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Invited Review

Sphingolipids in Infectious Diseases

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CONTENTS:

SUMMARY: Sphingolipids are ubiquitous constituents of membrane lipids in eukaryotes. Sphingolipid metabolites modulate various cellular events including proliferation, differentiation, and apoptosis. In addition, sphingolipids, along with cholesterol, form detergent-resistant membrane microdomains, so called 'lipid-rafts', which are implicated in signal transduction and membrane trafficking. Sphingolipids are also relevant to infectious diseases. Various types of pathogens exploit the sphingolipids of host cells as membrane receptors. Sphingolipid metabolites regulate pathogen infection and host defense: for instance, a specific glycosphingolipid acts as an endogenous ligand for activation of natural killer T cells. Lipid-rafts of host cells serve as platforms also for infection signaling and entry of intracellular parasites. Moreover, some post-infectious autoimmune diseases result from production of antibodies cross-reacting with mammalian sphingolipids. Differences in the pathways of sphingolipid metabolism between mammals and non-mammals are good clues for rational development of new anti-infectious disease drugs. This review summarizes recent advances in sphingolipid biology related to infectious diseases.

1. Introduction

Sphingolipids are ubiquitous constituents of membrane lipids in eukaryotes (1,2). Sphingolipids are essential for the growth of not only mammalian cells but also invertebrate and fungal cells (3-8), and modulate various cellular events including proliferation, differentiation, and apoptosis (9-12). In addition, sphingolipids, along with cholesterol, form detergent-resistant membrane microdomains, so called 'lipid-rafts', which are implicated in signal transduction and membrane trafficking (13-17). Besides the physiological roles, the pathological aspects of sphingolipids have also been receiving

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attention. Inborn dysfunctions of enzymes or accessory factors involved in the degradation of sphingolipids often cause infant lethality, suggesting that the abnormal accumulation of sphingolipids is toxic to cells or tissues (18,19). Another important pathological aspect of sphingolipids is relevant to infectious diseases. For example, various types of pathogens exploit sphingolipids of host cells as membrane receptors. In addition, lipid-rafts of host cells can be platforms also for infection signaling and entry of intracellular parasites. Differences in the pathways of sphingolipid metabolism between mammals and non-mammals are good clues for the rational development of new anti-infectious disease drugs. This review summarizes recent advances in sphingolipid biology related to infectious diseases.

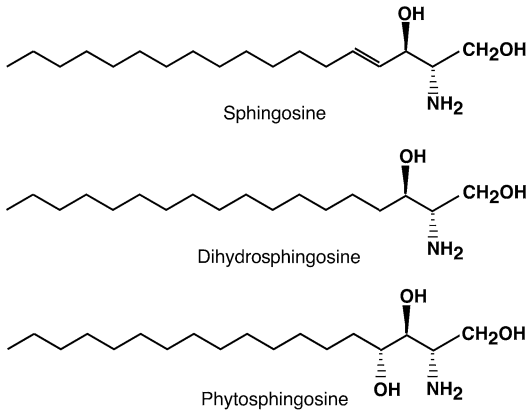

Fig. 1. Structures of sphingoid bases.

2. Structure and biosynthesis of sphingolipids

Sphingolipids are defined as lipids containing sphingoid bases (1,3-dihydroxy-2-amino-alkane and its derivatives) as a structural backbone. There are three major types of natural sphingoid bases (Fig. 1) (1). D-*Erythro*-sphingosine is the principal sphingoid base of sphingolipids in mammalian cells, and D-*erythro*-dihydrosphingosine is the second most abundant type. D-*Ribo*-phytosphingosine is the principal sphingoid base in plants and fungi, although some tissues including the kidney and stomach in mammals also have considerable amounts of phytosphingosine-containing sphingolipids. The alkyl chain length for natural sphingoid bases is predominantly 18, though it varies from 14 to 22 (1). *N*-Acylated forms of sphingoid bases are ceramides (more specifically, *N*-acylated forms of sphingosine, dihydrosphingosine, and phytosphingosine are ceramide, dihydroceramide, and phytoceramide, respectively). The fatty acids of ceramides vary in chain lengths (from 14 to 30), degree of unsaturation (but are mostly saturated), and presence or absence of a hydroxyl group at the α- or ω-carbon atom (2).

