally washed three times with 0.9% NaCl, frozen in solid CO_2 for 24 h, and subsequently stored at -30°C. Immediately after thawing, the viscous cell debris was dehydrated at -20°C with acetone, yielding 26 g of powder.

Butanol Extraction of Leukocytes

The acetone powder of leukocytes (21 g) was extracted three times with 2 l portions of *n*-butanol/0.02 M sodium phosphate buffer, pH 7.5, 1/1 by vol., as previously described [3]. The butanol and aqueous extracts were then separately pooled. The butanol extracts (I), the aqueous extracts (II) and the insoluble residue (III) were subsequently used

as starting materials for isolation of oligoglycosylceramides, polyglycosylceramides and polyglycosylpeptides, respectively.

Isolation of Glycoconjugates

Oligoglycosylceramides. The butanol extract (I) was evaporated to dryness under reduced pressure and the residue subjected to hydrolysis with 1 M KOH in methanol/water, 1/1 by vol., at 37°C for 18 h. After neutralisation with 1 M acetic acid the hydrolysate was dialysed. The retentate was partitioned in 600 ml of chloroform/methanol/water, 8/4/3 by vol. Lower phase glycolipids were then chromatographed on a 3 × 30 cm column packed with DEAE-Sephadex A-25, acetate form, as previously described [10]. Fractions eluted from the column with solvent 4 were pooled and evaporated to dryness under reduced pressure. Neutral glycolipid fractions GL 1-4 were obtained from this material by preparative TLC on silica gel using solvent 2.

Polyglycosylceramides. The aqueous phase (II) (2.4 g) was concentrated by ultrafiltration [3], dialysed, freeze-dried and acetylated with a mixture of 100 ml pyridine, 50 ml formamide and 40 ml acetic anhydride for 18 h [11]. Solvents were removed by dialysis and the retentate was centrifuged at 2 500 × *g* for 20 min at 4°C which gave a white sediment and a cloudy supernatant. The sediment was extracted successively with 50 ml portions of methanol/chloroform, 1/1 by vol. and methanol/chloroform, 1/2 by vol.

The pooled extracts were evaporated to dryness and then partitioned with 400 ml of chloroform/methanol/water, 8/4/3 by vol. The lower phase was collected and freed of solvents by evaporation under reduced pressure. This material separated on TLC in solvent 1 into two carbohydrate positive bands, one migrating ahead of, and the other below standard polyglycosylceramides from erythrocytes (Fig. 1). An additional amount of this material was obtained by partitioning the cloudy supernatant of the retentate with chloroform/methanol/water, 8/4/3 by vol. as above. Carbohydrate-positive bands of the pooled extracts were then purified and separated from each other by preparative TLC in solvent 1. They were designated GC1 and GC2.

Polyglycosylpeptides. The isolation method employed has been described previously [7, 8]. Briefly, the insoluble residue (III, 15 g) was digested with 100 ml of 0.15 M Tris-acetate buffer, pH 7.8 containing 150 mg of Pronase®, 0.0015 M calcium acetate, and 0.02% sodium azide. A fresh portion of 70 mg of Pronase was added daily and the pH readjusted to pH 7.8 with 0.15 M Tris. The clear supernatant was treated with cetylpyridinium chloride (289 mg) and sodium sulphate (108 mg). The mixture was incubated at 30°C for 20 min and the resulting precipitate removed by centrifugation at 25 000 × *g* for 30 min. The procedure was repeated three more times until further addition of cetylpyridinium chloride and sodium sulphate yielded no visible precipitate. Finally the excess of cetylpyridinium chloride was precipitated by two successive portions of 92 mg of potassium thiocyanate. After each addition the mixture was allowed to stand overnight at 4°C and the sediment was removed by centrifugation at 20 000 × *g* for 30 min. The supernatant was then concentrated under reduced pressure and filtered through a 1.9 × 70 cm column packed with Sephadex G-25. The excluded material was hydrolysed with alkaline

