Polyglycosylceramides, Poly-*N*-acetyllactosamine-Containing Glycosphingolipids: Methods of Analysis, Structure, and Presumable Biological Functions

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1. Introduction

The name polyglycosylceramides (PGCs) was introduced to designate highly glycosylated water-soluble sphingolipids containing from ca. 20 to ca. 50 monosaccharides per ceramide, not soluble in organic solvents used during traditional preparations of lipids and glycolipids.[1] PGCs constitute the most complex group in the large glycosphingolipid family, which has more than 250 members in total, as listed in a review from 1989.[2] Glycosphingolipids with 1−10 monosaccharides per ceramide occur frequently in biological material and are relatively well characterized. However, species with larger carbohydrate chains, including PGCs, have so far been isolated from a limited number of biological sources. A PGC molecule typically consists of a branched poly-*N*-acetyllactosamine chain which is linked via lactose unit to the lipid ceramide. The highly glycosylated PGC molecules are carriers of various structures, which is the basis for blood-group activities[1] and for binding of bacteria, viruses and proteins.[3,328] The recent progress in studies on glycoconjugate function has provided new evidence for the involvement of glycosphingolipids in transmembrane signaling.[4] The polyvalent PGCs with repeated binding epitopes may have a unique role among membrane components implicated in this process. The carbohydrate chains of PGCs are effective binders of ligands, and the

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ceramides contain fragments with potential signaling functions.[5] This paper will review the occurrence, structure, methods of preparation, and analysis of PGCs and will also discuss possible biological functions of these interesting molecules.

2. History

In 1973, Gardas and Koscielak[6] isolated from human erythrocytes a new form of water-soluble blood-group-active substances which differed from the known blood-group-active glycoproteins. The material contained 7% amino acids, 90% carbohydrates, 1−2% sphingosine, and expressed strong A-, B-, H-, and I-blood-group activities.[6−8,181] Using alkali degradation, it was possible to reduce the amino acid content to 0.2−0.3% and partial acid hydrolysis liberated from the material the organic solvent-soluble fragments which migrated on thin-layer plates as typical glycolipids.[7] On the basis of these results, the authors concluded that the new antigens were unusually complex glycosphingolipids (megaloglycolipids, later named polyglycosylceramides[1]) with ca. 20−40 sugar residues per mole of ceramide. The size of the molecules was judged from glucose and sphingosine contents of the preparations. Saccharide parts of the new antigens were reported by the same authors as branched poly-*N*-acetyllactosamines.[1,9,10] Highly glycosylated glycosphingolipids with 20 and more sugar residues per cermide were later isolated from other biological sources,[11−18] and the structure of highly complex, 40-sugar-containing glycolipid of rabbit erythrocytes was reported based on NMR spectroscopy.[14] Recently, we confirmed the presence of large oligo/polysaccharides linked to ceramides with up to and more than 40 monosaccharides per chain in heterogeneous mixtures of PGCs of human erythrocytes using MALDI-TOF mass spectrometry.[19]

3. Isolation of PGCs

Due to the presence of complex hydrophilic carbohydrate chains, PGCs are not extracted under conditions used for isolation of common lipids and glycolipids. The first methods of preparation of PGCs were based on extraction of membrane residues with mixtures of butanol and water.[1,6−10,20] PGCs were isolated from aqueous phases and purified using various types of ion-exchange chromatography. During this procedure a significant part of the material was lost due to the incomplete separation from proteins and incomplete elution from the gels. Other preparative methods included extraction with other mixtures of solvents,[21] use of detergents,[15,21] protease digestion,[22] reverse-phase chromatography,[22] and peracetylation.[23] Some of these approaches, e.g., use of detergents during column separations[21] or peracetylation of crude fractions,[23] allowed for better yields during isolation procedures. The peracetylated PGCs could be purified using organic solvents and simple silica gel chromatography. Later, a simplified method was introduced[24] in which PGCs were peracetylated before extraction from tissues. After peracetylation, which converts the water-soluble PGCs to organic solvent-soluble derivatives, the PGCs were extracted with chloroform and purified using phase partition, Sephadex LH-20, Sephadex LH-60, and silica gel chromatography.

4. Methods of PGC Analysis

A number of PGCs have now been characterized by combinations of chemical, biochemical, and other techniques. These include simple colorimetric assays, immunochemical assays, gas chromatography (GC), mass spectrometry (MS), (FAB MS, EI MS, MALDI-TOF MS), and nuclear magnetic resonance spectroscopy (NMR). For technical details and general strategies for analysis of glycoconjugates and complex carbohydrates see refs 25−27. The basic approaches are often used in combination with various degradation and derivatization techniques. For example, permethylated PGCs can be favored derivatives for some MS analyses. EI MS of the permethylated PGCs provides the information about terminal carbohydrate sequences and ceramide compositions,[16,28] and FAB MS after permethylation can be used for sequence analysis.[11,12,29] MS of permethylated and reduced glycosphingolipids is also a well-established method for the determination of carbohydrate sequence and ceramide structure of glycosphingolipids and had some application in analysis of complex species.[30,31] The reduction of permethylated glycosphingolipids is carried out with $LiAlH_4$. In this way amide groups of ceramide and amino sugars are converted into amines, whereas esterified carboxyl groups of sialic acids are transformed into primary hydroxyl functions. Recently developed MALDI-TOF MS was used for molecular fingerprinting of native PGC mixtures[19] (see also Figure 4). A valuable method for analysis of PGC also is NMR.[11,12,14,29,30,32,33] This approach provides information about various molecular features, and the structure of a 40-sugar glycolipid from rabbit erythrocytes has been reported based entirely on a two-dimensional ^1H NMR spectroscopy at 600 MHz.[14] The NMR approach is well suited for determination of the anomeric structures and may be used as a supplementary technique to MS studies.

A classical method for compositional analysis of complex carbohydrates is degradation by acid hydrolysis and the conversion of the resulting monosaccharides into their alditol acetates.[25−27] Each monosaccharide produces a single derivative, giving a chromatogram which may be quantitated and interpreted. For determination of linkage positions between monosaccharides, another common method, the analysis of partially methylated alditol acetates (PMAAs),[25−27] is often used. The molecule is permethylated and degraded, and the resulting partially *O*-methylated monosaccharides are reduced to alditols and acetylated. The resulting PMAAs are then analyzed by GC and EI MS. In this approach the methyl groups indicate free hydroxy groups in the native molecules. Other classical chemical degradation techniques such as Smith degradation,[15,30,34] nitrous acid deamination, or chromium trioxide oxidation[15,34] also had some application in PGC analysis. Smith degradation[25] allows a stepwise degradation

of poly-*N*-acetyllactosamine structures. The neighboring hydroxy groups of terminal sugars are oxidized by periodic acid, and the generated aldehyde groups are reduced by $NaBH_4$ to alcohols. The acetal bonds in polyalcohols can then be split by mild acid hydrolysis without cleaving the glycosidic bonds of the unoxidized sugars. The exposed hydroxyl groups may then be oxidized by periodate in another cycle.

Valuable information can be obtained from enzymatic degradations of PGCs. Exoglycosidases split off terminal sugars from oligo/polysaccharides, whereas endoglycosidases hydrolyze internal parts of the chains.[35] On the basis of the enzymatic specificities, it is possible to investigate sequence of sugars, linkage positions between monosaccharides, and anomerity of the glycosidic bonds. Among different endoglycosidases, endo-β-galactosidases are suited for analysis of PGCs because they hydrolyze Galβ4GlcNAc in linear but not branched chains.[36−37] The analysis of the released fragments using MS or NMR can give valuable information about the organization of monosaccharides and branches in complex chains.[38]

As will be discussed below, PGCs contain epitopes with binding affinities for antibodies, lectins, toxins, and microbes. For a fine dissection of binding epitopes, additional degradation and derivation methods are used in combination with MS[28] or NMR.[33] A classical example is mild periodate oxidation and reduction of sialylated molecules, which shortens the sialic acid glycerol side chain by one or two carbon atoms.[39] The chemically modified molecules are then tested for binding affinities to relate the structure with binding activity.[28] Purity and homogeneity of glycosphingolipids is often tested by thin-layer chromatography (TLC).[40] The TLC separation of PGCs usually results in "smears"; however, the immobilized fractions can be investigated for binding properties using overlay with labeled proteins,[16] bacterial cells,[42−45] or virus particles.[46] TLC is also used in combination with partial degradation techniques; for example, polysaccharides released from PGCs by ceramide glycanase can be analyzed using polar TLC solvent systems,[16,24] and simple glycolipids released by endo-β-galactosidase[24,47] or by chemical methods[1,34] are easily detected using less polar solvents. The analysis of the binding epitopes in complex mixtures of molecules can be difficult, especially if branching isomers are involved. In this case, the enzymatic synthesis of well-designed structures in combination with binding studies could be of value for final identification of the epitopes. A number of enzymes have been reported which are able to synthesize oligo-*N*-acetylactosamine structures in vitro.[48−50] For example, the in vitro synthesized branched polylactosamine backbone containing several sialyl−Lewis-x epitopes has been shown to be a highly potent antagonist of lymphocyte L-selectin.[50]

5. Structure and General Characterization of PGCs

5.1. Basic Chemistry of Glycosphingolipids[40,41]

Glycosphingolipids (GSLs) are composed of a ceramide part (lipid part) and a carbohydrate part (see structures in Figure 6). A ceramide consists of a fatty acid linked by an amide bond to the amino group of a long chain base (sphingosine). The carbohydrate part, which can contain from 1 to more than 40 monosacharides, is linked to the ceramide by a glycosidic bond. Glycosphingolipids are traditionally classified as neutral GSLs, gangliosides (sialic acid-containing), and sulfatides (sulfate-containing). In most GSLs of vertebrates the first monosaccharide linked to ceramide is Glc. GlcCer may be extended by addition of other sugars, and depending on the final structure, the GSLs are devided into series[2,40,41] designated as lacto (Lc) (with the first four monosaccharides linked to ceramide being Galβ3GlcNAcβ3Galβ4Glcβ), neolacto (nLc) (Galβ4GlcNAcβ3Galβ4Glcβ), globo (Gb) (GalNAcβ3Galα4Galβ4Glcβ), isoglobo or neoglobo (Gbi) (GalNAcβ3Galα3Galβ4Glcβ), and ganglio (Gg)(Galβ3GalNAcβ4Galβ4Glcβ). Less common are GSLs with repeated Gal units linked to Glc (muco series, Mc) or extended GSLs of the gala (Ga) series with Gal linked to ceramide. GSLs with Gal linked to ceramide are mainly known as monohexosylceramide GalCer and sulfatide SO_3GalCer. An unusual GSL was isolated from human colon carcinoma having fucose linked to ceramide (Fucα1−1Cer).[53] Invertebrates have the ability to synthesize Man (mannose)-containing GSLs which are classified as arthro- (At) (GalNAcβ4GlcNAcβ3Manβ4Glcβ)[2,191,192] and mollu- (Mu), (GlcNAcβ2Manα3Manβ4Glc)[2,191] series. Rare glycolipids with Man or GlcA (glucuronic acid) linked to ceramide were isolated from *Hyriopsis schlegelii* (freshwater bivalve)[2,193,194] and *Flavobacterium devorans*,[2,195] respectively. Mannose-containing GSLs were also found in plant material.[2,196]

Most GSLs contain one type of the core structure; however, there are examples of rare GSLs with combined structures, for example, GSLs with neolacto−ganglio,[51] neolacto−lacto,[18,52] or isoglobo−ganglio−neolacto hybrid carbohydrate chains.[210] Nomenclature of GSLs includes both trivial and systematic names. The systematic name of, for example, 3′-sialylparagloboside, Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcCer, is IV^3-α-Neu5Ac-nLc_4Cer (see recommendations of IUPAC−IUB Joint Commission on Biochemical Nomenclature, *Eur. J. Biochem.* **1998**, 257, 293). The name includes information about the glycolipid series (nLc), number of sugars in the core chain (nLc_4), terminal substitution (α-Neu5Ac), monosaccharide to which the terminal sugar is linked (IV), and terminal linkage position (IV^3). However, the naming of atypical and more complex species may be difficult and is of no practical use in the case of branched PGCs. Recently, a new terminology system has been proposed to describe carbohydrate histo-blood-group antigens,[326] which is currently being modified to better describe the polyglycosylceramides.

5.2. Basic Chemistry of PGCs

PGCs constitute the most complex group of glycosphingolipids which may contain up to 40−50 saccharides per molecule, as reported in original papers[1,6,14,15,17] and confirmed recently by molecular fingerprinting using MALDI TOF mass spectrometry,[19] see also Figure 4. PGCs typically belong to the

Table 1. Biological Activities of PGCs of Different Sources

source of PGCs (refs: structure and general information)	histo-blood-group-related determinants in PGCs	other binding activities
human erythrocyte (1, 6−10, 19−21, 23, 24, 34, 58, 60, 61, 181)	A, B, H, (1, 6−10, 21, 23, 58, 62, 181) I, i (7, 8, 23, 34, 58, 181) Le^x, Le^y (*)	*Helicobacter pylori* (16, 19, 38, 42−44) influenza viruses A and B (46) *Streptococcus suis* (142) *Mycoplasma pneumoniae* (143) *Ricinus communis* lectin (144)
human granulocyte (16, 45)	Le^x, sialyl-Le^x (*), (63−65)	*Helicobacter pylori* (45) influenza virus A (*)
human placenta (11)		*Helicobacter pylori* (*)
rabbit erythrocyte (12−15, 30−32)	B-like, I (12−14, 30, 32)	
rabbit small intestine mucosa (16)	B-like, A, B, H, Le^y (16)	heat-labile toxin of *Escherichia coli* (LT), (145, 180)
dog small intestine mucosa (16)	Le^x,Le^y, A, H (16)	
hog gastric mucosa (17, 18, 52, 67−69)	A, H (17, 18, 52, 67−69)	
human pancreatic carcinoma cell line PANC-1 (22)		

* Miller-Podraza, H., et al. Unpublished results.

neolacto series of glycolipids and contain a repeated structure Galβ4GlcNAcβ3 linked to lactosylceramide (Galβ4GlcCer). The saccharide chains are usually branched at Gal residues with side chains initiated by GlcNAcβ units. PGCs are carriers of a variety of blood-group determinants and other neutral and sialylated structures. They also display binding affinities for proteins, bacteria, and viruses (Table 1 and Figure 1). In humans the identified sialic acid is Neu5Ac, while other species may contain Neu5Ac and/or Neu5Gc.

Analysis of carbohydrates as partially methylated alditol acetates typically reveals in PGCs three main carbohydrate components:[1,9−11,15,16] (a) 1,4-linked GlcNAc (detected as 3,6-di-*O*-methyl-1,4,5-tri-*O*-acetyl-2-deoxy-2-*N*-methylacetamidoglucitol), (b) 1,3,6-linked Gal (branching Gal), (2,4-di-*O*-methyl-1,3,5,6-tetra-*O*-acetylgalactitol), and (c) 1,3-linked Gal (2,4,6-tri-*O*-methyl-1,3,5-tri-*O*-acetylgalactitol), present in linear fragments of poly-*N*-acetyllactosamine chains and in many terminal structures. A characteristic minor component of PGCs is also 1,4-linked Glc (2,3,6-tri-*O*-methyl-1,4,5-tri-*O*-acetylglucitol), being part of the LacCer unit. Terminal monosaccharides may differ between PGC preparations, but typical examples are Gal (2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetylgalactitol), Fuc (2,3,4-tri-*O*-methyl-1,5-di-*O*-acetylfucitol), and sialic acid. In dog intestine PGCs, a significant part of GlcNAc is substituted at C3 with αFuc (1,3,4-linked GlcNAc), giving after permethylation and degradation appreciable amounts of 6-*O*-methyl-1,3,4,5-tetra-*O*-acetyl-2-deoxy-2-*N*-methylacetamidoglucitol.[16]

Organization of branches in poly-*N*-acetyllactosamine backbones of PGCs remains in most cases unclear. Two possibilities of branching arrangements are being discussed in the literature:[34,54] (a) arrangements with linear core chains and multiple side linear branches (A−D in Figure 2) and (b) dendritic arrangements with tree-like structures (E and F in Figure 2). The first model assumes that only one antenna provides conditions for branching, while in the second model both 3- and 6-antennae can be multiply branched. Both models include fully branched molecules with short (monolactosamine) branches (A and E in the figure) and molecules with linear extensions in branches and/or between branches (B−D and F).