Polar head groups of sphingolipids show marked structural diversity among phylogenetically separate organisms. In mammalian cells, choline phosphoceramide (sphingomyelin; SM) accounts for 5 - 10% of membrane phospholipids, while various lower animal cells have ethanolamine phosphoceramide and its *N*-mono or dimethyl derivatives in place of SM, and fungal and plant cells produce phosphoinositol-containing sphingolipids (4). For glycosphingolipids (GSLs), great variety in the glycosyl head structure exists even in the same organism (Tables 1 and 2; see also below).

De novo biosynthesis of sphingolipids in mammalian cells proceeds as shown in Figure 2 (20). The first step is the condensation of L-serine and palmitoyl CoA to generate 3-ketodihydrosphingosine, which is reduced to dihydrosphingosine. Dihydrosphingosine undergoes *N*-acylation followed by desaturation to generate ceramide. These reactions to produce ceramide occur at the cytosolic surface of the endoplasmic reticulum (ER). Then, ceramide is delivered to the lumenal side of the Golgi apparatus, and converted to SM by SM synthase catalyzing the transfer of phosphocholine from phosphatidylcholine to ceramide. Ceramide is also converted to glucosylceramide (GlcCer) by GlcCer synthase catalyzing the transfer of glucose from UDP-glucose to ceramide at the cytosolic surface of the ER or *cis* Golgi region. After being transported to the lumen of the Golgi apparatus, GlcCer is converted to lactosylceramide (LacCer), which is converted to varieties of more complex GSLs (Fig.

Table 1. Abbreviations, structures, and semi-systematic names of neutral glycosphingolipids

Abbreviation[1)]	Structure[2)]	Semi-systematic name
GlcCer	Glcβ1'Cer	Glucosylceramide
GalCer	Galβ1'Cer	Galactosylceramide
LacCer	Galβ4Glcβ1'Cer	Lactosylceramide
Gb3Cer	Galα4Galβ4Glcβ1'Cer	Globotriaosylceramide
Gb4Cer	GalNAcβ3Galα4Galβ4Glcβ1'Cer	Globotetraosylceramide
iGb3Cer	Galα3Galβ4Glcβ1'Cer	Isoglobotriaosylceramide
iGb4Cer	GalNAcβ3Galα3Galβ4Glcβ1'Cer	Isoglobotetraosylceramide
Mc3Cer	Galβ4Galβ4Glcβ1'Cer	Mucotriaosylceramide
Mc4Cer	Galβ3Galβ4Galβ4Glcβ1'Cer	Mucotetraosylceramide
Lc3Cer	GlcNAcβ3Galβ4Glcβ1'Cer	Lactotriaosylceramide
Lc4Cer	Galβ3GlcNAcβ3Galβ4Glcβ1'Cer	Lactotetraosylceramide
nLc4Cer	Galβ4GlcNAcβ3Galβ4Glcβ1'Cer	Neolactotetraosylceramide
Gg3Cer[3)]	GalNAcβ4Galβ4Glcβ1'Cer	Gangliotriaosylceramide
Gg4Cer[3)]	Galβ3GalNAcβ4Galβ4Glcβ1'Cer	Gangliotetraosylceramide
Ga2Cer	Galα4Galβ1'Cer	Galabiaosylceramide

[1)]: abbreviations recommended by IUPAC-IUB Joint Commission on Biochemical Nomenclature (205,206).
[2)]: Cer, ceramide; Glc, glucose; Gal, galactose; NeuAc, *N*-acetylneuraminic acid; GalNAc, *N*-acetylgalactosamine; GlcNAc, N-acetylglucosamine.
[3)]: Gg3Cer and Gg4Cer are alternatively named asialoGM2 and asialoGM1, respectively.

Table 2. Abbreviation and structures of gangliosides

Abbreviation[1)]	Structure[2)]
GM4[3)]	NeuAcα3Galβ1'Cer
GM3	NeuAcα3Galβ4Glcβ1'Cer
GM2	GalNAcβ4(NeuAcα3)Galβ4Glcβ1'Cer
GM1a	Galβ3GalNAcβ4(NeuAcα3)Galβ4Glcβ1'Cer
GM1b	NeuAcα3Galβ3GalNAcβ4Galβ4Glcβ1'Cer
GD3	NeuAcα8NeuAcα3Galβ4Glcβ1'Cer
GD2	GalNAcβ4(NeuAcα8NeuAcα3)Galβ4Glcβ1'Cer
GD1a	NeuAcα3Galβ3GalNAcβ4(NeuAcα3)Galβ4Glcβ1'Cer
GalNAc-GD1a	GalNAcβ4NeuAcα3Galβ3GalNAcβ4(NeuAcα3)Galβ4Glcβ1'Cer
GD1b	Galβ3GalNAcβ4(NeuAcα8NeuAcα3)Galβ4Glcβ1'Cer
GT1a	NeuAcα8NeuAcα3Galβ3GalNAcβ4(NeuAcα3)Galβ4Glcβ1'Cer
GT1b	NeuAcα3Galβ3GalNAcβ4(NeuAcα8NeuAcα3)Galβ4Glcβ1'Cer
GQ1b	NeuAcα8NeuAcα3Galβ3GalNAcβ4(NeuAcα8NeuAcα3)-Galβ4Glcβ1'Cer