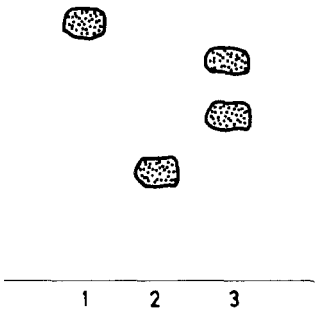

Figure 1. TLC of acetylated fractions GC1 and GC2. 1, GC1; 2, GC2; 3, standard acetylated polyglycosylceramides from erythrocytes. Solvent 1, Polygram Sil-G plate.

borohydride, desalted and subjected to affinity chromatography on a Con A-Sepharose column exactly as described in [7]. The carbohydrate-containing fraction which was not retained on the column was concentrated under reduced pressure and passed through a 1.9 × 70 cm column packed with Sephadex G-50. The fraction eluted in the void volume was collected.

Analytical and Structural Methods

Sugars were determined as alditol acetates [12] using modified conditions for acetolysis at 90°C [13]. Sialic acid-containing glycoconjugates were quantified also as trimethylsilyl derivatives by GLC [14] using a 0.4 × 200 cm glass column packed with 3% SE-30 on Gas Chrom Q. The column was heated at 130°C for 5 min and then programmed to 220°C at 1°C/min. The gas chromatograph was a GCV model (Pye Unicam) equipped with a flame ionization detector and DP-80 digital integrator. In some instances sialic acid was determined colorimetrically [15]. Sphingosine was determined by the method of Lauter and Trams [16]. Glycoconjugates were methylated in dimethyl sulfoxide, with sodium methylsulfinyl carbanion and methyl iodide [17]. Purification of the methylated materials, acetolysis, reduction and acetylation were performed as described [6]. Partially methylated alditol acetates were quantitatively determined by GLC [6] and identified by GLC-MS with a Hewlett-Packard 9995A mass spectrometer. Columns used in GLC-MS analyses were a 12 m long OV-1 capillary column made of fused silica, and a 20 m long glass capillary column coated with OV-225. The columns were connected to the mass spectrometer with a glass restrictor. Conditions for MS were: ionization potential 70 eV, temperature of ion source 148°C, and temperature of transfer line 220°C.

Chromium trioxide degradation of glycolipids was performed by the method of Laine and Renkonen [18, 19], but under harsher conditions i.e. at 50°C for 60 min.

Hydrolysis of Glycolipids with Glycosidases

α-Galactosidase. The reaction mixture contained the following components in a final volume of 50 μl: sodium phosphate buffer, adjusted to pH 3.9 with equimolar citric acid, 5 μmol; α-galactosidase, 1.0 U; glycolipid, 15 μg; sodium cholate, 66 μg. The mixture was incubated at 37°C for 72 h in the presence of toluene vapour.

β-N-Acetylhexosaminidase. The reaction mixture contained the following components in a total volume of 50 μl; NaCl, 50 μmol; sodium phosphate buffer, adjusted to pH 4.0 with equimolar citric acid, 5 μmol; glycolipid 15 μg; *Turbo cornutus* β-*N*-acetylhexosaminidase, 30 mU or *Charonia lampas* β-*N*-acetylhexosaminidase, 40 mU; sodium taurocholate, 50 μg. Incubation lasted for 70 h and 48 h at 37°C employing the enzyme from *Turbo cornutus* and *Charonia lampas* respectively.

β-Galactosidase. The reaction mixture (200 μl) contained 25 mU of the enzyme and 30 μg of lacto-*N*-*neo*tetraosylceramide. Concentrations of the other components of the reaction mixture were the same as in experiments with β-*N*-acetylhexosaminidases. Incubation time was 70 h at 37°C. In all instances reaction products were isolated as described [20].

Results

Oligoglycosylceramides

The glycolipid fractions GL1 to GL4 migrated on TLC plates in solvent 1 as doublets (Fig. 2a and b). Carbohydrate compositions and the results of chromium trioxide degradation of these fractions are shown in Table 1, and methylation data in Table 2. Presence of terminal glucose and galactose in GL1 as indicated by the results of methylation analysis and the complete oxidation of the hexosyl residues by CrO_3 allows identification of this fraction as a mixture of glucosyl- and galactosylceramides. GL2 contained terminal galactopyranosyl and 4-*O*-substituted glucopyranosyl residues in roughly equimolar proportions. Both hexosyl residues were destroyed by CrO_3 oxidation. Therefore GL2 was lactosylceramide.