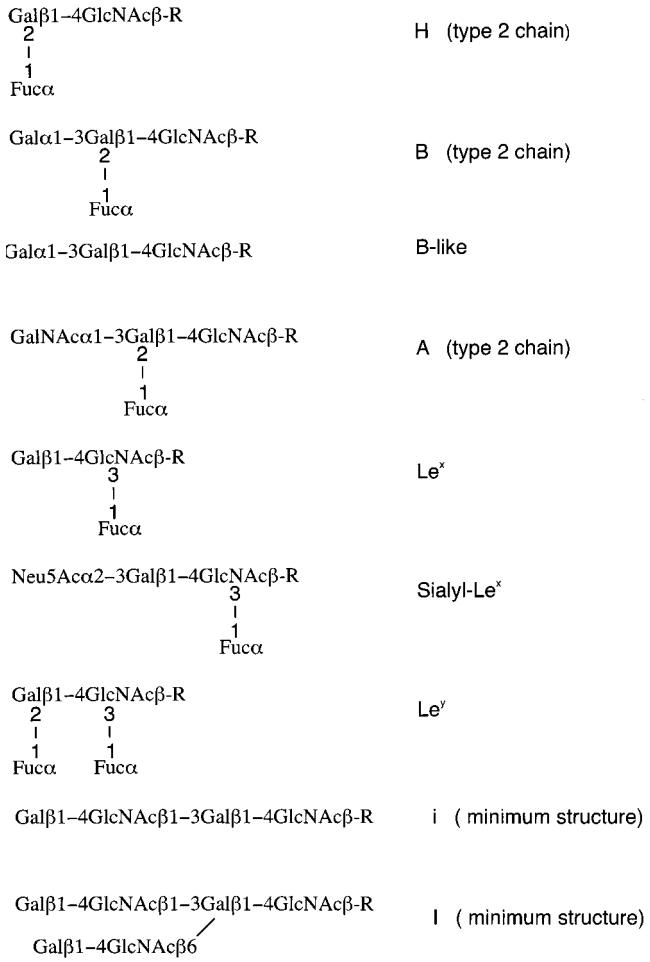

Figure 1. Structures of histo-blood-group-related determinants found in PGCs (based on refs 55, 146, and 325 and references in Table 1).

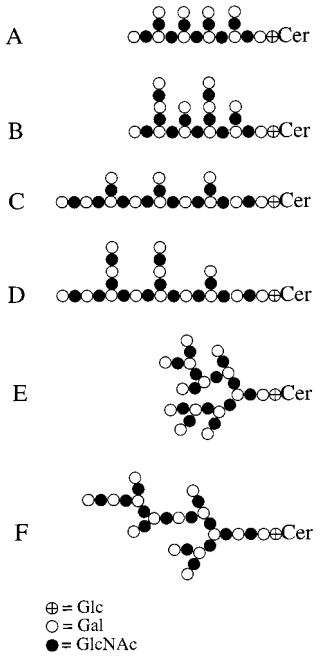

Figure 2. Different models of branched poly-*N*-acetyllactosamine chains.

PGCs from rabbit erythrocytes contain multiple Galα3Galβ4GlcNAcβ branches linked by 1,6-glycosidic linkages to all galactoses of the linear (Galβ4GlcNAcβ3)$_n$ chains, which is in agreement with model A of Figure 2. This regular structure was confirmed first by NMR spectroscopy for a 15-sugar glycolipid after enzymatic removal of terminal α-Gal and penultimate β-Gal residues.[32] The analysis allowed a differentiation between GlcNAcs of the core linear chain and the exposed GlcNAcs of the branches and showed that all side GlcNAcs were linked to the main chain by 1,6-linkages. This regular structure was later confirmed for a 40-sugar glycolipid of the same source based entirely on NMR analysis.[14] Recent sudies on one of the β4galactosyltransferases, the β4Gal-GTI, and other glycosyltransferases have demonstrated that the synthesis of structures with linear (Galβ4GlcNAcβ3)$_n$ cores and short β-1,6-linked branches is possible in vitro.[48,49] On the other hand, it has been demonstrated that different isomeric branching arrangements are present in mixtures of PGCs prepared from human placenta.[11] This had been shown for a 15-sugar ganglioside, Neu5Ac$_3$Hex$_7$-HexNAc$_5$Cer, which was degraded sequentially with three exoglycosidases: sialidase, β-galactosidase, and β-glucosaminidase. One cycle of this triple hydrolysis converted the 15-sugar glycolipid to a 6-sugar fraction. The latter was analyzed by permethylation and degradation, and both 1,3- and 1,6-linked Gal derivatives were detected among partially methylated alditol acetates (2,4,6-tri-*O*-Me-Gal-ol and 2,3,4-tri-*O*-Me-Gal-ol, respectively). This implies that the 15-sugar fraction was a mixture of isomers with side branches linked by either 1,3- or 1,6-glycosidic linkages.

Branch isomers are also highly possible in mixtures of PGCs from human erythrocytes. As will be discussed later, PGCs of this source display high structural heterogeneity. The molecules may be fully or incompletely branched, and the linear extensions may be present in both in 3- and-6-linked antennae.[38] Earlier studies had shown that four cycles of Smith degradation are not enough to completely destroy the branches in these complex glycolipids.[34] Human erythrocyte PGCs may contain dendritic carbohydrate structures (E and F in Figure 2); however, further studies are necessary to confirm this conclusion. The existence of multiple enzymes capable of synthesizing poly-*N*-acetyllactosamine chains (see section 6) may indicate structural differences between different PGCs and other poly-*N*-acetyllactosamine-containing molecules.

Poly-*N*-acetyllactosamine is a developmentally regulated saccharide which carries i and I antigenic structures.[55,324,325] The i epitope is associated with linear polylactosamine chains and characteristic for some embryonic tissues and tumor tissues, while the I epitope occurs in branched polylactosamines and appears later during development. It has been shown that human umbilical cord blood erythrocytes contain less complex and less branched PGCs than erythrocytes of adult individuals.[23] More information about PGC fractions isolated from different biological sources is given below.

5.3. PGCs of Human Erythrocytes

PGCs from human erythrocyte membranes may contain up to 40−50 saccharide residues per ceramide.[1,9,10,19,21] The complete structures have been determined for less complex glycolipids with up to 16 monosaccharides[29,57] (Figure 3) and for species with 22-[9] and 23-sugar[10] residues. However, most of the analytical work has been performed on mixtures of molecules. PGCs of human erythrocytes display considerable structural heterogeneity in contrast with PGCs from rabbit erythrocytes which form a series of components with a 5-sugar interval between species.

PGCs of human erythrocytes carry epitopes of ABH, Ii, and Le^x/Le^y histo-blood-group systems (Table 1 and Figure 1). Epitopes of ABH and Ii systems are abundant; however, the type-2 Lewis structures are minor and only trace amounts of 1,3,4-linked GlcNAc have been detected by analysis of PMAAs (Miller-Podraza. H., et al., Unpublished results). Some PGC molecules of human erythrocytes are sialylated, which is the basis for binding of some pathogenic microbes, such as *Helicobacter pylori, Streptococcus suis, Mycoplasma pneumoniae,* and influenza virus (Table 1). The content of sialic acid in unseparated mixtures is close to 2 mol per 1 mol of ceramide.[24] High-pH anion-exchange chromatography (using Dionex Corporation ion-exchange equipment) of polysaccharides released from the molecules by ceramide glycanase shows the presence of a major neutral fraction and several other fractions with increasing Neu5Ac/Hex molar ratio.

Molecular masses of highly heterogeneous mixtures of neutral and monosialylated PGCs (blood-group O) and the corresponding polysaccharides have recently been resolved by MALDI TOF MS,[19] see Figure 4. On the basis of the accuracy of measurements, the exact number and type of sugars could

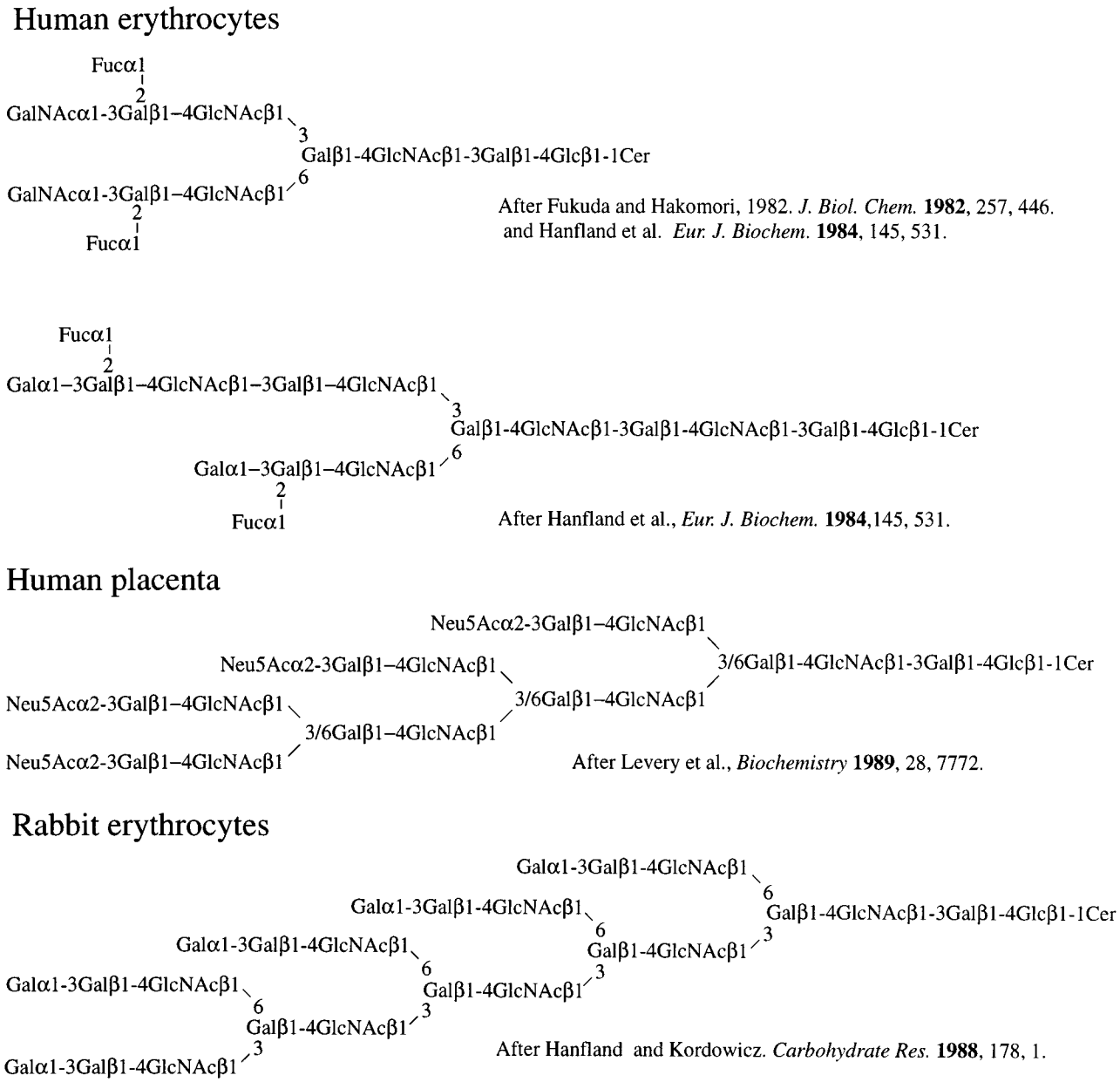

Figure 3. Selected structures of complex glycosphingolipids.

be concluded from these analyses. The compositions of the PGC molecules agreed with the general formula of $Fuc_{(0-7)}Neu5Ac_{(0-1)}Hex_{(x+2)}HexNAc_{(x)}Cer_{(1)}$, with x varying between 4 and 17. The most complex sialylated PGC was found at m/z 8057.4 (M−H), which could be assigned to a structure composed of $Fuc_{(4)}NeuAc_{(1)}Hex_{(19)}HexNAc_{(17)}Cer_{(1)}$ (ceramide d18:1−24:0/1; Table 4 in ref 19). From this and similar compositions, it is evident that fucose (a characteristic part of ABH and Lewis histo-blood-group antigens) and sialic acid may be present in the same molecules.

The poly-*N*-acetyllactosamine chains in the PGC molecules are typically branched. Only in humans of the rare i phenotype,[58] in infants,[23] and in individuals with some rare glycosylation defects[59] are the chains mostly linear. According to permethylation−degradation analyses of PGCs (adult individuals), the branching Gal (1,3,6-linked Gal) constitutes ca. $^1/_3$ of the total Gal of the saccharide chains.[1,24,34] This agrees with branching on every second lactosamine unit.[34] However, recent MALDI-TOF MS showed that the distribution of branches is not necessarily even. The MALDI analysis revealed that both completely and incompletely branched structures are present in mixtures of PGCs isolated from human erythrocytes.[19] For example, highly fucosylated PGCs like $Fuc_{(5)}Hex_{(11)}HexNAc_{(9)}Cer_{(1)}$ (described in ref 19 as fraction 9.5) indicate fully branched chains. This particular chain should contain four branching points and five FucGalGlcNAc terminals (H epitopes) (Figure 5). On the other hand, molecules with even numbers of lactosamine units, like $Fuc_{(2)}Hex_{(12)}HexNAc_{(10)}Cer_{(1)}$, should contain at least one linear dilactosamine structure. Analysis of fragments released by endo-β-galactosidase confirmed the presence of both fully branched and incompletely branched domains, and the linear extensions were found in both 3- and 6-linked antennae.[38] This is different from structures established for PGCs of rabbit eryth-

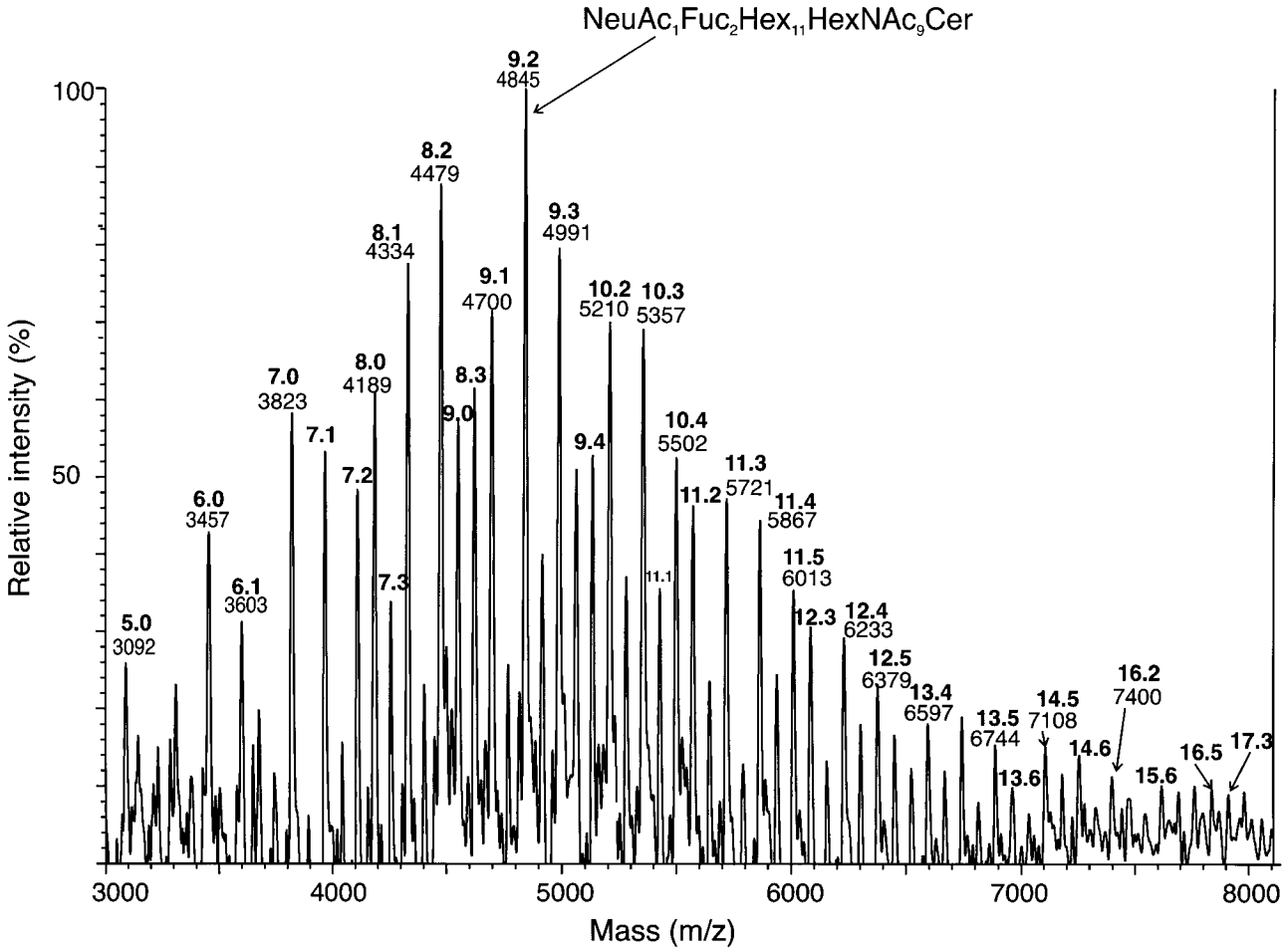

Figure 4. Example of the complexity of PGCs from human erythrocyte membranes (group O): MALDI-TOF mass spectrum, obtained in negative-ion linear mode, showing the monosialylated species (M−H) containing from 13 (*m*/*z* 3092) to 40 (*m*/*z* 7912, labeled as 17.3) monosaccharides per ceramide. All marked components followed the general formula of $NeuAc_{(1)}$-$Fuc_{(y)}Hex_{(x+2)}HexNAc_{(x)}Cer$ (d18:1−24:0/1), with *x* varying from 5 to 17 and *y* from 0 to 6. The annotations are made as *x*.*y* (HexNAc.Fuc). Minor species with d18:1-C22:0/1ceramide present in the mixture are not marked in the picture. The analysis was performed on a TofSpec-E time-of-flight mass spectrometer (Micromass, Manchester, England). For technical details and more information about identified species, see ref 19.