Methylation analysis of GL3 revealed the presence of terminal galactosyl and *N*-acetylglucosaminyl residues in a 1:2 molar ratio. Di-*O*-substituted hexosyl residues were absent. Thus GL3 was a mixture of two glycolipids. The molar ratio of terminal *N*-acetylglucosamine: 3-*O*-substituted galactosyl residues was 0.9 and that of terminal galactose: 4-*O*-substituted galactosyl residues was 1.0, suggesting that one of the glycolipids contained terminal *N*-acetylglucosamine and 3-*O*-substituted galactose whereas the other contained terminal galactose and a 4-*O*-substituted galactosyl residue. An attempt was made to resolve GL3 into homogeneous glycolipids by rechromatography on silica gel-H plates in solvent 2. Two apparently homogeneous glycolipids (GL3a and GL3b) were

Table 1. Yields, carbohydrate composition and results of CrO_3 degradation of neutral oligoglycosylceramides of leukocytes. Results are given as molar ratios of sugars relative to glucose. Values in parentheses indicate % recovery of a particular sugar after degradation of a glycolipid with CrO_3.

Fraction	Yield (μmol/200 ml)	Gal	Glc	GlcNAc
GL1	9.5	0.3 (0)	1 (0)	—
GL2	50.9	1.1 (0)	1 (0)	—
GL3	4.4	1.5 (nd)	1 (nd)	0.7 (nd)
GL4	14.1	2.3 (23)	1 (0)	1.3 (0)

n.d. = not determined.

Table 2. Methylation data for fractions GL1-4. Results are expressed as molar ratios relative to 2,3,6-tri-*O*-methylglucitol (GL2-4) or 2,3,4,6-tetra-*O*-methylglucitol (GL1).

O-Acetyl derivative of	GL1	GL2	GL3	GL4
2,3,4,6-Tetra-*O*-Me-GlcOL	1.0	—	—	—
2,3,4,6-Tetra-*O*-Me-GalOL	0.3	1.1	0.3	1.0
2,3,6-Tri-*O*-Me-GlcOL	—	1.0	1.0	1.0
2,4,6-Tri-*O*-Me-GalOL	—	—	0.7	1.0
2,3,6-Tri-*O*-Me-GalOL	—	—	0.3	—
3,4,6-Tri-*O*-Me-GlcNAc(Me)OL	—	—	0.6	—
3,6-Di-*O*-Me-GlcNAc(Me)OL	—	—	—	1.1

Table 3. Fatty acid compositions of fractions GL4a and GL4b

Fatty acid	% of total GL4a	% of total GL4b
C16:0	21.1	79.0
C16:1	2.3	0.3
C18:0	15.7	9.7
C18:1	7.1	11.0
C22:0	22.1	—
C24:0	26:8	—
C24:1	4.9	—

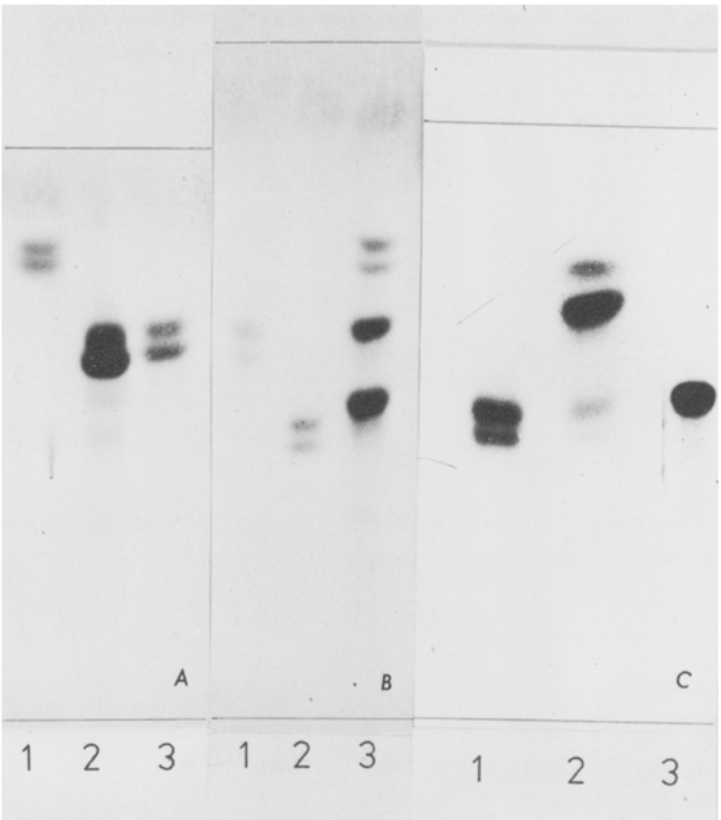

Figure 2. TLC of leukocyte oligoglycosylceramides. (A) 1, GL1; 2, GL2; 3, standard lactosylceramide from erythrocytes. (B) 1, GL3; 2, GL4; 3, standard glycolipid mixture from erythrocytes containing (from top to bottom) lactosylceramide, globotriaosylceramide and globotetraosylceramide. (C) 1, GL3; 2, GL3 after treatment with β-*N*-acetylhexosaminidase from *Turbo cornutus*; 3, globotriaosylceramide from erythrocytes. Solvent 2, Polygram Sil-G plates. Spots were stained with orcinol reagent at 105°C.

obtained. Methylation data for these subfractions were, however, the same as for unfractionated GL3, indicating that components GL3a and GL3b were still mixtures. Unresolved GL3 was then treated separately with coffee bean α-galactosidase and β-*N*-acetylhexosaminidase from *Turbo conutus*. Each of the enzymes released a glycolipid from GL3 with the mobility of GL2 but neither degraded components GL3a or GL3b completely (see Fig. 2c for the effect of β-*N*-acetylhexosaminidase). Thus the terminal *N*-acetylglucosamine residue was β-linked while the terminal galactose was α-linked. The results are consistent with the assumption that GL3 was a mixture of lactotriaosylceramide (GlcNAcβ1-3Galβ1-4GlcCer) and globotriaosylceramide (Galα1-4Galβ1-4GlcCer) in a molar ratio of 2:1.

GL4 contained terminal galactose, 3-*O*-substituted galactose, 4-*O*-substituted glucose and 4-*O*-substituted *N*-acetylglucosamine residues in approximately equimolar proportions. The *N*-acetylglucosamine, glucose and most of the galactose were destroyed

Table 4. Composition and results of CrO_3 degradation of fraction GC1. Total yield 1.9 mg

	Molar ratios	% recovery after CrO_3 treatment
Fucose	1.7	60
Galactose	4.0	0
Glucose	1.0	14
N-Acetylglucosamine	3.3	0
Sialic acid	0.3	nd
Sphingosine	0.9	nd
Mannose	trace	trace

n.d. = not determined

Table 5. Molar ratios of partially methylated alditol and 2-acetamido-2-deoxyalditol acetates obtained by hydrolysis of permethylated fraction GC1.

O-Acetyl derivative of	Molar ratio
2,3,4-Tri-*O*-Me-FucOL	1.1
2,3,4,6-Tetra-*O*-Me-GalOL	0.9
2,4,6-Tri-*O*-Me-GalOL	3.1
2,3,6-Tri-*O*-Me-GlcOL	1.0
3,6-Di-*O*-Me-GlcNAc(Me)OL	2.3
6-*O*-Me-GlcNAc(Me)OL	1.2