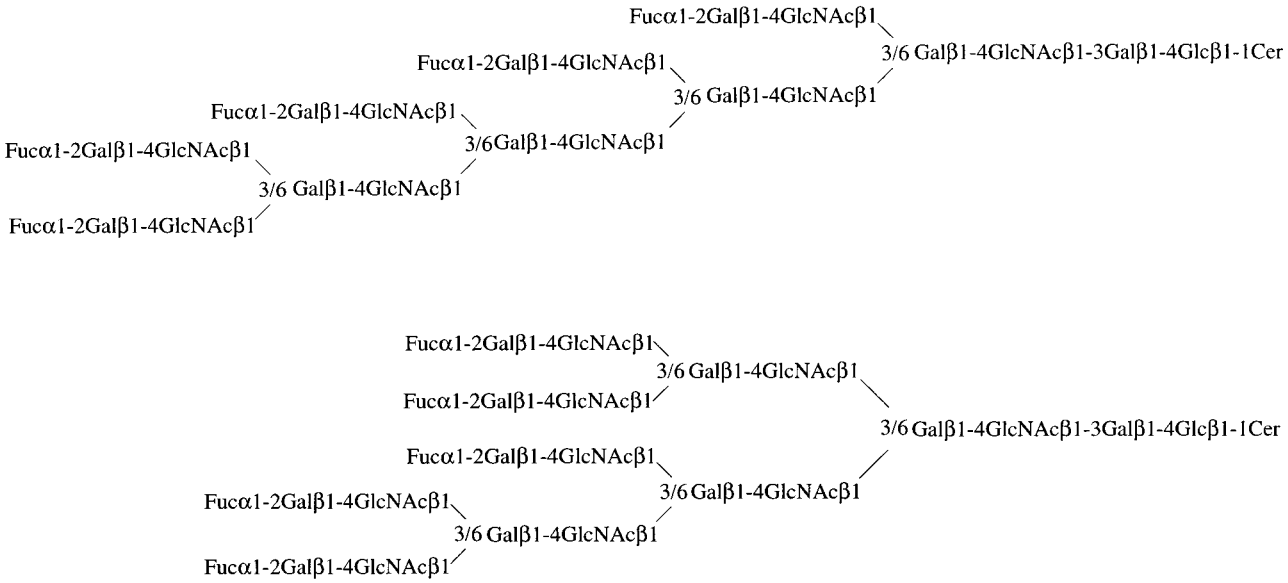

Figure 5. Two variants of branching arrangements proposed for a PGC species with a composition of $Fuc_5Hex_{11}HexNAc_9$-Cer, identified by MALDI-TOF MS in a mixture of PGCs prepared from human erythrocytes (group O).[19]

rocytes and suggests that dendritic chain arrangements may be involved in PGCs of human erythrocytes (E and F in Figure 2). Different possibilities of branching arrangements in human erythrocyte PGCs

has earlier been discussed based on the observation that the elimination of branching points by Smith degradation is gradual and not complete even after four cycles of degradation.[34] The understanding of complete structures of PGCs from human erythrocytes requires further investigations. Zdebska and Koscielak[60] reported the presence of chitobiose (GlcNAcβ4GlcNAc) fragments, which was not confirmed by MALDI-TOF MS fingerprinting. However, these structures may be present in highly sialylated subfractions, which have not yet been analyzed.

PGCs can be isolated from human erythrocytes in yields of 2−5 mg per unit of blood (400 mL).[21,23,24] In these cells the PGCs carry ca. 20% of all poly-*N*-acetyllactosamine chains associated with the membrane,[61] and their contribution to the total ABH blood-group activity has been estimated as 10−15%.[62] For comparison, ca. 5% of the activity has been shown to be associated with less complex glycosphingolipids and ca. 80% with glycoproteins. The predominant ceramides in human erythrocyte PGCs are made up of the sphingosine d18:1 and fatty acids C24 and C22, saturated and monounsaturated.

5.4. PGCs of Human Placenta

Complex gangliosides with 15 and 20 saccharides per ceramide were isolated from human placenta in 1989 by Levery et al.[11] The 20-sugar glycolipid contained the saccharide chain with three branching points and four termini substituted with Neu5Ac (Figure 3). As shown by a combined analysis using NMR, mass spectrometry, and degradation tests, the branches were arranged according to the linear chain with short branches (model A in Figure 2). However, the sequential enzyme degradation of terminal sequences followed by analysis of PMMAs indicated the presence of isomeric gangliosides with different distribution of 1,3- and 1,6-glycosidic linkages. The main ceramides of placenta PGCs were shown to contain sphingosine d18:1 and fatty acids C22:0 and C24:0.

5.5. PGCs of Human Granulocytes

Human granulocytes contain a series of oligofucosylated monosialo neolacto gangliosides with up to 15 monosaccharides per molecule[63−65] called myeloglycans. Recently the presence of a highly sialylated fraction of complex glycolipids has been reported, which migrated on TLC plates in the chromatographic region of PGCs.[44,45] This material has not yet been structurally characterized, but its glycosphingolipid nature has been proven by use of ceramide glycanase. This enzyme specifically cleaves the glycosidic bond between ceramides and carbohydrate chains in glycolipids which can be monitored by TLC. The glycolipids contain very little fucose and have ceramides with mainly d18:1 sphingosine and C16 and C24 fatty acids. Like PGCs from human erythrocytes, the PGC fraction of human granulocytes is receptor-active for the human gastric pathogen *Helicobacter pylori* (45). The content of these complex glycolipids in granulocytes is ca. 0.8 nmol per 10^8 cells, which is ca. 20 times less compared to levels of less complex gangliosides in this material.[45]

5.6. PGCs of Rabbit Erythrocytes

Complex mixtures of glycolipids containing on average 30 sugar residues per molecule were isolated in 1981 by Honma et al.[15] The material contained highly branched poly-*N*-acetyllactosamine chains with Galα3Galβ4GlcNAc (Figure 1, B-like structure) sequences at nonreducing termini. At the same time, Hanfland et al.[29] reported the presence in rabbit erythrocytes of a 10 sugar neolacto glycolipid with one branching point and two B-like terminal structures. Later, the complete structure was resolved for glycolipids with 15,[30,32] 20 and 25,[12,31] 30 and 35,[13] and 40[13,14] saccharide units per molecule, thus revealing a series of molecules with a five-sugar interval between molecular species (see also Figure 3). The structures were determined by a combined methodology which included various mass spectrometry techniques, degradation tests, and NMR. The investigated fractions contained saccharides with short branches (Galα3Galβ4GlcNAcβ) on all internal lactosamine units, which is in accord with model A in Figure 2. Serologically, the PGCs were described as B and I blood-group active.[12−14,29,30,32] Using ^{1}H NMR spectroscopy at 600 MHz, the regular branched structure was confirmed even for the 40 sugar glycolipid.[14] The major ceramides identified in rabbit erythrocyte PGCs corresponded to species with sphingosine d18:1 and fatty acids C24:0 and C24:1.

5.7. PGCs of Rabbit and Dog Small Intestines[16]

PGCs isolated from these tissues display considerable structural heterogeneity at the nonreducing termini. Binding tests using monoclonal antibodies and EI MS after permethylation revealed that PGCs of rabbit intestine contain B-like epitopes as well as complete epitopes of the ABO and type 2 Lewis histo-blood-group systems (Table 1 and Figure 1). This is different from PGCs of rabbit erythrocytes which are terminated entirely with B-like structures (see section 5.6). In addition, these PGCs contain side chains terminated with HexHexNAc (unsubstituted branches) and branches terminated by sialic acid in the form of Neu5Ac or Neu5Gc. A series of ceramides was released from PGCs of rabbit intestine by ceramide glycanase, which contained sphingosine d18:1 and C18−C26 fatty acids.

PGC fractions isolated from dog intestine mucosa were interesting in this respect in that they contained high amounts of fucose linked to C3 of GlcNAc residues, as shown by analysis of PMMAs. These PGCs were highly reactive with anti Le^x and anti Le^y monoclonal antibodies and also displayed activities of the H and A blood-group systems. Similar to PGCs of rabbit intestine, the dog intestine PGCs also contained unsubstituted terminal structures and structures substituted with Neu5Ac or Neu5Gc. The presence of highly fucosylated PGC molecules in dog intestine was confirmed by MALDI TOF MS (Miller-Podraza, H., et al., Unpublished results). The main ceramides identified in dog intestine PGCs contained phytosphingosine and C16−C24 hydroxy fatty acids.

5.8. PGCs of Hog Gastric Mucosa

Hog gastric mucosa contains a series of complex glycosphingolipids with A- and H-blood-group-activities.[17,18,52,66−69] On the basis of molar ratios of sphingosine and carbohydrates, the presence of glycosphingolipids with up to 36 sugar residues per ceramide has been reported,[17] and the complete structure has been proposed for species with up to 20 saccharides.[18,69] Some of the reported sequences were uncommon, for example, Galβ3(Galβ6)Gal branches in some less complex species[66,68] or the hybrid lactoneo/lacto[18,52] or chitobiose fragments[18,52] in more complex species. These structures were reported based on old methodology, and it might be worthwhile to reexamine them using more advanced techniques. Ceramides of PGCs of hog gastric mucosa contained sphingosine d18:1 and C16 and C18 fatty acids.[17,18]

5.9. PGCs of Cultured Cells

Polyglycosylceramides were isolated also from pancreatic carcinoma cell line PANC-1 using a combination of detergent solubilization, protease digestion, and reverse-phase chromatography.[22] The glycolipids were degraded by ceramide glycanase, and the released saccharides were analyzed by methylation and degradation tests and by lectin binding. The structures reported for these PGCs were in agreement with a linear chain with short side branches (model A in Figure 2), with 16−18 monosaccharides in polylactosamine core chains and 1−2 mol of sialic acid in α2−3 linkage to penultimate galactose. The material also contained some fucose probably linked to the internal GlcNAc, as judged from binding to lectins.

5.10. Complex Glycosphingolipids of Other Sources

Indications of the presence of PGCs were obtained for human colon mucosa,[16] rat small intestine mucosa, and wild boar small intestine mucosa.[44] GSLs with saccharide chains with up to 10−15 monosaccharides per molecule were isolated also from some other tissues such as bovine erythrocytes,[71,72] porcine kidney,[73] rabbit and sheep thymus,[74] or some human cancer tissues.[75]

A rich source of highly polar gangliosides are brains of lower vertebrates, such as fish and amphibians.[76−79] These animals contain appreciable amounts of gangliosides containing up to five sialic acid residues linked to Galβ3GalNAcβ4Galβ4GlcCer (ganglio structure). Tetra- and pentasialogangliosides were isolated from human brain[80−83] and brains of some higher animals[84,85] in only minor amounts. Higher vertebrates mainly contain less sialylated species with 1−3 sialyl residues per molecule.[86−88] It should also be mentioned that GSLs with up to nine monosaccharides per Cer, belonging to the arthro-series of GSLs, were isolated from insects.[191] The glycolipids of lower animals may have unusual substitutions, e.g., phosphoryletanolamine[191] or phosphorylcholine.[192,197,198]

5.11. PGCs and Poly-*N*-acetyllactosamine-Containing Glycoproteins

There are indications that poly-*N*-acetyllactosamine chains differ in glycoproteins and glycolipids. In human erythrocytes, which are well characterized regarding cell surface carbohydrates, polylactosamines are associated with membrane glycoproteins known as Band-3 and Band-4.5[139,140,317−321] and with PGCs. The poly-*N*-acetyllactosamine chains on Band-3 resemble the carbohydrate moieties of PGCs in that they carry the same blood-group determinants and like PGCs contain sialic acid. However, endo-β-galactosidase releases from Band-3 Neu5Acα3Galβ4GlcNAcβ3Gal and Neu5Acα6Galβ4GlcNAcβ3Gal,[54,141] whereas the simplest sialylated product detected after this enzymatic digestion of PGCs is a heptasaccharide with a possible branched structure, Neu5AcHexHexNAc(HexNAc)HexHexNAcHex.[38] This indicates that in Band-3 Neu5Ac may terminate linear extensions based on more than one lactosamine units, whereas in PGCs the sialic acid is present on short (monolactosamine-based) branches. Different presentation of Neu5Ac in Band-3 and PGCs is supported by studies with *H. pylori*, where it has been shown that the PGCs are receptor-active for the bacterium whereas Band-3 is not.[42] The difference in binding was shown by overlay of the glycoconjugates separated on artificial surfaces with radiolabeled bacteria.

The differences between protein- and glycolipid-associated poly-*N*-acetyllactosamines was indicated also by structural studies. On the basis of enzymatic degradation, the Band-3 glycoprotein is believed to have linear chains with short branches.[54] One round of digestion with exo-β-galactosidase and exo-β-glucosaminidase eliminated branches in Band-3 poly-*N*-acetyllactosamines. On the other hand, several rounds of Smith degradation of PGCs could not destroy the branches.[34] The latter results were discussed with precaution because of the possibility of incomplete oxidation; however, recent experiments with endo-β-galactosidase indicate that linear extensions may be present on both 3- and 6-linked antennae in PGCs.[38] Various ways of synthesis of different polylactosamine glycoconjugates seem to be probable in view of the recent discovery of different branching enzymes (see section 6). As discussed, the β6cGnT and β6dGnT introduce β1,6 branches in oligo/polylactosamine chains in a different way. Also, the extending enzymes may be different, and it has been demonstrated that two different β4-galactosyltransferases, β4GalTI and β4GalTIV, are involved in the synthesis of poly-*N*-acetyllactosamines in *N*-och *O*-glycans.[104]

6. Biosynthesis of PGCs

Biosynthesis of GSLs proceeds in a stepwise manner by the sequential addition of glycosyl units to the lipid ceramide.[89−93] Ceramide part is build up at the cytosolic surface of the endoplasmic reticulum from D-erythrosphinganine and acyl-CoA.[89,90] Dihydroceramide which is formed in the first step is converted to ceramide by introduction of the double

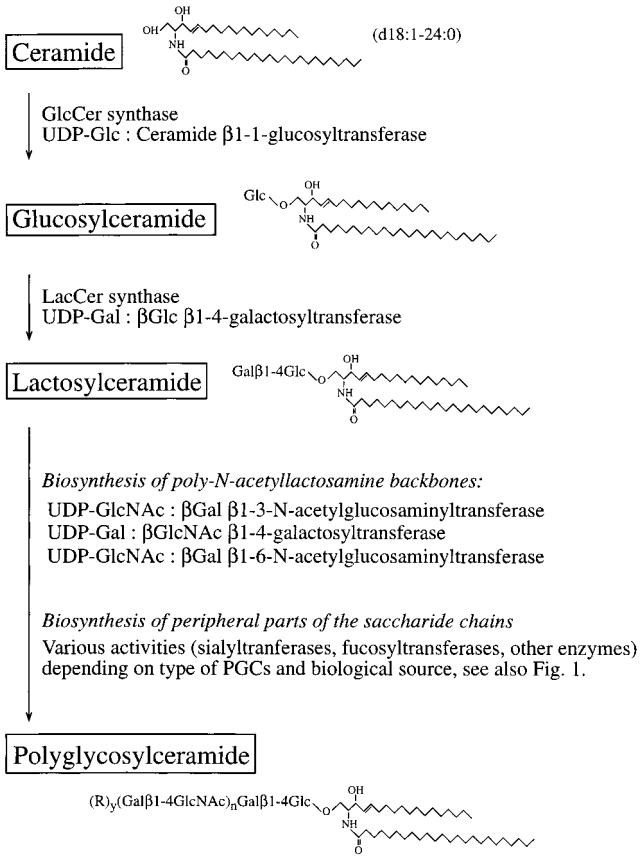

Figure 6. Enzymatic activities involved in biosynthesis of carbohydrate parts of polyglycosylceramides. R_y indicates terminal substitutions and $(Gal\beta1\text{-}4GlcNAc)_n$ repeated LacNAc units of the core chain.

bond.[91] Ceramide constitutes the acceptor for monosaccharides which are transferred by specific glycosyltransferases from appropriate sugar nucleotides to the growing molecule. The first steps of glycosylation may take place in the endoplasmic reticulum,[94,95] but most of the glycosyltranferases involved in the biosynthesis of GSLs are localized in the Golgi complex.[89,91,92]

The biosynthesis of PGCs (Figure 6) proceeds via Glcβ1Cer (GlcCer) and Galβ4Glcβ1Cer (LacCer). GlcCer synthase, UDP-Glc:Ceramide β1,1-glucosyltransferase,[96−98] and LacCer synthase, UDP−Gal:βGlc β1,4-galactosyltransferase (enzyme designated as GalTII belonging to the β4GalT family),[99−101] are important for synthesis of different series of glycosphingolipids. The further formation of poly-*N*-acetyllactosamine backbones of PGCs requires three enzyme activities: (a) UDP-GlcNAc:βGal β1,3-*N*-acetylglucosaminyltransferase activity (β3GnT family of enzymes), (b) UDP−Gal:βGlcNAc β1,4-galactosyltransferase activity (β4GalT family), and (c) UDP-GlcNAc:βGal β1,6-*N*-acetylglucosaminyltransferase activity (β6GnT family). The first two activities contribute, by alternate action, to the formation of linear poly-*N*-acetyllactosamine chains with 3-*O*-substituted Gal and 4-*O*-substituted GlcNAc,[102] and the third activity transfers GlcNAc to Gal residues of the chain forming GlcNAcβ1,6-branches.[103] Additional enzymes introduce terminal blood-group structures, sialic acid, etc. The latter enzymes may differ depending on the kind of PGC and biological source.