by CrO_3 oxidation indicating that the sugars were joined in β-glycosidic linkages. The results suggested that GL4 was paragloboside. To elucidate why GL4 migrated on TLC as a doublet, the fraction was resolved by preparative TLC into a faster components (GL4a) and a slower component (GL4b). The subfractions were analysed by methylation and sequential enzymic digestion, and their fatty acid composition was determined. Methylation data for the subfractions were identical to those for the unresolved GL4. When the subfractions were separately digested with β-galactosidase of *Charonia lampas*, they were converted to glycolipids with the chromatographic mobility of triglycosylceramides. These products were re-isolated from the respective reaction mixtures and treated with the *Charonia lampas* β-*N*-acetylhexosaminidase. Unexpectedly they decomposed to glycolipids with chromatographic mobilities of monoglycosylceramides (data not shown). Control experiments revealed that standard lactotriaosylceramide from erythrocytes but not globotriaosylceramide was hydrolysed under the same conditions to glucosylceramide indicating that the enzyme preparation was contaminated with β-galactosidase. Finally the fatty acid composition of subfractions GL4a and GL4b were determined and the results are shown in Table 3. The predominance of higher fatty acids in GL4a and the total absence of these acids in GL4b provides the explanation for their different chromatographic mobilities.

At least five glycolipid bands with $R_{globoside}$ values of 0.1, 0.46-0.56, 0.62-0.66, 0.88 and 0.92 were visible on TLC of the "upper phase" oligoglycosylceramides (see under Materials and Methods). Separation of these bands was not attempted. Gross examination of this fraction by GLC as their trimethylsilyl ethers revealed the presence of galactose, *N*-acetylglucosamine, glucose and sialic acid in the molar proportions 2.5 : 1 : 1 : 1. Fucose was present, albeit in small amounts. On methylation analysis the galactopyranosyl residues were found to be terminal or 3-*O*-substituted while residues of *N*-acetylglucosamine were substituted at position 4. Glucose was also substituted at position 4. Di-*O*-substituted residues of galactose were not detected. The results are consistent with the assumption that the "upper phase" oligoglycosylceramides were largely gangliosides based on the "*neo*lacto" unbranched core.

Polyglycosylceramides. Yield, carbohydrate composition and methylation data for fraction GC1 (see under Materials and Methods) are shown in Tables 4 and 5. Methylation analysis was also performed on this fraction after defucosylation by partial acid hydrolysis with 0.1 M trichloroacetic acid for 2 h at 100° C [21]. 2,3, 4-tri-*O*-methyl-fucitol and 6-*O*-methyl-*N*-methyl-acetamido-glucitol were absent from the defucosylated material. Other partially methylated alditol and hexosaminitol acetates were the same as in the intact material. The results suggest that GC1 was a straight chain glycolipid of the *neo*-lacto type with fucosyl residues attached by α1-3 glycosidic linkages to *N*-acetylglucosamine (see also in the Discussion).

Fraction GC2 (yield 0.8 mg) contained mannose in addition to constituents present in GC1. Molar ratios of carbohydrates for GC2 relative to glucose were as follows: fucose 2.3; galactose 5.9; *N*-acetylglucosamine 6.1; mannose 1.5; glucose 1.0. Methylation analysis revealed the same partially methylated galactitol and hexosaminitol derivatives as in GC1 plus small amounts of 4,6-di-*O*-methyl-galactitol and 2,4-di-*O*-methyl-galactitol. The following mannose derivatives were identified: 2,3,4,6-tetra-*O*-methyl-mannitol, 3,4,6-tri-*O*-methyl-mannitol, 2,4-di-*O*-methyl-mannitol and 2,6-di-*O*-methyl-mannitol. A small amount of 2,3,4,6-tetra-*O*-methyl-glucitol was detected.

Polyglycosylpeptides. Polyglycosylpeptides were obtained from leukocytes with a yield of only 0.15 mg of hexose as determined by the phenol-sulfuric acid method. Using the GLC-MS technique the following partially methylated alditol and hexosaminitol acetates were identified in the hydrolysates of the permethylated material: 2,3,4,6-tetra-*O*-methyl-galactitol; 2,4,6-tri-*O*-methyl-galactitol; 3,6-di-*O*-methyl-*N*-methylacetamido-glucitol; 6-*O*-methyl-*N*-methylacetamido-glucitol (trace); 2,3,6-tri-*O*-methyl-glucitol; 3,6-di-*O*-methyl-mannitol; 2,4-di-*O*-methyl-mannitol. 2,4-di-*O*-methyl-galactitol was not detected.

Discussion

During the course of the current study we examined the major classes of glycolipids of leukocytes including neutral oligoglycosylceramides, polyglycosylceramides and gangliosides. In addition we analysed glycopeptides, obtained after Pronase digestion of leukocytes, for the presence of erythroglycan. We found that the most abundant glycoli-

pids of human leukocytes are mono-, di- and tetraglycosylceramides. Our data are consistent with the assumption that the monoglycosylceramide fraction is a mixture of glucosyl- and galactosylceramide, that the diglycosylceramide is lactosylceramide, and the tetraglycosylceramide is paragloboside. These findings confirm the results of previous studies [22-27]. The triglycosylceramide fraction of leukocytes probably consists of globotriaosylceramide and lactotriaosylceramide. The glycolipids were not separated from each other but their identities were adequately determined on the basis of carbohydrate composition, methylation data, chromium trioxide degradation and susceptibility to the action of β-*N*-acetyl-hexosaminidase and α-galactosidase. Since lactotriaosylceramide and globotriaosylceramide have been found in neutrophils [22-24] and lymphocytes [25-27] respectively, our findings indicated that both types of cells must have been present in our original packed leukocyte preparation.

Thus neutrophils were not destroyed during the interferon production procedure. The only tetraglycosylceramide we found was paragloboside. It migrated on TLC plates as a doublet of subfractions with identical glycans but different fatty acid compositions. Unexpectedly we have not found globoside. This attests to the absence in the leukocyte preparations of erythrocytes which contain a high concentration of globoside. It contradicts, however, earlier claims on the presence of small amounts of globoside in lymphocytes [25-27]. However, the possibility that a particular subpopulation of lymphocytes rich in globoside was lost during the interferon production procedure cannot be excluded.

The major finding of this study is the presence in leukocytes of a straight chain fucoglycolipid of the lacto-*N*-*neo* series containing nine sugar residues with fucose attached by α1-3 glycosidic linkages to *N*-acetylglucosamine. This sequence is the immunodominant structure of stage specific embryonic antigen - 1 (SSEA-1) of mouse embryo [28, 29]. This fucoglycolipid (GC1) probably has the structure: Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer.

We assume that there is only one fucosyl residue in the molecule although by alditol acetate analysis 1.7 fucosyl residues were found. We ascribe this result to impurities which in our experience sometimes migrate on gas chromatograms together with fucitol tetra-acetate. The conclusion is based upon only 60% recovery of fucose after CrO_3 oxidation of the glycolipid and the result of methylation analysis. It was not possible to determine which *N*-acetylglucosamine residue was substituted by fucose, but this was ascribed by analogy to other fucoglycolipids of this type [1, 30]. Except for a recently described fucoganglioside from human cancer [31] these fucoglycolipids never have fucosyl residues located at internal *N*-acetylglucosaminyl residues when the more distal ones are not substituted with this sugar. A fucoglycolipid with an identical structure to that reported here has been isolated from human erythrocytes [30].

The other fucose-containing fraction (GC2) also contained glucose and mannose which suggested it was a mixture or a complex of glycolipids and glycopeptides. The glycan of this material was also unbranched at the galactopyranosyl residues and consisted of five to six Galβ1-4GlcNAcβ1-3 units per residue of glucose or 1.5 residues of mannose. Fucose was linked to *N*-acetylglucosamine but was present at a higher molar ratio. Therefore some glycans of this fraction should be difucosylated. The fucoglycoconjugates are probably derived from neutrophils because only these cells react strongly

with anti-My-1 monoclonal antibody to human HL60 promyelocytic leukemia cells [32] which is specific for the Galβ1-4(Fucα1-3)GlcNAc structure [33].

Lymphocytes, monocytes, platelets and erythrocytes do not react with anti-My-1 [34-36]. Interestingly when neutrophil glycolipid extracts were screened for reactivity with anti-My-1 by an autoradiographic technique the reactive glycolipids migrated on TLC plates as closely packed bands with mobilities intermediate between those of branched glycolipid standards containing 10 and 12 glycosyl residues [33], which is largely in keeping with the results of the current study. Other reactive glycolipids behaved on TLC plates as heptaglycosylceramides. We have not isolated the latter glycolipids but nevertheless observed the presence of a small amount of fucose in leukocyte extracts containing less complex glycolipids than GC1.