There are several known β4GalT enzymes, and two of them, β4GalTI and β4GalTIV, were reported to be efficient in the synthesis of polylactosamine chains.[48,104] They are different from β4GalTII involved in the synthesis of LacCer (see above). The β4GalTI has recently been shown to have, under defined laboratory conditions, higher preference to β1,3-linked GlcNAc compared with β1,6-linked GlcNAc,[48] which may be of importance for final arrangement of the branched structures in some PGC preparations. On the other hand, earlier studies by Vilkman et al.[122] demonstrated that the 1−6 branches of oligo-*N*-acetyllacosaminoglycan backbones can be enzymatically elongated as well as the 1−3 branches, thus allowing the formation of a large number of different branched structures. Concerning the β3GnT family, there are indications that at least two different enzymes are involved. The recently described β3GnTs with structural similarity to β3GalTs[105] had the same activity as β3GnT described by Kawashima.[106] However, this enzyme showed no structural similarity to the β3GnT reported by Sasaki.[107] Three variants have also been reported for β6GnTs: (a) terminally acting enzymes which transfer GlcNAc to C6 of terminal Gal of a growing chain, (reviewed in 103); (b) distally acting enzymes (β6dGnT)[108−115] which transfer GlcNAc to C6 of the peridistal Gal residue of a sugar chain (GlcNAcβ3**Gal**β4GlcNAcβ-R) being active immediately after addition GlcNAcβ3 to the branching Gal (shown in bold), and (c) centrally acting enzyme (β6cGnT) which transfers GlcNAc residue to midchain Gal residues, being active after attachment of at least one full lactosamine unit to the branching Gal, as proposed for the following acceptor oligosaccharides: Galβ4GlcNAcβ3**Gal**β4GlcNAc−R,[113,116,117] Galβ4GlcNAcβ3**Gal**β4GlcNAcβ3**Gal**β4GlcNAcβ-R,[114,118−121] and Galβ4GlcNAcβ3**Gal**β4GlcNAcβ3**Gal**β4GlcNAcβ**Gal**β4GlcNAcβ-R.[49]

According to current concepts on the glycoconjugate biosynthesis, the formation of saccharide chains is controlled by the substrate specificities of individual transferases and by the order in which they act. On the basis of the transferase affinities for the growing oligolactosamine chains, the following sequence of reactions was proposed by Ujita et al.[48] First, a GlcNAc residue is added by the β6cGnT to a linear polylactosamine chain (Galβ4GlcNAcβ3Galβ4-R) producing the 6-branch at the peridistal LacNAc, giving Galβ4GlcNAcβ3(**GlcNAcβ6**)Galβ4-R (extended structure shown in bold). As a next step, another GlcNAc residue is added by β3GnT to the 3-linked arm resulting in **GlcNAc**β3Galβ4GlcNAcβ(**GlcNAcβ6**)-3Gal-R. This reaction is followed immediately by galactosylation of the 3-linked branch producing **Galβ4GlcNAc**β3Galβ4GlcNAcβ(**GlcNAc**β6)3Gal-R. Finally, galactosylation of the 6-linked GlcNAc results in **Galβ4GlcNAc**β3Galβ4GlcNAc(**Galβ4GlcNAcβ6**)Gal-R. The extended 3-linked branch may then serve as a substrate for β6cGnT and another cycle of branching may take place. In this type of synthesis, the βGalTI has a preference for the 3-linked arm compared with 6-linked arm which is

in agreement with the synthesis of polylactosamines with multiple short 6-linked branches. Such structures have been identified in natural glycoconjugates such as PGCs of rabbit erythrocytes or Band-3 of human erythrocytes. If the branching enzyme acting on the linear poly-*N*-acetyllactosamine chain is not active enough, the result would be an incompletely branched chain with linear extensions between branches. As discussed previously, the incompletely branched domains were identified in polylactosamine chains of PGCs from human erythrocytes. Ujita et al.[48] emphasized the preference of β6cGnT for the penultimate distal Gal; however, other investigators[49,118−121] have shown that the same centrally acting enzyme has the ability to transfer GlcNAc to all internal galactoses of linear polylactosamine primers. The latter finding may explain the in vivo conversion of the developmentally regulated i antigens (with linear lactoneo structures) into I antigens (with branched lactoneo structures), see Figure 1. The sequence of reactions described in ref 48 is in line with polylactosamine structure based on linear $(Gal\beta4GlcNAc\beta3)_n$ chains with multiple, short branches (A in Figure 2). However, the analysis of products obtained by endo-β-galactosidase digestion indicates that in PGCs of human erythrocytes, linear extensions are present on both 3- and 6-linked arms. In agreement with this are reports on the in vitro elongation of the 6-arm[50,122] and on the synthesis of dendritic poly-*N*-acetyllactosamine structures.[50,123] Besides this, different branching arrangements were reported for PGCs from human placenta.[11] It seems that biosynthesis of polylactosamines may differ depending on the type of tissue and type of the glycoconjugate.

Essential for final structures of PGC molecules are also enzymes which participate in the biosynthesis of peripheral parts of the saccharide chains. It has been shown that α1,3-Fuc residues in poly-*N*-acetyllactosamine chains inhibit the β6cGnT reaction at the fucosylated LacNAc unit and at other LacNAc units upstream (synthesized earlier than the fucosylated LacNAc) but not downstream from the fucosylated locus.[121] This implies that site-directed branching in poly-*N*-acetyllactosamines may be possible by linkage/removal of α3Fuc units. An interesting observation was also made for α2fucosyltransferase and human erythrocytes of rare Bombay and para-Bombay phenotypes.[124] Individuals with these phenotypes were shown to contain less complex polylactosamine chains than individuals with complete ABH structures. In PGCs of Bombay and para-Bombay individuals, the sum of carbohydrate residues was found to be ca. 12, whereas in PGCs of other blood groups this value is around 25. Since in Bombay and para-Bombay phenotypes there is a deficiency of the GDP−Fuc:βGal α-1,2-fucosyltransferase, it has been suggested that this enzyme promotes the complexity of polyglycosyl conjugates of erythrocyte membranes. On the other hand, studies by Henry et al.[327] indicate that fucosylation may inhibit in the human small intestine the extending and branching of carbohydrate chains during biosynthesis. These authors showed that fucosyltransferases responsible for synthesis of ABO and Lewis histo-blood-group determinants compete during biosynthesis with other glycosyltranserases, thus cutting off alternative pathways.

At present very little is known about in vivo pathways leading to the formation of different polylactosamine-containing glycoconjugates. The evidence from related fields shows, however, that different types of molecules may be synthesized at different places and that specific sorting mechanisms regulate the intracellular transport of these molecules.[125−128] Recent multi-enzyme kinetic analysis of ganglioside biosynthesis indicate that CMP−Neu5Ac:GM3 2−8 sialyltransferase and UDP−*N*-acetylgalactosaminyl:GM3 *N*-acetylgalactosaminyltransferase in some cell lines do not compete for the same pool of GM3.[129] This may imply that two closely related gangliosides, GD3 and GM2, are synthesized at different sites.

7. Degradation of PGCs

According to conventional models[130,131,227,237] of catabolism of GSLs, PGCs should be degraded in lysosomes by a stepwise action of specific acidic glycosidases. Glycosidases distinguish the configuration α/β in glycosidic linkages but exhibit varying degrees of preference toward different types of monosaccharides and linkage positions. The enzymatic hydrolysis of GSLs requires the presence of protein cofactors (sphingolipid activator proteins, SAPs) which bind GSLs and facilitate their digestion in an aqueous environment.[56,131,132,227,237] GSLs are generally not freely soluble in water because of the presence of the lipophilic ceramide moiety. SAPs can also interact with glycosidases and stimulate enzymatic activities.[56] Several sphingolipid activator proteins have been described, and these include the GM2 activator[182,227] encoded in humans by a gene located on chromosome 5[183] and four saposins, SAP A, SAP B, SAP C, and SAP D, derived from a common precursor (prosaposin) encoded by a gene located on chromosome 10.[56] GSL activator proteins exibit varying degrees of specificity toward GSLs and glycosidases; for example, SAP B (called also SAP-1) is known as sulfatide and GM1 activator protein and SAP C as GlcCer activator protein (Gaucher factor). The GSL activators are small glycoproteins with a high content of hydrophobic amino acid residues, which may be of importance for interactions with lipophilic parts of GSLs.[56] Of clinical importance is that mutations in the genes that encode glycosidases or GSL activator proteins may lead to GSL storage disease.[131,184−190] To facilitate enzymatic degradation of GSLs in vitro, various detergents must be used, and this requirement applies even to apparently water-soluble PGCs.

A number of glycosidases have been described which may be involved in the degradation of complex poly-*N*-acetyllactosamine chains of PGCs. These include both exoglycosidases and endoglycosidases. The first group comprises enzymes which split off monosaccharides from the nonreducing end, like fucosidases, galactosidases, sialidases, glucosaminidases, etc.[35] Among endoglycosidases there are several endo-β-galactosidases which are able to hydrolyze Galβ4GlcNAc structures in poly-*N*-acetyllactosamine

chains in various glycoconjugates including PGCs[38,133] and ceramide glycanases,[134−136] which hydrolyze the β1,1-glycosidic linkage in the Glcβ1,1Cer part of the glycolipids.[16,24] Fatty acid may be split off from GSLs with the aid of a sphingolipid ceramide-*N*-deacylase.[137,138] For a review on glycosidases involved in GSL degradation, see ref 35.

8. Biological Functions of PGCs

8.1. Presumable Biological Functions of GSLs

It is reasonable to assume that GSLs serve more than one function in nature. These amphipathic compounds constitute a large group of cellular components which comprise a wide variety of carbohydrate and ceramide structures. One function may be stabilization and shape determination of lipid bilayers. It has been proven by studies in vitro that GSLs can form extensive hydrogen bonding with phospholipids which influences both the transition temperature and structural integrity of the membranes.[250−253] Negatively charged gangliosides and sulfatides are believed to be involved in ion binding and transport in membranes.[256] Gangliosides can effectively bind Ca^{2+} ions, which is of importance for various cellular events including synaptic transmission.[254,255,259,282,316] Renal glycosphingolipids have been reported to be involved in transport of various positively and negatively charged ions,[256] and in one of the recent approaches it has been demonstrated that the GlcCer synthase inhibitor PDMP increases Na/Pi cotransport activity in cultured kidney cells.[257]

GSLs are carriers of various binding epitopes and, as such, believed to be implicated in a variety of vital biological processes. They can interact with antibodies (e.g., anti-blood-group antigens antibodies[55]), lectins, toxins, and other proteins such as selectins, integrins, or fibronectin.[156,256,314] There are many indications that GSLs are involved in cellular recognition and cell adhesion.[256,296,298] Interaction with extracellular matrix proteins may be of importance for latter processes. For example, fibronectin has been shown to bind to gangliosides on the surface of cultured cells.[297] Recent studies using artificial phospholipid membranes with embedded ligands have demonstrated that sialyl-Le^x-active GSLs can mediate rolling and firm adhesion of selectin-containing hamster ovarian cells.[296] The same sialyl-Le^x ligands are involved in rolling of leukocytes along endothelial cells.[152,153,299]

New reports on the involvement of GSLs in receptor functions continually appear,[263−278] and there is a growing body of evidence that GSLs play a role in signal transduction.[4,154−156] It seems that GSLs occur in plasma membranes in the form of clusters (GSL-enriched microdomains, GEMs), which is currently the subject of extensive research.[4,70,155,161−164,315] GEMs seem to be associated with caveolae,[160] small invaginations of the plasma membranes, and sphingolipid-cholesterol clusters functioning as mobile rafts within the fluid bilayer.[159] It is believed that GEMs, which also contain GPI (glycosylphosphatidylinositol)-anchored proteins and other signaling proteins,[315] are involved in glycosignaling. Some glycosphingolipids have been described as receptor modulators and effectors of protein kinases, enzymes involved in signaling cascade. For example, ganglioside GM3 has been shown to bind to EGF (epidermal growth factor) receptor tyrosine kinase[157] and GM1 to Trk A kinase (receptor for NGF, nerve growth factor).[158] In addition, GSLs can induce biochemical changes in cells. For example, sulfatides induce lymphocyte activation and elevation of cytosolic Ca^{2+},[278] stimulate generation of intracellular oxygen radicals in neutrophils,[279] and cause induction of phagocytosis and phagosome−lysosome fusion in these cells.[280] Of importance is that GSLs metabolites (ceramide, sphingosine-1-phosphate) can function as second messengers.[5,165,295]

Many discoveries have been made which show that GSLs are involved in cellular growth and differentation.[156,228,281,323] Using exogenously added GSLs, it has been shown that GSLs influence growth rate and saturation density of various cultured cells.[283−286] This has been confirmed by more advanced techniques, and in one recent report based on genetic engineering, it was demonstrated that transfection of a cDNA coding for GD3 synthase into Nuro2A cells causes neurite outgrowth and cholinergic differentiation of these cells.[287] In another study, the importance of GSLs for embryonic development has been proven by elimination of the major synthesis pathway of GSLs through targeted disruption of the gene encoding GlcCer synthase in mice.[228] Considerable attention has been devoted to glycosphingolipids and malignant transformation.[288,289,291−293] Early studies indicated that cell transformation was associated with a simplification in various glycolipid structures due to blocked synthesis.[290,291] Changes in gangliosides and other GSLs have been observed in various tumors including melanoma, neuroblastoma, astrocytoma, lung cancer, and sarcoma. In melanoma, the transformed cells synthesize large amounts of ganglioside GD3,[294] which replaces ganglioside GM3, the main ganglioside of normal skin melanocytes. It has been suggested that ganglioside GD3 may enhance the metastatic potency of melanoma cells by influencing the attachment of the malignant cells to extracellular matrix proteins such as fibronectin and laminin.[258]

GSLs are also known binders of microbes[3,300] and toxic proteins.[301,302] They have been described as receptors for important pathogens such as influenza virus,[303] *Helicobacter pylori*,[3,300] *Haemophilus influenzae*,[304] *Neisseria meningitidis* [304] or *Escherichia coli*.[305] GSLs seem also to be implicated in HIV infections,[306,307,311] most probably as immunoregulatory molecules.[306] Regarding toxins, a classical example is the ganglioside GM1 which has been described as a receptor for cholera toxin B-subunit.[308] Other examples are ganglioside GD1b which binds to tetanus toxin,[310] paragloboside, nLc$_4$Cer, with affinity for heat-labile enterotoxin of *Escherichia coli*,[309] or ganglioside GT1b which has been suggested to be a component of the receptor complex for *Clostridium botulinum* type B neurotoxin (BoNT/B).[312] On the other hand, it has been suggested that GSLs play a role in receptor activity for interferon.[260] Interferons are species-specific proteins which induce

antiviral and antiproliferative responses in cells. Although studies with ganglioside-deficient transformed mice did not prove that gangliosides are necessary for interferon action,[261] a recent study showed that globotriaosylceramide, Gb_3Cer, may mediate interferon-dependent antiviral activity.[262]

There is extensive literature on GSLs roles, and many aspects are beyond the scope of this review. The biological significance of GSLs remains in many cases unproven; however, the future is promising for the development of new techniques and for probing biological roles of these interesting molecules.

8.2. Presumable Biological Functions of PGCs

PGCs display antigenic and receptor properties. They are carriers for blood-group determinants and are effective binders of some microbes and toxic proteins (Table 1). PGCs of human blood cells have been shown to bind to such pathogens as *Helicobacter pylori*,[42−45] *Streptococcus suis*,[142] influenza virus,[46] and *Mycoplasma pneumoniae*.[143] PGCs of human red cells have also been reported to interact with *Ricinus communis* lectin (ricin),[144] and PGCs from rabbit intestine have been shown to interact with heat-labile toxins of *Escherichia coli*.[145,180] The latter activity seemed to be developmentally regulated because PGCs from adult rabbits but not from infant rabbits were binding.[145] PGCs are also likely candidates as receptors for ligands of nonpathogenic nature. A variety of molecular interactions of PGCs with yet undefined molecules may be expected because the branched poly-*N*-acetyllactosamine chains are potential carriers of many binding structures.

One interesting piece of evidence for receptor properties of PGCs comes from studies on ricin-resistant BHK (baby hamster kidney) cultured fibroblasts and PGCs of human erythrocytes.[144] Ricin (*Ricinus communis* lectin) is toxic to animal cells, and one of its effects is the inhibition of cell protein synthesis. The resistant BHK cells contained a reduced number of receptors for ricin at the surface and did not fully respond to the cytotoxicity of the ricin protein. When these cells were incubated with PGCs, the increased binding of ricin to the cells was observed and there was a significantly greater sensitivity of the cells to the ricin-mediated inhibition of cell protein synthesis, as measured by reduced uptake of radiolabeled leucine. Thus, in these studies, the exogeneously incorporated PGCs caused the phenotypic reversion of the ricin-resistant cells to sensitive cells.

The binding of pathogenic agents to PGCs may be to sialylated or neutral sequences. The dependence on the presence on sialic acid was well proven for *H. pylori* and PGCs of human erythrocytes and granulocytes. In these systems the binding disappeared after mild periodate treatment of the receptor carbohydrates.[28] The mild periodate oxidation modifies selectively the sialic acid glycerol side chain. Binding to sialylated sequences of PGCs was also proven for *Mycoplasma pneumoniae*[143] and *Streptococcus suis*.[142] On the other hand, binding of *H. pylori* to PGCs of rat intestine mucosa and wild boar intestine mucosa was in a sialic acid independent way, as proven by experiments with different bacterial strains.[43]

The majority of known PGCs display blood-group activities (Table 1). Blood-group antigens are sometimes seen as markers of differentiation and maturation,[55,146] especially for tumor cells.[147] However, the actual role of the blood-group saccharides is in most cases not known.[325] Varki[148] emphasized that saccharides serve as binding sites for pathogenic microorganisms and, as such, are subject to structural variation because of ongoing host−pathogen interactions during evolution. Koscielak[149] suggested that blood-group determinants of ABH, Lewis, and P group systems in erythrocytes are noninformative structures which confer biological inertness on otherwise functionally active carbohydrate structures. Sialic acid should serve the same purpose, which is protection from proteolysis, denaturation, and protection from recognition of potentially antigenic sites by the defense system of the body. In this hypothesis, glycolipids were considered as energetically cheaper alternatives for coating material compared to glycoproteins. It is true that carbohydrates may have a protective role; however, there is a growing body of evidence that some may participate actively in biological phenomena.[148,150,151] For example, the histo-blood-group determinant sialyl-Le^x (Neu5Acα3Galβ4-(Fucα3)GlcNAcβ) serves as a ligand for E-selectin which is of importance for recruitment of leukocytes to inflammatory sites.[152,153] The functions of saccharides are diverse, and the same oligosaccharide sequence may mediate different functions at different locations within the same organism or at different times in its ontogeny or life cycle.[148] The actual biological significance of PGCs is at present not defined. Studies on PGCs in different tissues are hampered by their low abundance in cells and by difficulties in their separation from other water-soluble glycoconjugates.

The biological significance of PGCs may have a connection with their lipid parts. GSLs are primarily known as cell membrane components and are believed to be anchored in the lipid bilayer of plasma membrane through the hydrophobic ceramide moiety. The polar saccharide chains extend into the surrounding water milieu providing conditions for interaction with ligands. As previously mentioned, GSLs are not distributed randomly in cell membranes but rather are present in GSL-enriched domains, GEMs,[4,70,155,161−164] which are believed to be implicated in signal transduction due to the presence of concentrated receptors for interaction with ligands.[155,159] The mechanism by which GSLs could be implicated in this process is not defined. One possibility is the interaction of GSL clusters with transducer proteins. The alterations of the transducer molecule conformation would then trigger signal transduction by an as yet unexplained mechanism.[155] It should again be emphasized that GSLs themselves contain fragments which upon release display biological activities important for cell regulation and signal transduction.[5,165] Ceramide has been shown to be an important regulatory component of apoptosis, and sphingosine-1-phosphate has been considered as a second messenger implicated in vital cellular

processes associated with cell growth and cell survival.[5]

9. Relationship of PGCs with Disease

The majority of known binding sites for microbes are glycoconjugates, and the binding to target cells is important for establishment of infections and development of clinical symptoms.[3,328] As discussed in the previous section, PGCs display binding activities for various microbes and toxins. This indicates that PGCs may serve as mediators of pathological processes, but it may also mean that they contribute to a barrier for the entry of pathogens and toxic proteins.

Of recent medical interest is the recognition of human PGCs by *Helicobacter pylori*, which is a human-specific pathogen implicated in common gastric diseases such as gastritis and ulcer and cancer.[166] This microbe colonizes more than 50% of the population in the world, causing chronic gastric inflammation in all infected individuals.[167] Overt clinical symptoms appear in ca. 10−20% of colonized individuals,[168] resulting in medical problems on a global scale. The bacterium is uniquely complex in its recognition of carbohydrate structures with ca. 10 different specificities detected.[3,328] At least two different sialic acid-dependent activities were identified,[42,43] and one of them had been shown to be associated with PGCs of human origin.[16,42−45] The *H. pylori*-binding molecules were found in different tissues including granulocytes,[44,45] which are inflammatory cells, and phagocytes actively engaged in *H. pylori*-associated diseases.[169−171] The pathogenesis and mechanisms of *H. pylori* diseases are not clear, but there are indications that contact of the bacterial cells with granulocytes is essential for development of chronic inflammations.[167] It has even been suggested that sialic acid-dependent binding of *H. pylori* is of importance for resistance of the bacterium to phagocytosis.[171] The sialylated epitope recognized by *H. pylori* in human PGCs has not yet been characterized. The major sialylated sequence in *H. pylori*-binding PGCs of human erythrocytes is a side branch Neu5Acα3Galβ4GlcNAc.[38] This trisaccharide is, however, inactive in linear chains and in branched PGCs of animal origin. It is possible that *H. pylori* recognizes branch isomers which are common in humans but not in animals. It is also possible that the bacterium binds to di- or oligosialylated molecules which are present in the mixtures in minor amounts[24] but which have not yet been characterized.

PGCs of human erythrocytes have also been described as the most potent of known natural binders of influenza viruses A and B.[46] The virus-binding PGCs were later found in human granulocytes (Miller-Podraza, H., et al., Unpublished results), and this, again, may be of medical importance because influenza viruses mediate dysfunction[172] and apoptosis[173] of these cells. PGCs are also likely contributors to the binding of a protozoal parasite *Plasmodium falciparum*, which invades human red blood cells causing malaria. It has been shown that polylactosaminyl carbohydrates obtained from human red cells exhibit high levels of inhibition of invasion by this parasite.[174,175] However, these studies were done on poly-*N*-acetyllactosamines derived from glycoproteins, and the role of PGCs remains to be explained. The reports on the role of polylactosamines in *P. falciparum* infections are somewhat contradictory, and it has later been suggested that these structures are not obligate receptors for invasion of this parasite.[176]

It seems that in some diseases PGCs may compensate for poly-*N*-acetyllactosamine chains of glycoproteins. This has been shown for a rare type of an inherited anaemia, the congenital dyserythropoietic anaemia type II (CDA II), in which a 6-fold increase of PGCs was reported.[59] The CDA II disease (referred to also as HEMPAS, hereditary erythroblastic multinuclearity with positive acidified serum[177,322]) is characterized by defective glycosylation of glycoproteins and glycolipids. The molecular basis of HEMPAS anaemia seems to vary between cases, and the level of PGCs was reported to be lowered in another case of this disease.[178]

Finally, it should be noted that blood-group-active PGCs may contribute, together with other blood-group-active molecules, to incompatibility in pregnancy and transplantation. Certain blood-group antigens were reported to be important for the rejection of solid organ transplants.[179]

10. Future Directions

One of the major challenges in glycobiology is to understand the significance of structural diversity of complex carbohydrates and to explain the actual biological functions of complex glycoconjugates. Studies on PGCs are still hampered by laborious purification procedures on the one hand and by the lack of effective analytical techniques on the other. A recent success was the resolution of mixtures of PGCs from human erythrocytes using MALDI-TOF MS.[19] This technique was excellent for mass fingerprinting; however, it gave little structural information. For advanced investigation of PGCs, more powerful methods are still needed, possibly with MS/MS (tandem mass spectrometry[235]) applications. In MS/MS, one mass spectrometer separates the components and another records product ion mass spectra of a selected precursor. A promising technique is ESI-MS/MS, which has already been applied for structural studies of less complex glycosphingolipids[200,201] and glycoprotein-derived carbohydrates.[202,203] Another important developing area is NMR spectroscopy. Improvements in high-field NMR instrumentation provide opportunities to analyze chemical bonding, structure, and dynamics in complicated molecular systems including PGCs.[14] This method is nondestructive to samples and provides direct information about many structural features including anomerity of glycosidic bonds.

Considering the heterogeneous nature of PGC preparations, a combination of methods for structural elucidation are required, such as MS/MS, NMR, high-performance liquid chromatography (LC), or capillary electrophoresis (CE). These powerful techniques are now under development in many laboratories, and one can anticipate that they will gradually replace

traditional analytical approaches used for analysis of complex mixtures of molecules. New reports constantly appear on various applications of LC-MS,[204,205,208] CE-MS,[206] and LC NMR.[207] However, at present, laborious combinations of traditional and modern techniques are still in practical use, including chemical and enzymatic fragmentations, other chemical modifications, traditional methylation analysis, MS and/or NMR of separated molecules or fragments, etc. (as exemplified in refs 209 and 210).

No clear picture has yet emerged concerning the actual biological roles of PGCs. If PGCs have receptor functions, an important future effort would be to identify the corresponding binding ligands. This seems to be a complicated task; however, the recent development of glycobiology, molecular biology, and other sciences may help to rationalize difficult approaches. By use of specific carbohydrate-containing photoaffinity probes,[211,212,244] several carbohydrate-binding proteins and binding peptide domains have been identified.[212−216] Of great importance for current science is the increasing volume of information about genome sequences of living species[217] including humans.[218,219] For some species, like *Haemophilus influenzae*[245] or *Helicobacter pylori* (bacteria),[246] *Saccharomyces cervisiae* (yeast),[247] or *Caenorhabditis elegans* (nematode),[248] the entire genome sequences have been reported. This progress, together with the development of proteomics approaches, provide better opportunities to identify functional molecules. Proteomics is an emerging area that interlinks protein and DNA sequence and deals with the global analysis of gene expression.[232−234,249] It has recently been shown that it is possible to correlate carbohydrate epitopes to minor binding proteins using carbohydrate-based photoaffinity probes, electrophoresis, MALDI-TOF MS, and database searching.[216] This has been proven by the identification in the database of the recently cloned[212] membrane-bound Le^b-recognizing adhesin of *H. pylori*. This protein is expressed in low numbers, ca. 500 copies per bacterial cell.[212]

It is well-known that the structures of complex carbohydrates are changed in various diseases, and this concerns even cancer disease (for more information, see Molecular Basis of Glycoconjugate Disease. *Biochim. Biophys Acta*, **1999**, 1455). The role of PGCs in cancer progression remains to be explored; however, it has been shown that protein-linked polylactosamines and other complex carbohydrates change with malignant transformation.[220−223] It has been demonstrated, for example, that *N*-acetylglucosaminyltransferases, which play a pivotal role in the processing of N-linked glycans, are involved in cancer progression and metastasis during hepatocarcinogenesis.[223] The GnT-V, which catalyzes the formation of GlcNAcβ1−6 branches at the Man α1−6 side of the trimannosyl cores of complex type *N*-glycans, is overexpressed in the tumor cells, which results in increased branching. Other conditions where PGCs should be taken into consideration as possible pathogenic factors are autoimmune diseases,[224] inherited diseases,[225] or rejection of transplants.[179,226] The current topic is pig-to-human xenotransplantation,[226] which is considered to be a potential solution to the shortage of human donor organs. However, a better knowledge of cellular glycoconjugates including PGCs, in both humans and pigs, is required for development of approaches to prevent carbohydrate-mediated rejection.

Of great importance for future research is genetic engeneering. Many enzymes responsible for synthesis of carbohydrates and glycolipids have been cloned, and many genes have been localized in chromosome systems of the living species.[238,240−243] Genetic manipulation and gene knockout animals have been applied to investigate the biological roles of many cellular components including GSLs and saccharides. Vimentin-knockout mice have been used to study biosynthesis and intracellular transport of GSLs in fibroblasts,[231] and GlcCer synthase-deprived mice have been applied to investigate the role of GSLs in development and differentiation of embryonic cells.[228] The role of GalCer and sulfatide in myelin of the central nervous was investigated in the UDP−galactose−ceramide galactosyltransferase knockout mice.[239] A genetic animal model has also been developed to study the biological significance of the Le^b structure for gastric colonization by a human pathogen *Helicobacter pylori*.[236] In these studies a human α-1,3/4-fucosyltransferase (enzyme necessary for synthesis of the Le^b structures) was expressed in transgenic mice which normally lack this enzyme. The genetically changed animals were then used to study the molecular pathogenesis of diseases caused by *H. pylori* infection.

Genetic models are also of great importance for investigation of glycosphingolipid storage diseases and for development of new therapeutic approaches.[227,229,230,237] One example is Fabry's disease, which is caused by a deficiency of the lysosomal hydrolase α-galactosidase A and is characterized by progressive deposition of globotriaosylceramide (Galα4Galβ4GlcCer, Gb_3Cer) in tissues.[237] Gene transfer has been applied in mice to test the possibility of a gene replacement therapy against this disease. Recombinant adenoviral vectors encoding human α-galactosidase A were injected intravenously into Fabry's knockout mice, which resulted in the elevation of the α-galactosidase activity in tissues and, consequently, in the reduction in Gb_3Cer content. On the basis of these and other results, the gene replacement has been discussed as a viable approach for the treatment of Fabry's disease and potentially other lysosomal storage disorders. These examples show that genetic manipulation provides a powerful means for future exploration of vital biological questions. The role of polylactosamine and PGCs could be tested, for example, by using β6dGnT- or β6cGnT-knockout animals or cells or by using genetic models with deficiencies of other enzymes essential for biosynthesis or degradation of these components. Alternatively, the use of specific inhibitors may be applied for elimination of selected metabolic pathways, as exemplified by the use of inhibitors of ceramide synthesis.[231]

11. Abbreviations[328]

MALDI-TOF MS	matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
FAB MS	fast atom bombardment mass spectrometry
EI MS	electron ionization mass spectrometry
ESI MS	electrospray-ionization mass spectrometry
TLC	thin-layer chromatography
PGCs	polyglycosylceramides
GSLs	glycosphingolipids
Neu5Ac	*N*-acetylneuraminic acid
Neu5Gc	*N*-glycolylneuraminic acid
PMAAs	partially methylated alditol acetates