Another important finding of this study is the almost total absence of branching at carbon atom 6 of the 3-*O*-substituted galactopyranosyl residues of leukocyte glycolipids and glycopeptides i.e. the structure involved in I antigenic activity [37]. Only one fraction analyzed (GC2) contained this structure, albeit in trace amounts. In contrast, comparable fractions from erythrocytes such as polyglycosylceramides [3] or polyglycosylpeptides [7, 8] contain on the average 3-4 branching points per ceramide or 1.5 residues of mannose respectively. Furthermore we have not found in these glycoconjugates significant amounts of 2-*O*- and 2,3-di-*O*- substituted residues of galactose which should be derived from ABH immunodominant groups [38]. The latter structure was found in small amounts only in fraction GC2. Thus it seems that in the course of differentiation from a common stem cell, erythrocytes acquire branched glycans terminated with ABH immunodominant structures which are not present (or present in trace amounts) in cells of myeloid and lymphoid lineages. On the other hand fucopyranosyl residues attached to position 3 of *N*-acetylglucosamine occur predominantly in glycoconjugates of leukocytes (see also Table 6). The meaning of these findings is difficult to assess.

Superficially our results are at variance with those of numerous immunological investigations in which Ii [39, 40] and ABH antigens [41] were demonstrated in leukocytes. Immunological techniques like immunofluorescence, immunoadherence and agglutination of cytotoxicity assays are, however, more sensitive than chemical methods and are not quantitative. According to the results of this as well as a previous study [44], leukocytes should exhibit predominantly i antigenic activity, which depends on a repeating Galβ1-4GlcNAcβ1-3 sequence [37].

We have not found gangliosides of the ganglio series in leukocytes although the glycolipids are detected therein with the use of specific antibodies [42] or cholera toxin [43]. Again high sensitivity of the latter two techniques may account for this discrepancy. In keeping with previous studies [44] we have found that the major gangliosides of leukocytes should belong to the *neo*lacto series of glycolipids.

Our results do not support the claim of Fukuda *et al.* [45] that human neutrophils contain significant amounts of lactosaminoglycan with the GlcNAcβ1-3(GlcNAcβ1-6)Gal branched sequence. Evidence for the claim was based upon the gel filtration profile of oligosaccharides released from the cells by the action of endo-β-galactosidase from *Escherichia freundii*. This technique detects oligosaccharides branched at galactopyranosyl residues which are resistant to the enzyme [46]. Recently it has been demonstrated, however, that galactopyranosyl residues flanked on both sides with fucosylated resi-

Table 6. Antigenic carbohydrate structures in blood cells according to present and previous [3, 10, 22-27, 30, 32, 40-44] data. Assignment of residual ABH structures to lymphocytes was made on the basis of studies in which the H gene-specified transferase was found in lymphocytes but not in neutrophils [47].

	ABH	I	i	SSEA-1
Erythrocytes	+++	+++	+	+
Neutrophils	—	—	+	+++
Lymphocytes	+	—	+	—

dues of *N*-acetylglucosamine are also resistant to the enzyme [30]. Therefore endo-β-galactosidase resistant oligosaccharides of neutrophils as observed by Fukuda *et al.* [45] may have contained the structure of the latter type.

One problem in our study remains unanswered, namely a possibility that the infection of leukocytes with Sendai virus affected their glycoconjugate composition. However, with the exception of globoside we have found oligoglycosylceramides in leukocytes which have been described by others [22-27, 33] and this suggests that the gross composition of glycolipids has not changed. In studies performed on tumour cells of neural origin it has been demonstrated that although the biosynthesis and translocation of glycolipids to cell surface occurs within 20-30 min, their turnover rates are much slower with half-lives of surface bound glycolipids amounting from 44 h to several days [48]. Thus there is only a remote possibility that the absence of branched or ABH-active glycolipids in leukocytes is due to their complete degradation during the interferon production cycle.

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