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13. References

(1) Koscielak, J.; Miller-Podraza, H.; Krauze, R.; Piasek, A. *Eur. J. Biochem.* **1976**, *71*, 9.
(2) Stults, C. L. M.; Sweeley, C. C.; Macher, B. A. *Methods Enzymol.* **1989**, *179*, 167.
(3) Karlsson, K.-A. *Mol. Microbiol.* **1998**, *29*, 1.
(4) Hakomori, S.-i.; Yamamura, S.; Hanada, K. *Ann. N.Y. Acad. Sci.* **1998**, *845*, 1.
(5) Spiegel, S.; Cuvillier, O.; Edsall, L.; Kohama, T.; Menzeleev, R.; Olah, Z.; Oliviera, A.; Pirianov, G.; Thomas, D. M.; Tu, Z.; Van Brocklyn, J. R.; Wang, F. *Ann. N.Y. Acad.* Sci. **1998**, *845*, 11.
(6) Gardas, A.; Koscielak, J. *Eur. J. Biochem.* **1973**, *32*, 178.
(7) Gardas, A.; Koscielak, J. *FEBS Lett.* **1974**, *42*, 101.
(8) Gardas, A.; Koscielak, J. *Vox. Sang.* **1974**, *26*, 27.
(9) Gardas, A. *Eur. J. Biochem.* **1976**, *68*, 177.
(10) Gardas, A. *Eur. J. Biochem.* **1978**, *89*, 471.
(11) Levery, S. B.; Nudelman, E. D.; Salyan, M. E. K.; Hakomori, S.-i. *Biochemistry* **1989**, *28*, 7772.
(12) Hanfland, P.; Kordowicz, M.; Peter-Katalinic, J.; Egge, H.; Dabrowski, J.; Dabrowski, U. *Carbohydr. Res.* **1988**, *178*, 1.
(13) Kordowicz, M.; Hanfland, P.; Witzel, G.; Dabrowski, U.; Dabrowski, J.; Peter-Katalinic, J.; Egge, H. *Abstracts, XIIIth International Carbohydrate Symposium*, Aug 10−15, 1986, Ithaca, NY, Cornell University; p 265.
(14) Dabrowski, J.; Dabrowski, U.; Bermel, W.; Kordowicz, M.; Hanfland, P. *Biochemistry* **1988**, *27*, 5149.
(15) Honma, K.; Manabe, H.; Tomita, M.; Hamada, A. *J. Biochem.* **1981**, *90*, 1187.
(16) Miller-Podraza, H.; Stenhagen, G.; Andersson, C.; Karlsson, K.-A. *Glycoconjugate J.* **1997**, *14*, 231.
(17) Slomiany, B. L.; Slomiany, A.; Murty, V. L. N. *Biochem. Biophys. Res. Commun.* **1979**, *88*, 1092.
(18) Slomiany, B. L.; Slomiany, A.; Galicki, N.; Kojima, K. *Eur. J. Biochem.* **1980**, *113*, 27.
(19) Karlsson, H.; Johansson, L.; Miller-Podraza, H.; Karlsson, K.-A. *Glycobiology* **1999**, *9*, 765.
(20) Koscielak, J.; Miller-Podraza, H.; Zdebska, E. *Methods Enzymol.* **1978**, Vol L, 211.
(21) Dejter-Juszynski, M.; Harpaz, N.; Flowers, H. M.; Sharon, N. *Eur. J. Biochem.* **1978**, *83*, 363.
(22) Barnett, T.; Clark, G. F. *J. Biol. Chem.* **1992**, *267*, 11760.
(23) Koscielak, J.; Zdebska, E.; Wilczynska, Z.; Miller-Podraza, H.; Dzierzkowa-Borodej, W. *Eur. J. Biochem.* **1979**, *96*, 331.
(24) Miller-Podraza, H., Andersson, C.; Karlsson, K.-A. *Biochim. Biophys. Acta* **1993**, *1168*, 330.
(25) *Glycobiology, A Practical Approach*; Fukuda, M., Kobata, A., Eds.; Oxford University Press (IRL Press): Oxford, New York, Tokyo, 1993.
(26) *A Laboratory Guide to Glycoconjugate Analysis*; Jackson, P., Gallagher, J. T., Eds.; Birkhäuser Verlag: Basel, Boston, Berlin, 1997.
(27) *Clinical Biochemistry, Principles, Methods, Applications: 1. Mass Spectrometry*; Lawson, A. M., Ed.; Walter de Gruyter: Berlin, New York, 1989.
(28) Miller-Podraza, H.; Larson, T.; Nilsson, J.; Teneberg, S.; Matrosovich, M.; Johansson, L. *Acta Biochim. Pol.* **1998**, *45*, 439.
(29) Hanfland, P.; Egge, H.; Dabrowski, U.; Kuhn, S.; Roelcke, D.; Dabrowski, J. *Biochemistry* **1981**, *20*, 5310.
(30) Egge, H.; Kordowicz, M.; Peter-Katalinic, J.; Hanfland, P. *J. Biol. Chem.* **1985**, *260*, 4927.
(31) Breimer, M. E.; Hansson, G. C.; Karlsson, K.-A.; Larson, G.; Leffler, H.; Pimlott, W.; Samuelsson, B. E.; Strömberg, N.; Teneberg, S.; Thurin, J. *Int. J. Mass Spectrosc. Ion Phys.* **1983**, *48*, 113.
(32) Dabrowski, U.; Hanfland, P.; Egge, H.; Kuhn, S.; Dabrowski, J. *J. Biol. Chem.* **1984**, *259*, 7648.
(33) Johansson, L.; Johansson, P.; Miller-Podraza, H. *Eur. J. Biochem.* **1999**, *266*, 559.
(34) Zdebska, E.; Krauze, R.; Koscielak, J. *Carbohydr. Res.* **1983**, *120*, 113.
(35) Li, Y.-T.; Li, S.-C. *Anal. Biochem.* **1999**, *273*, 1.
(36) Scudder, P.; Uemura, K.-i.; Dolby, J.; Fukuda, M. N.; Feizi, T. *Biochem. J.* **1983**, *213*, 485.
(37) Scudder, P.; Hanfland, P.; Uemura, K.-i.; Feizi, T. *J. Biol. Chem.* **1984**, *259*, 6586.
(38) Miller-Podraza, H.; Karlsson, H.; Larsson, T.; Karlsson, K.-A. *Glycoconjugate J. (Abstracts of the XV International Symposium on Glycoconjugates)* **1999**, *16*, S15.
(39) Veh, R. W.; Corfield, A. P.; Sander, M.; Schauer, R. *Biochim. Biophys. Acta* **1977**, *486*, 145.
(40) Hakomori, S.-i. In *Sphingolipid Biochemistry*; Hanahan, D. J., Ed.; Plenum Press: New York and London, 1983; pp 1−165.
(41) In *Essentials of Glycobiology*, Varki, A., et al., Eds.; Cold Spring Harbor Laboratory Press: La Jolla, CA, 1999; Chapter 9, pp 115−129.
(42) Miller-Podraza, H.; Abul Milh, M.; Bergström, J.; Karlsson, K.-A. *Glycoconjugate J.* **1996**, *13*, 453.
(43) Miller-Podraza, H.; Bergström, J.; Abul Milh, M.; Karlsson, K.-A. *Glycoconjugate J.* **1997**, *14*, 467.
(44) Miller-Podraza, H.; Abul Milh, M.; Teneberg, S.; Karlsson, K.-A. *Infect. Immun.* **1997**, *65*, 2480.
(45) Miller-Podraza, H.; Bergström, J.; Teneberg, S.; Abul Milh, M.; Longard, M.; Olsson, B.-M.; Uggla, L.; Karlsson, K.-A. *Infect. Immun.* **1999**, *67*, 6309.
(46) Matrosovich, M.; Miller-Podraza, H.; Teneberg, S.; Robertson, J. S.; Karlsson, K.-A. *Virology* **1996**, *223*, 413.
(47) Nakagawa, H.; Yamada, T.; Chien, J.-L.; Gardas, A.; Kitamikado, M.; Li, S.-C.; Li, Y.-T. *J. Biol. Chem.* **1980**, *255*, 5955.
(48) Ujita, M.; McAuliffe, J.; Suzuki, M.; Hindsgaul, O.; Clausen, H.; Fukuda, M. N.; Fukuda, M., *J. Biol. Chem.* **1999**, *274*, 9296.
(49) Leppänen, A.; Zhu, Y.; Maaheimo, H.; Helin, J.; Lehtonen, E.; Renkonen, O. *J. Biol. Chem.* **1998**, *273*, 17399.
(50) Renkonen, O.; Toppila, S.; Penttilä, L.; Salminen, H.; Helin, J.; Maaheimo, H.; Costello, C. E.; Turunen, J. P.; Renkonen, R. *Glycobiology* **1997**, *7*, 453.
(51) DeGasperi, R.; Koerner, T. A. W.; Quarles, R. H.; Ilyas, A. A.; Ishikawa, Y.; Li, S.-C.; Li Y.-T. *J. Biol. Chem.* **1987**, *262*, 17149.
(52) Slomiany, B. L.; Slomiany, A. *Eur. J. Biochem.* **1978**, *90*, 39.
(53) Watanabe, K.; Matsubara, T.; Hakomori, S. *J. Biol. Chem.* **1976**, *251*, 2385.
(54) Fukuda, M.; Dell, A.; Oates, J. E.; Fukuda, M. N. *J. Biol. Chem.* **1984**, *259*, 8260.
(55) King, M.-J. *Biochim. Biophys. Acta* **1994**, *1197*, 15.
(56) Kishimoto, Y.; Hiraiwa, M.; O'Brien, J. S. *J. Lipid. Res.* **1992**, *33*, 1255.
(57) Fukuda, M. N.; Hakomori, S.-i. *J. Biol. Chem.* **1982**, *257*, 446.
(58) Zdebska, E.; Magnuska, A.; Kusnierz, G.; Seyfried, H.; Koscielak, J. *FEBS Lett.* **1980**, *120*, 33.
(59) Zdebska, E.; Anselstetter, V.; Pacuszka, T.; Krauze, R.; Chelstowska, A.; Heimpel, H.; Koscielak, J. *Br. J. Haematol.* **1987**, *66*, 385.
(60) Zdebska, E.; Koscielak, J. *Eur. J. Biochem.* **1978**, *91*, 517.
(61) Viitala, J.; Finne, J. *Eur. J. Biochem.* **1984**, *138*, 393.
(62) Wilczynska, Z.; Miller-Podraza, H.; Koscielak, J. *FEBS Lett.* **1980**, *112*, 277.
(63) Müthing, J. *Carbohydr. Res.* **1996**, *290*, 217.
(64) Stroud, M. R.; Handa, K.; Salyan, M. E. K.; Fang, H.; Levery, S. B.; Hakomori, S.-i.; Reinhold: B. B.; Reinhold: V. N. *Biochem. Biophys. Res. Commun.* **1995**, *209*, 777.
(65) Stroud, M. R.; Handa, K.; Salyan, M. E. K.; Ito, K.; Levery, S. B.; Hakomori, S.-i. *Biochemistry* **1996**, *35*, 770.
(66) Slomiany, B. L.; Slomiany, A. *Chem. Phys. Lipids* **1977**, *20*, 57.
(67) Slomiany, B. L.; Slomiany, A. *FEBS Lett.* **1977**, *73*, 175.
(68) Slomiany, B. L.; Slomiany, A. *Biochim. Biophys. Acta* **1977**, *486*, 531.
(69) Slomiany, A.; Slomiany, B. L. *Biochem. Biophys. Res. Commun.* **1980**, *93*, 770.
(70) Hooper N. M. *Curr. Biol.* **1998**, *8*, R114.
(71) Miller-Podraza, H.. *Biochim. Biophys. Acta* **1979**, *586*, 209.
(72) Dasgupta, S.; Hogan, E. L.; Glushka, J.; Van Halbeek, H. *Arch. Biochem. Biophys.* **1994**, *310*, 373.
(73) Bouhours, D.; Liaigre, J.; Lemoine, J.; Mayer-Posner, F.; Bouhours, J.-F. *Glycoconjugate J.* **1998**, *15*, 1001.
(74) He, P.; Hu, J.; Macher, B. A. *Arch. Biochem. Biophys.* **1993**, *305*, 350.

(75) Nudelman, E. D.; Mandel, U.; Levery, S. B.; Kaizu, T.; Hakomori, S.-i. *J. Biol. Chem.* **1989**, *264*, 18719.
(76) Ishizuka, I.; Wiegandt, H. *Biochim. Biophys. Acta* **1972**, *260*, 279.
(77) Yu, R. K.; Ando, S. *Adv. Exp. Med., Biol.* **1980**, *125*, 33.
(78) Rahman, H.; Hilbig, R. *J. Comput. Physiol.* **1983**, *151*, 215−224.
(79) Nakamura, K.; Tamai, Y.; Kasama, T. *Neurochem. Int.* **1997**, *30*, 593.
(80) Suzuki, K. *J. Neurochem.* **1965**, *12*, 969.
(81) Kuhn, R.; Wiegandt, H. *Chem. Ber.* **1963**, *96*, 866.
(82) Fredman, P.; Månsson, J.-E.; Svennerholm, L.; Karlsson, K.-A.; Pascher, I.; Samuelsson, B. *FEBS Lett.* **1980**, *110*, 80.
(83) Miller-Podraza, H., Månsson, J.-E.; Svennerholm, L. *FEBS Lett.* **1991**, *288*, 212.
(84) Sonnino, S.; Ghidoni, R.; Chigorno, V.; Masserini, M.; Tettamanti, G. *Anal. Biochem.* **1983**, *128*, 104.
(85) Ando, S.; Yu, R. K. *J. Biol. Chem.* **1979**, *254*, 12224.
(86) Fishman, P. H.; Miller-Podraza, H. In *Structural Carbohydrates in the Liver* (*Falk Symposium*); Popper, H., et al., Eds.; PTP Press Limited: Boston, 1983; Vol. 34, p 99.
(87) Kracun, I.; Rösner, H.; Cosovic, C.; Stavljenic, A. *J. Neurochem.* **1984**, *43*, 979.
(88) Thomas, P. D.; Brewer, G. J. *Biochim. Biophys. Acta* **1990**, *1031*, 277.
(89) Sandhoff, K.; Van Echten G. *Adv. Lipid Res.* **1993**, *26*, 119.
(90) Van Echten-Deckert, G.; Sandhoff, K. In *Comprehensive Natural Products Chemistry*, Pinto, B. M., Ed.; Pergamon Elsevier Science: New York, 1998; Vol. 3, Chapter 5.
(91) Rother, J.; Van Echten, G.; Schwarzmann, G.; Sandhoff, K. *Biochem. Biophys. Res. Commun.* **1992**, *189*, 14.
(92) Schwarzmann G.; Sandhoff, K. *Biochemistry.* **1990**, *29*, 10865.
(93) Lloyd, K. O.; Furukawa, K. *Glycoconjugate J.* **1998**, *15*, 627.
(94) Burger, K. N.; Van der Bijl, P.; Van Meer, G. *J. Cell Biol.* **1996**, *133*, 15.
(95) Sprong. H.; Kruithof, B.; Leijendekker, R.; Slot, J. W.; Van Meer, G.; Van der Sluijs, P. *J. Biol. Chem.* **1998**, *273*, 25880.
(96) Basu, S.; Kaufmann, B.; Roseman, S. *J. Biol. Chem.* **1968**, *243*, 5802.
(97) Paul, P.; Kamisaka, Y.; Marks, D. L., and Pagano, R. E. *J. Biol. Chem.* **1996**, *271*, 2287.
(98) Ichikawa, S.; Hirabayashi, Y. *Trends Cell Biol.* **1998**, *8*, 198.
(99) Chatterjee, S.; Ghosh, N.; Khurana, S. *J. Biol. Chem.* **1992**, *267*, 7148.
(100) Basu, M.; De, T.; Das, K. K.; Kyle, J. W.; Chon, H.-C.; Schaeper, R. J.; Basu, S. *Methods Enzymol.* **1987**, *138*, 575.
(101) Trinchera, M.; Fabbri, M.; Ghidoni, R. *J. Biol. Chem.* **1991**, *266*, 20907.
(102) Van den Eijnden, D. H.; Koenderman, A. H. L.; Schiphorst, W. E. C. M. *J. Biol. Chem.* **1988**, *263*, 12461.
(103) Van den Eijnden, D. H.; Joziasse, D. H. *Curr. Opin. Struct. Biol.* **1993**, *3*, 711.
(104) Ujita, M.; McAuliffe, J.; Schwientek, T.; Almeida, R.; Hindsgaul, O.; Clausen, H.; Fukuda, M. *J. Biol. Chem.* **1998**, *273*, 34843.
(105) Zhou, D.; Dinter, A.; Gutiérrez Gallego, R.; Kamerling, J. P.; Vliegenthart, J. F. G.; Berger, E. G.; Hennet, T. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 406.
(106) Kawashima, H.; Yamamoyo, K.; Osawa, T.; Irimura, T. *J. Biol. Chem.* **1993**, *268*, 27118.
(107) Sasaki, K.; Kurata-Miura, K.; Ujita, M.; Angata, K.; Nakagawa, S.; Sekine, S.; Nishi, T.; Fukuda, M. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14294.
(108) Yeh, J.-C.; Ong, E.; Fukuda, M. *J. Biol. Chem.* **1999**, *274*, 3215.
(109) Piller, F.; Cartron, J.-P.; Maranduba, A.; Veyrieres; A.; Leroy, Y.; Fournet, B. *J. Biol. Chem.* **1984**, *259*, 13385.
(110) Brockhausen, I.; Matta, K. L.; Orr, J.; Schachter, H.; Koenderman, A. H.; Van den Eijnden, D. H. *Eur. J. Biochem.* **1986**, *157*, 463.
(111) Koenderman, A. H. L.; Koppen, P. L.; Van den Eijnden, D. H. *Eur. J. Biochem.* **1987**, *166*, 199.
(112) Ropp, P.; Little, M. R.; Cheng, P.-W. *J. Biol. Chem.* **1991**, *266*, 23863.
(113) Gu, J.; Nishikawa, A.; Fujii, S.; Gasa, S.; Taniguchi, N. *J. Biol. Chem.* **1992**, *267*, 2994.
(114) Helin, J.; Penttilä, L.; Leppänen, A.; Maaheimo, H.; Laura, S.; Costello, C. E.; Renkonen, O. *FEBS Lett.* **1997**, *412*, 637.
(115) Seppo, A.; Penttilä, L.; Makkonen, A.; Leppänen, A.; Niemelä, R.; Jäntti, J.; Helin, J.; Renkonen, O. *Biochem. Cell. Biol.* **1990**, *68*, 44.
(116) Leppänen, A.; Penttilä, L.; Niemelä, R.; Helin, J. S.; Seppo, A.; Lusa, S.; Renkonen, O. *Biochemistry* **1991**, *30*, 9287.
(117) Niemelä, R.; Rabinä, J.; Leppänen, A.; Maaheimo, H.; Costello, C. E.; Renkonen, O. *Carbohydr. Res.* **1995**, *279*, 331.
(118) Mattila, P.; Salminen, H.; Laura, H.; Niittymäki, J.; Salo, H.; Niemela, R.; Fukuda, M.; Renkonen, O.; Renkonen, R. *J. Biol. Chem.* **1998**, *273*, 27633.
(119) Sakamoto, Y.; Taguchi, T.; Tano, Y.; Ogawa, T.; Leppänen, A.; Kinnunen, M.; Aitio, O.; Parmanne, P.; Renkonen, O.; Taniguchi, N. *J. Biol. Chem.* **1998**, *273*, 27625.
(120) Leppänen, A.; Salminen, H.; Zhu, Y.; Maaheimo, H.; Helin, J.; Costello, C. E.; Renkonen, O. *Biochemistry* **1997**, *36*, 7026.
(121) Leppänen, A.; Niemelä, R.; Renkonen, O. *Biochemistry* **1997**, *36*, 13729.
(122) Vilkman, A.; Niemela, R.; Pentila, L.; Helin, J.; Leppanen, A.; Seppo, A.; Maaheimo, H.; Lusa, S.; Renkonen, O. *Carbohydr. Res.* **1992**, *226*, 155.
(123) Turunen, J. P.; Majuri, M. L.; Seppo, A.; Tiisala, S.; Paaronen, T.; Miyasaka, M.; Lemstrom, K.; Pentila, L.; Renkonen; O. Renkonen, R. *J. Exp. Med.* **1995**, *182*, 1133.
(124) Zdebska, E.; Chelstowska, A.; Zielenski, J.; Lenkiewicz, B.; Koscielak, J. *Biochim. Biophys. Acta* **1982**, *717*, 1.
(125) Miller-Podraza, H.; Fishman, P. H. *Biochim. Biophys. Acta* **1984**, *805*, 44.
(126) Schwarzmann, G.; Sandhoff, K. *Biochemistry* **1990**, *29*, 10865.
(127) Gillard, B. K.; Thurman, L. T.; Marcus, D. M. *Glycobiology* **1993**, *3*, 57.
(128) Hoekstra, D.; Kok, J. W. *Biochim. Biophys. Acta* **1992**, *1113*, 277.
(129) Bieberich, E.; Yu, R. K. *Biochim. Biophys. Acta* **1999**, *1432*, 113.
(130) Sandhoff, K.; Kolter, T. *Trends Cell. Biol.* **1996**, *6*, 98.
(131) Sandhoff, K.; Kolter, T. *Acta Biochem. Pol.* **1998**, *45*, 373.
(132) Li, Y.-T.; Li, S.-C. *Adv. Exp. Med. Biol.* **1984**, *174*, 213.
(133) Nakagawa, H.; Yamada, T.; Chien, J.-L.; Gardas, A.; Kitamikado; M., Li, S.-C.; Li, Y.-T. *J. Biol. Chem.* **1980**, *255*, 5955.
(134) Li, S.-C.; DeGasperi, R.; Muldrey, J. E.; Li, Y.-T. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 346.
(135) Zhou, B.; Li, S.-C.; Laine, R. A.; Huang, R. T.; Li, Y.-T. *J. Biol. Chem.* **1989**, *264*, 12272.
(136) Ito, M.; Yamagata, T. *J. Biol. Chem.* **1986**, *261*, 14278.
(137) Hirabayashi, Y.; Kimura, M.; Matsumoto, M.; Yamamoto, K.; Kadowaki, S.; Tochikura, T. *J. Biochem.* **1988**, *103*, 1.
(138) Ito, M.; Kurita, T.; Kita, K. *J. Biol. Chem.* **1995**, *270*, 24370.
(139) Finne, J. *Eur. J. Biochem.* **1980**, *104*, 181.
(140) Fukuda, M.; Fukuda, M. N.; Papayannopoulou, T.; Hakomori, S. I. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3474.
(141) Fukuda, M.; Dell, A.; Fukuda, M. N. *J. Biol. Chem.* **1984**, *259*, 4782.
(142) Liukkonen, J.; Haataja, S.; Tikkanen, K.; Kelm, S.; Finne, J. *J. Biol. Chem.* **1992**, *267*, 21105.
(143) Loomes, L. M.; Uemura, K.; Childs, R. A.; Paulson, J. C.; Rogers, G. N.; Scudder, P. R.; Michalski, J.-C.; Hounsell, E. F.; Taylor-Robinson, D.; Feizi, T. *Nature* **1984**, *307*, 560.
(144) Hughes, R. C.; Gardas, A. *Nature* **1976**, *264*, 63.
(145) Karlsson, K.-A.; Teneberg, S.; Ångström, J.; Kjellberg, A.; Hirst, T. R.; Bergström, J.; Miller-Podraza, H. *Bioorg. Med. Chem.* **1996**, *4*, 1919.
(146) Clausen, H.; Hakomori, S.-i. *Vox. Sang.* **1989**, *56*, 1.
(147) Hakomori, S.-i. *Acta Anat.* **1998**, *161*, 79.
(148) Varki, A. *Glycobiology* **1993**, *3*, 97.
(149) Koscielak, J. *Glycoconjugate J.* **1986**, *3*, 95.
(150) Kelm, S.; Schauer, R. *Int. Rev. Cytol.* **1997**, *175*, 137.
(151) Blithe, D. L. *Trends Glycosci. Glycotechnol.* **1993**, *5*, 81.
(152) Walz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132.
(153) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-I.; Paulson, J. C. *Science* **1990**, *250*, 1130.
(154) Hakomori, S. *Biochem. Soc. Trans.* **1993**, *21*, 583.
(155) Hakomori, S.-i.; Handa, K.; Iwabuchi, K.; Yamamura, S.; Prinetti, A. *Glycobiology* **1998**, *8*, xi.
(156) Yates, A. J.; Rampersaud, A. *Ann. N.Y. Acad. Sci.* **1998**, *845*, 57.
(157) Hanai, N.; Nores, G. A.; MacLeod, C.; Torres-Mendez, C.-R.; Hakomori, S. *J. Biol. Chem.* **1988**, *263*, 10915.
(158) Mutoh, T.; Tokuda, A.; Miyada, T.; Hamaguchi, M.; Fujiki, N. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5087.
(159) Simons, K.; Ikonen, E. *Nature* **1997**, *387*, 569.
(160) Parton, R. G. *Curr. Opin. Cell Biol.* **1996**, *8*, 542.
(161) Sargiacomo, M.; Sudol, M.; Tang, Z.; Lisanti, M. P. *J. Cell Biol.* **1993**, *122*, 789.
(162) Tillack, T. W.; Allietta, M.; Moran, R. E.; Young, W. W., Jr. *Biochim. Biophys. Acta* **1983**, *733*, 15.
(163) Sorice, M.; Parolini, I.; Sansolini, T.; Garofalo, T.; Dolo, V.; Sargiacomo, M.; Tai, T.; Peschle, C.; Torrisi, M. R.; Pavan, A. *J. Lipid Res.* **1997**, *38*, 969.
(164) Brown, D. A.; Rose, J. K. *Cell* **1992**, *68*, 533.
(165) Hannun, Y. A.; Bell, R. M. *Science* **1989**, *243*, 500.
(166) Dunn, B. E.; Cohen, H.; Blaser, M. J. *Clin. Microbiol. Rev.* **1997**, *10*, 720.
(167) Blaser, M. J. *Gastroenterol.* **1992**, *102*, 720.
(168) Telford, J. L.; Covacci, A.; Rappuoli, R.; Ghiara, P. *Curr. Opin. Immunol.* **1997**, *9*, 498.
(169) Mooney, C.; Keenan, J.; Munster, D.; Wilson, I.; Allardyce, R.; Bagshaw, P.; Chapman, B.; Chadvick, V. *Gut* **1991**, *32*, 853.
(170) Rautelin, H.; Blomberg, B.; Fredlund, H.; Järnerot, G.; Danielsson, D. *Gut* **1993**, *34*, 599.
(171) Chmiela, M.; Lelwala-Guruge, J.; Wadström, T. *FEMS Immunol. Med. Microbiol.* **1994**, *9*, 41.
(172) Abramson, J. S.; Mills, E. L. *Rev. Infect. Dis.* **1988**, *10*, 326.

(173) Colamussi, M. L.; White, M. R.; Crouch, E.; Hartshorn, K. L. *Blood* **1999**, *93*, 2395.
(174) Friedman, M. J.; Fukuda, M.; Laine R. A. *Science* **1985**, *228*, 75.
(175) Okoye, V. C. N.; Bennett, V. *Science* **1985**, *227*, 169.
(176) Dhume, S. T.; Adams-Burton, C. R.; Shumak, K. H.; Laine, R. A. *Glycobiology* **1994**, *4*, 903.
(177) Fukuda, M. N. *Glycobiology* **1990**, *1*, 9.
(178) Fukuda, M. N.; Masri, K. A.; Dell, A.; Thonar, E. J.-M.; Klier, G.; Lowenthal, R. M. *Blood* **1989**, *73*, 1331.
(179) Ramsey, G. *Transfusion* **1991**, *31*, 76.
(180) Bäckström, M.; Shahabi, V.; Johansson, S.; Teneberg, S.; Kjellberg, A.; Miller-Podraza, H.; Holmgren, J.; Lebens, M. *Mol. Microbiol.* **1997**, *24*, 489.
(181) Gardas, A. *Archiv. Biochem. Biophys.* **1982**, *216*, 440.
(182) Li, Y.-T.; Mazzotta, M. Y.; Wan, C.-C.; Orth, R.; Li, S.-C. *J. Biol. Chem.* **1973**, *248*, 7512.
(183) Burg, J.; Conzelman, E.; Sandhoff, K.; Solomon, E.; Swallow, D. M. *Ann. Hum. Genet.* **1985**, *49*, 41.
(184) Christomanou, H.; Aignesberger, A.; Linke, R. P. *Biol. Chem. Hoppe-Seylers* **1986**, *367*, 879.
(185) O'Brien, J. S.; Kishimoto, Y. *FASEB J.* **1991**, *5*, 301.
(186) Schnabel, D.; Schröder, M.; Sandchoff, K. *FEBS Lett.* **1991**, *284*, 57.
(187) Brady, R. O. *Braillieres Clin. Haematol.* **1997**, *10*, 621.
(188) Callahan, J. W. *Biochim. Biophys. Acta* **1999**, *1455*, 85.
(189) Kolter, T.; Sandhoff, K. *Brain. Pathol.* **1998**, *8*, 79.
(190) Schuette, C. G.; Doering, T.; Kolter, T.; Sandhoff, K. *Biol. Chem.* **1999**, *380*, 759.
(191) Wiegandt, H. *Biochim. Biophys. Acta* **1992**, *1123*, 117.
(192) Gerdt, S.; Dennis, R. D.; Borgonie, G.; Schnabel, R.; Geyer, R. *Eur. J. Biochem.* **1999**, *266*, 952.
(193) Hori, T.; Sugita, M.; Shimizu, H. *Biochim. Biophys. Acta* **1981**, *665*, 170.
(194) Itasaka, O.; Sugita, M.; Kataoka, H.; Hori, T. *Biochim. Biophys. Acta* **1983**, *751*, 8.
(195) Yamamoto, A.; Yano, I.; Masui, M.; Yabuuchi, E. *J. Biochem (Tokyo)* **1978**, *83*, 1213.
(196) Laine, R. A.; Renkonen, O. *Biochemistry* **1974**, *13*, 2837.
(197) Noda, N.; Tanaka, R.; Miyahara, K.; Kawasaki, T. *Biochim. Biophys. Acta* **1993**, *1169*, 30.
(198) Sugita, M.; Fujii, H.; Inagaki, F.; Suzuki, M.; Hayata, C.; Hori, T. *J. Biol. Chem.* **1992**. *267*, 22595.
(199) Harvey, D. J. *Mass. Spectrom. Rev.* **1999**, *18*, 349.
(200) Toledo, M. S.; Levery, S. B.; Strauss, A. H.; Suzuki, E.; Momany, M.; Glushka, J.; Multon, J. M.; Takahashi, H. K. *Biochemistry* **1999**, *38*, 7294.
(201) Levery, S. B.; Toledo, M. S.; Strauss, A. H.; Takahaski, H. K. *Biochemistry* **1998**, *37*, 8764.
(202) Melo, N. S.; Nimtz, M.; Conradt, H. S.; Feveiro, P. S.; Costa, J. *FEBS Lett.* **1997**, *415*, 186.
(203) Morelle, W.; Lemoine, J.; Strecker, G. *Anal. Biochem.* **1998**, *259*, 16.
(204) Déziel, E.; Lepine, F.; Dennie, D.; Boismenu, D.; Mamer, O. A.; Villemur, R. *Biochim. Biophys. Acta* **1999**, *1440*, 244.
(205) Saba, J. A.; Shen, X.; Jamieson, J. C.; Perreault, H. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 704.
(206) Monsarrat, B.; Brando, T.; Condouret, P.; Nigou, J.; Puzo, G. *Glycobiology* **1999**, *9*, 335.
(207) Bäcker, A. E.; Thobert, S.; Rakotonirainy, O.; Hallberg, E. C.; Olling, A.; Gustavsson, M.; Samuelsson, B. E.; Soussi, B. *Glycoconjugate J.* **9991**, *16*, 45.
(208) Suzuki, S.; Kakehi, K.; Honda, S. *Anal. Chem.* **1996**, *68*, 2073.
(209) Bahia, M. C.; Vieira, R. P.; Mulloy, B.; Hartmann, R.; Bergter, E. B.; *Mycopathologia* **1997**, *137*, 17.
(210) Yamamoto, H.; Iida-Tanaka, N.; Kasama, T.; Ishizuka, I.; Kushi, Y.; Handa, S. *J. Biochem.* **1999**, *125*, 923.
(211) Hatanaka, Y.; Nakayama, H.; Kanaoka, Y. *Rev. Heteroatom. Chem.* **1996**, *14*, 213.
(212) Ilver, D.; Arnqvist, A.; Ögren, J.; Frick, I.-M.; Kersulyte, D.; Incecik, E. T.; Berg, D. E.; Covacci, A.; Engstrand, L.; Borén, T. *Science* **1998**, *279*, 373.
(213) Koumanov, F.; Yang, J.; Jones, A. E.; Hatanaka, Y. *Biochem. J.* **1998**, *330*, 1209.
(214) Hashimoto, M.; Hatanaka, Y. *Chem. Pharm. Bull. Tokyo* **1999**, *47*, 667.
(215) Nashihira, J.; Sakai, M.; Nishi, S.; Hatanaka, Y. *Eur. J. Biochem.* **1995**, *232*, 106.
(216) Larsson, T.; Berdström, J.; Nilsson, C., Karlsson, K.-A. *FEBS Lett.* **2000**, *469*, 155.
(217) Skupski, M. P.; Brooker, M.; Farmer, A.; Harpold, M.; Huang, W.; Inman, J.; Kiphart, D.; Kodira, C.; Root, S.; Schilkey, F.; Schwertfeger, J.; Siepel, A.; Stampel, D.; Thayer, N.; Thompson, R.; Wortman, J.; Zhuang, J. J.; Harger, C. *Nucleic Acids Res.* **1999**, *27*, 35.
(218) Collins, F. S.; Patrinos, A.; Jordan, E.; Chakravarti, A.; Gesteland, R.; Walters, L. *Science* **1998**, *282*, 682.
(219) The Sanger Centre, Wellcome Trust, Cambridge. *Genome Res.* **1998**, *8*, 1097.
(220) Yousefi, S.; Higgins, E.; Doaling, Z.; Hindsgaul, O.; Pollex-Kruger, A.; Dennis, J. W. *J. Biol. Chem.* **1991**, *266*, 1772.
(221) Shimodaira, K.; Nakayama, J.; Nakamura, N.; Hasebe, O.; Katsuyama, T.; Fukuda, M. *Cancer Res.* **1997**, 52011.
(222) Dennis, J. W.; Granovsky, M.; Warren, C. E. *Biochim. Biophys. Acta* **1999**, *1473*, 21.
(223) Taniguchi, N.; Miyoshi, E.; Ko, J. H.; Ikeda, Y.; Ihara, Y. *Biochim Biophys. Acta* **1999**, *1455*, 287.
(224) Axford, J. S. *Biochim. Biophys. Acta* **1999**, *1455*, 219.
(225) Michalski, J.-C.; Klein, A. *Biochim. Biophys. Acta* **1999**, *1455*, 69.
(226) Joziasse, D. H.; Oriol, R. *Biochim. Biophys. Acta* **1999**, *1455*, 403.
(227) Kolter, T.; Sandhoff, K. *J. Inherit. Metab. Dis.* **1998**, *21*, 548.
(228) Yamashita, T., Wada, R.; Sasaki, T.; Deng, C.; Bierfreund, U.; Sandhoff, K.; Proia, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9142.
(229) Liu, Y.; Wada, R.; Kawai, H.; Sango, K.; Deng, C.; Tai, T.; McDonald, M. P.; Araujo, K.; Crawley, J. N.; Bierfreund, U.; Sandhoff, K.; Suzuki, K.; Proia, R. L. *J. Clin. Invest.* **1999**, *103*, 497.
(230) Ziegler, R. J.; Yew, N. S.; Li, C.; Cherry, M.; Berthelette, P.; Romanczuk, H.; Ioannou, Y. A.; Zeidner, K. M.; Desnick, R. J.; Cheng, S. H. *Hum. Gene Ther.* **1999**, *10*, 1667.
(231) Gillard, B. K.; Clement, R.; Colucci-Guyon, E.; Babinet, C.; Schwarzman, G.; Taki, T.; Kasama, T.; Marcus, D. M. *Exp. Cell. Res.* **1998**, *242*, 561.
(232) Wilkins, M. R.; Sanchez, J. C.; Gooley, A. A.; Appel, R. D.; Humphrey-Smith, I.; Hochstrasser, D. F.; Williams, K. L. *Biotech. Genet. Eng. Rev.* **1996**, *13*, 19.
(233) Mann, M.; Hojrup, P.; Roepstorff, P. *Biol. Mass Spectrom.* **1993**, *22*, 338.
(234) Celis, J. E.; Ostergaard, M.; Jensen, N. A.; Gromova, I.; Holm-Rasmunsen, H.; Gromov, P. **1998**, *FEBS Lett. 430*, 64.
(235) Proctor, B. L. *Annu. Rev. Pharmacol. Toxicol.* **1983**, *23*, 171.
(236) Falk, P. G.; Bry, L.; Holgersson, J.; Gordon, J. I. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 1515.
(237) Sandhoff, K.; Kolter, T.; Van Echten-Deckert, G. *Ann. N. Y. Acad. Sci.* **1998**, *845*, 139.
(238) Ichikawa, S.; Hirabayashi, Y. *Methods Enzymol.* **1999**, *311*, 303.
(239) Bosio, A.; Bussow, H.; Adam, J. Stoffel, W. *Cell Tissue Res.* **1998**, *292*, 199.
(240) Amado, M.; Almeida, R.; Schwientek, T.; Clausen, H. *Biochim. Biophys. Acta* **1999**, *1473*, 35.
(241) Xu, H.; Storch, T.; Yu, M.; Elliott, S. P.; Haslam, D. B. *J. Biol. Chem.* **1999**, *274*, 29390.
(242) Lee, Y. C.; Kaufmann, M.; Kitazume-Kawaguchi, S.; Kono, M.; Takashima, S.; Kurosawa, N.; Liu, H.; Pircher, H.; Tsuji, S. *J. Biol. Chem.* **1999**, *274*, 11958.
(243) Kono, M.; Takashima, S.; Liu, H.; Inoue, M.; Kojima, N.; Lee, Y. C.; Hamamoto, T.; Tsuji, S. *Biochem. Biophys. Res. Commun.* **1998**, *253*, 170.
(244) Lauc, G.; Lee, R. T.; Dumiae, J.; Lee, Y. C. *Glycobiology* **2000**, *10*, 357.
(245) Fleischmann, R. D.; Adams, M. D.; White, O.; Clayton, R. A.; Kirkness, E. F.; Kerlavage, A. R.; Bult, C. J.; Tomb, J. F.; Dougherty, B. A.; Merrick, J. M., et al. *Science* **1995**, *269*, 496.
(246) Tomb, J.-F.; White, O.; Kerlavage, A. R.; Clayton, R. A.; Sutton, G. G.; Fleischmann, R. D.; Ketchum, K. A.; Klenk, H. P.; Gill, S.; Dougherty, B. A.; Nelson, K.; Quackenbush, J.; Zhou, L.; Kirkness, E. F.; Peterson, S.; Loftus, B.; Richardson, D.; Dodson, R.; Khalak, H. G.; Glodek, A.; McKenny, K.; Fitzegerald, L. M.; Lee, N.; Adams, M. D.; Venter, J. C., et al. *Nature* **1997**, *388*, 539.
(247) Bauer, B. E.; Wolfger, H.; Kuchler, K. *Biochim. Biophys. Acta* **1999**, *1461*, 217.
(248) The *C. elegans* Sequencing Consortium. *Science* **1998**, *282*, 2012.
(249) Frishman, D.; Mewes, H. W. *Prog. Biophys. Mol. Biol.* **1999**, *72*, 1.
(250) Lee, A. G. *Biochim. Biophys. Acta* **1977**, *472*, 285.
(251) Curatolo, W. *Biochim. Biophys. Acta* **1987**, *906*, 111.
(252) Grant, C. W. M.; Jarrell, H. C.; Florio, E. *Biochem. Soc. Trans.* **1990**, *18*, 827.
(253) Bagatolli, L. A. Gratton, E.; Fidelio, G. D. *Biophys. J.* **1998**, *75*, 331.
(254) Svennerholm, L. *Adv. Exp. Med. Biol.* **1980**, *125*, 533.
(255) Rahman, H. *Behav. Brain Res.* **1995**, *66*, 105.
(256) Curatolo, W. *Biochim. Biophys. Acta* **1987**, *906*, 137.
(257) Zajicek, H.; Wang, H.; Kumar, V.; Wilson, P.; Levi, M. *Kidney Int. Suppl.* **1997**, *61*, S-32.
(258) Nakano, J.; Yasui, H.; Lloyd, K. O.; Muto, M. *J. Investig. Dermatol. Symp. Proc.* **1999**, *4*, 173.
(259) Lekman, A.; Skjeldahl, O.; Sponheim, E.; Svennerholm, L. *Acta Paediatr.* **1995**, *84*, 787.
(260) Bensancon, F.; Ankel, H. *Nature* **1974**, *252*, 478.
(261) Schiffmann, D.; Koschel, K. Med. *Microbiol. Immunol.* **1981**, *169*, 281.
(262) Khine, A. A.; Lingwood, C. A. *J. Cell. Physiol.* **2000**, *182*, 97.

(263) Bast, D. J.; Banerjee, L.; Clark, C.; Read, R. J.; Brunton, J. L. *Mol. Microbiol.* **1999**, *32*, 953.
(264) Mylvaganam, M.; Lingwood, C. A. *Biochem. Biophys. Res. Commun.* **1999**, *257*, 391.
(265) Ardila-Osorio, H.; Clausse, B.; Mishal, Z.; Wiels, J.; Tursz, T.; Busson, P. *Int. J. Cancer* **1999**, *81*, 645.
(266) Evans, S. V.; Roger-MacKenzie, C. *J. Mol. Recognit.* **1999**, *12*, 155.
(267) Wakshull, E.; Brunke-Reese, D.; Lindermuth, J.; Fisette, L.; Nathans, R. S.; Crowley, J. J.; Tufts, J. C.; Zimmerman, J.; Mackin, W.; Adams, D. S. *Immunopharmacology* **1999**, *41*, 89.
(268) Grange, P. A.; Erickson, A. K.; Levery, S. B.; Francis, D. H. *Infect. Immun.* **1999**, *67*, 165.
(269) Lanne, B.; Schierbeck, B.; Ångström, J. *J. Biochem. (Tokyo)* **1999**, *126*, 226.
(270) Minke, W. E.; Roach, C.; Hol, W. G.; Verlinde, C. L. *Biochemistry* **1999**, *38*, 5684.
(271) Tzeng, S. F.; Deibler, G. E.; DeVries, G. H. *Neurochem. Res.* **1999**, *24*, 255.
(272) Markotic, A.; Lumen, R.; Marusic, A.; Jonjic, S.; Muthing, J. *Carbohydr. Res.* **1999**, *321*, 75.
(273) Crain, S. M.; Shen, K. *Brain. Res.* **2000**, *856*, 227.
(274) Kawakami, K.; Ahmed, K.; Utsunomiya, Y.; Rikitomi, N.; Hori, A.; Oishi, K.; Nagatake, T. *Microbiol. Immunol.* **1998**, *42*, 697.
(275) Rolsma, M. D.; Kuhlenschmidt, T. B.; Gelberg, H. B.; Kuhlenschmidt, M. S. *J. Virol.* **1998**, *72*, 9079.
(276) Vital, M. A.; Florio, J. C.; Frussa-Filho, R.; DeLucia, R.; Tufik, S.; Palermo-Neto, J. *Life Sci.* **1998**, *62*, 1161.
(277) Rusnati, M.; Tanghetti, E.; Urbinati, C.; Tulipano, G.; Marchesini, S.; Ziche, M.; Presta, M. *Mol. Biol. Cell* **1999**, *10*, 313.
(278) Ding, Z.; Kawashima, H.; Suzuki, Y.; Suzuki, T.; Ward, P. A. Miyasaka, M. *FEBS Lett.* **1997**, *418*, 310.
(279) Bengtsson, T.; Grenegard, M.; Olsson, A.; Sjogren, F.; Stendahl, O.; Zalavary, S. *Biochim. Biophys. Acta* **1996**, *1313*, 119.
(280) Yamaguchi, S.; Miyazaki, Y.; Oka, S.; Yano, I. *FEMS Immunol. Med. Microbiol.* **1996**, *13*, 107.
(281) Yates, A. J. *Neurochem. Pathol.* **1986**, *5*, 309.
(282) Thomas, P. D.; Brewer, G. J. *Biochim. Biophys. Acta* **1990**, *1031*, 277.
(283) Keenan, T. W.; Schmid, E.; Franke, W. W.; Wiegandt, H. *Exp. Cell. Res.* **1975**, *92*, 259.
(284) Icard-Liepkalns, C.; Liepkalns, V. A.; Yates, A. J.; Rodriguez, Z. R.; Stephens, R. E. *J. Cell. Physiol.* **1982**, *113*, 186.
(285) Yates, A. J.; Stephens, R. E.; Elder, P. J.; Markowitz, D. L.; Rice, J. M. *Cancer Res.* **1985**, *45*, 1033.
(286) Paller, A. S.; Arnsmeier, S. L.; Alvarez-Franco, M.; Bremer, E. G. *J. Invest. Dermatol.* **1993**, *100*, 841.
(287) Liu, H.; Nakagawa, T.; Kanematsu, T.; Uchida, T.; Tsuji, S. *J. Neurochem.* **1999**, *72*, 1781.
(288) Hakomiri, S. *Annu. Rev. Immunol.* **1984**, *2*, 103.
(289) Feizi, T. *Nature* **1985**, *314*, 53.
(290) Hakomori, S.; Murakami, W. T. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *59*, 254.
(291) Hakomori, S. *Biochim. Biophys. Acta* **1975**, *417*, 55.
(292) Lloyd K. O. *J. Brazilian Assoc. Adv. Sci.* **1994**, *46*, 228.
(293) Li, R.; Gage, D.; McKallip, R.; Ladish, S. *Glycoconjugate J.* **1996**, *13*, 385.
(294) Pukel, C. S.; Lloyd, K. O.; Travassos, L. R.; Dippold, W. G.; Oettgen, H. F.; Old, L. J. *J. Exp. Med.* **1982**, *155*, 1133.
(295) Ogura, M.; Handa, S. *Biochim. Biophys. Acta* **2000**, *1483*, 111.
(296) Vogel, J.; Bendas, G.; Bakowsky, U.; Hummel, G.; Schmidt, R. R.; Kettman, U.; Rothe, U. *Bichim. Biophys. Acta* **1998**, *1372*, 205.
(297) Yamada, K. M.; Critchley, D. R.; Fishman, P. H.; Moss, J. *Exp. Cell Res.* **1983**, *143*, 295.
(298) Hakomori, S. *J. Biol. Chem.* **1990**, *265*, 18713.
(299) Springer, T. A. *Annu. Rev. Physiol.* **1995**, *57*, 827.
(300) Lingwood, C. A. *Biochim. Biophys. Acta* **1999**, *1455*, 375.
(301) Eidels, L.; Proia, R. L.; Hart, D. A. *Microbiol. Rev.* **1983**, *47*, 596.
(302) Sprangler, B. D. *Microbiol. Rev.* **1992**, *56*, 622.
(303) Suzuki, Y. *Prog. Lipid Res.* **1994**. *33*, 429.
(304) Hugosson, S.; Ångström, J.; Olsson, B.-M.; Bergström, J.; Fredlund, H.; Olcen, P.; Teneberg, S. *J. Biochem (Tokyo)* **1998**, *124*, 1138.
(305) Lanne, B.; Uggla, L.; Stenhagen, G.; Karlsson, K.-A. *Biochemistry* **1995**, *34*, 1845.
(306) Berenson, C. S.; Gallery, M. A. Katari, M. S.; Foster, E. W.; Pattoli, M. A. *J. Leukocyte Biol.* **1998**, *64*, 311.
(307) Liu, X.-H.; Lingwood, C. A.; Ray, P. *Kidney Int.* **1999**, *55*, 554.
(308) Fishman, P. H.; *Chem Phys. Lipids* **1986**, *42*, 137.
(309) Teneberg, S.; Hirst, T. R.; Ångström, J.; Karlsson, K.-A. *Glycoconjugate J.* **1994**, *11*, 533.
(310) Holmgren, J.; Elwing, H.; Fredman, P.; Svennerholm, L. *Eur. J. Biochem.* **1980**, *106*, 371.
(311) Fantini, J.; Hammache, D.; Delezay, O.; Pieroni, G.; Tamalet, C.; Yahi, N. *Virology* **1998**, *246*, 211.
(312) Kozaki, S.; L Kamata, Y.; Watarai, S.; Nishiki, T.; Mochida, S. *Microb. Pathog.* **1998**, *25*, 91.
(313) Futerman, A. H.; Boldin, S.; Brann, A, B.; Schwarz, A.; Zisling, R. *Ann. N.Y. Acad. Sci.* **1998**, *845*, 176.
(314) Schnaar, R. *Trends Glycosci. Glycotechnol.* **1992**, *4*, 90.
(315) Horejsi, V.; Drbal, K.; Cebecauer, M.; Cerny, J.; Brdicka, T.; Angelisova, P.; Stockinger, H. *Immunol. Today* **1999**, *20*, 356.
(316) Ando, S.; Tanaka, Y.; Waki, H.; Kon, K.; Iwamoto, M.; Fukui, F.; *Ann. N.Y. Acad. Sci.* **1998**, *845*, 232.
(317) Järnefelt, J.; Rush, J.; Li, Y.-T.; Laine, R. A. *J. Biol. Chem.* **1978**, *253*, 8006.
(318) Krusius, T.; Finne, J.; Rauvala, H. *Eur. J. Biochem.* **1978**, *92*, 289.
(319) Rauvala, H.; Finne, J. *FEBS Lett.* **1979**, *97*, 1.
(320) Tsuji, T.; Irimura, T.; Osawa, T.; *Biochem. J.* **1980**, *187*, 677.
(321) Laine, R. A.; Rush, J. S. *Adv. Exp. Med. Biol.* **1988**, *228*, 331.
(322) Fukuda, M. N. *Biochim. Biophys. Acta* **1999**, *1455*, 231.
(323) Ariga, T.; Yu, R. K. *Neurochem. Res.* **1998**, *23*, 291.
(324) Feizi, T. *Immunol. Commun.* **1981**, *10*, 127.
(325) Henry, S. M.; Samuelsson, B. E. In *Human Blood Cells*; King M.-J., Ed.; Imperial College Press: London, 2000; Chapter 2, pp 15−103.
(326) Henry, S. M.; Moulds, J. J. *Immunohematology* **2000**, *16*, 49.
(327) Henry, S.; Oriol, R.; Samuelsson, B. *Vox Sang.* **1995**, *69*, 166.
(328) Karlsson, K.-A. *Glycobiology* **2000**, *10*, 761.
(329) The carbohydrate and glycosphingolipid nomenclatures are according to recommendations of the Joint Commission on Biochemical Nomenclature (JCBN). Nomenclature of carbohydrates: *Carbohydr. Res.* **1997**, 297, 1. Nomenclature of glycolipids: *Carbohydr. Res.* **1998**, 312, 167 and *Eur. J. Biochem.* **1998**, 257, 293.

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