[1)]: nomenclature recommended by Svennerholm (207).
[2)]: NeuAc, *N*-acetylneuraminic acid. For symbols for other monosaccharides, see the footnote of Table 1.
[3)]: Although Neu5Acα3Galβ4Cer does not belong to the ganglio-series of glycosphingolipids, it is often referred to as GM4 ganglioside.

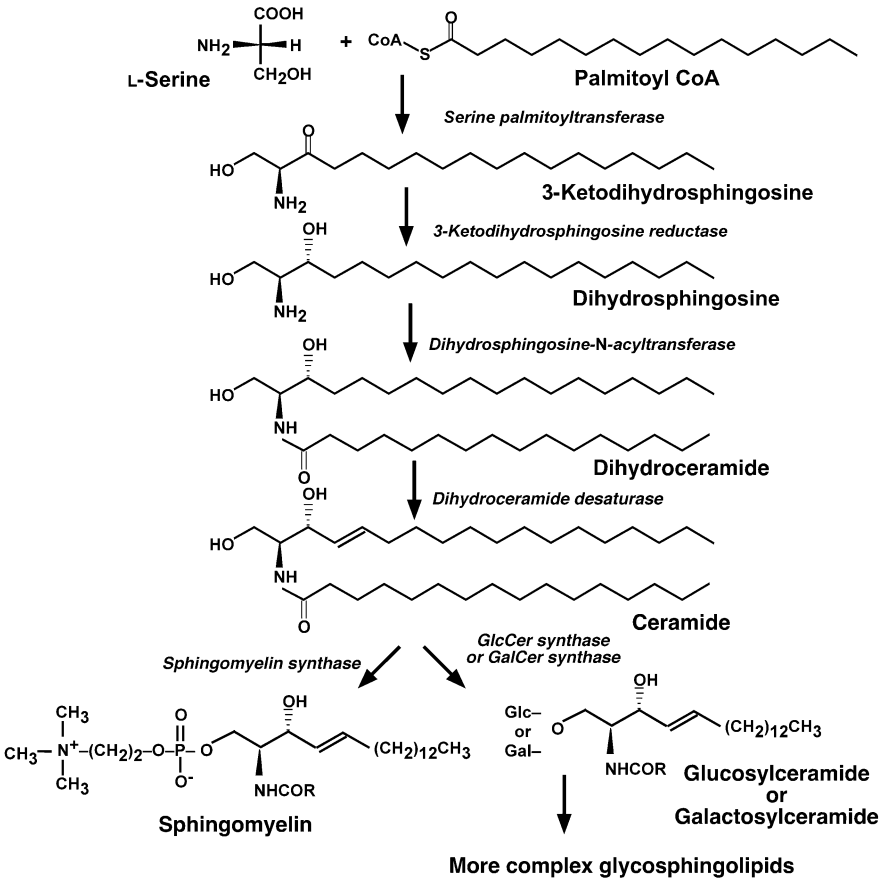

Fig. 2. Biosynthetic pathway of sphingolipids in mammalian cells.

3). Ceramide is also converted to galactosylceramide (GalCer) at the lumen of the ER, and GalCer is sulfated (to form the sulfatide I^3-sulfo-GalCer) or further glycosylated (Fig. 3). Note that the acyl chain of ceramide used for GalCer synthesis is predominantly α-hydroxylated. After the synthesis, these complex sphingolipids are ultimately delivered to the plasma membrane. SM and GlcCer are ubiquitously produced in mammalian tissues, while substantial production of GalCer occurs in limited types of tissues such as the brain and kidney.

3. Sphingolipids act as membrane receptors of various pathogens

3-1. Receptors for protein toxins

3-1-1. AB toxins

The AB toxins are protein toxins consisting of two different types of subunits: enzymatically active A-subunits and receptor-binding B-subunits. Some members (e.g., cholera toxin and Shiga toxin) of this group have A- and B-subunits at a molecular ratio of 1 to 5, and, therefore, are sub-classified

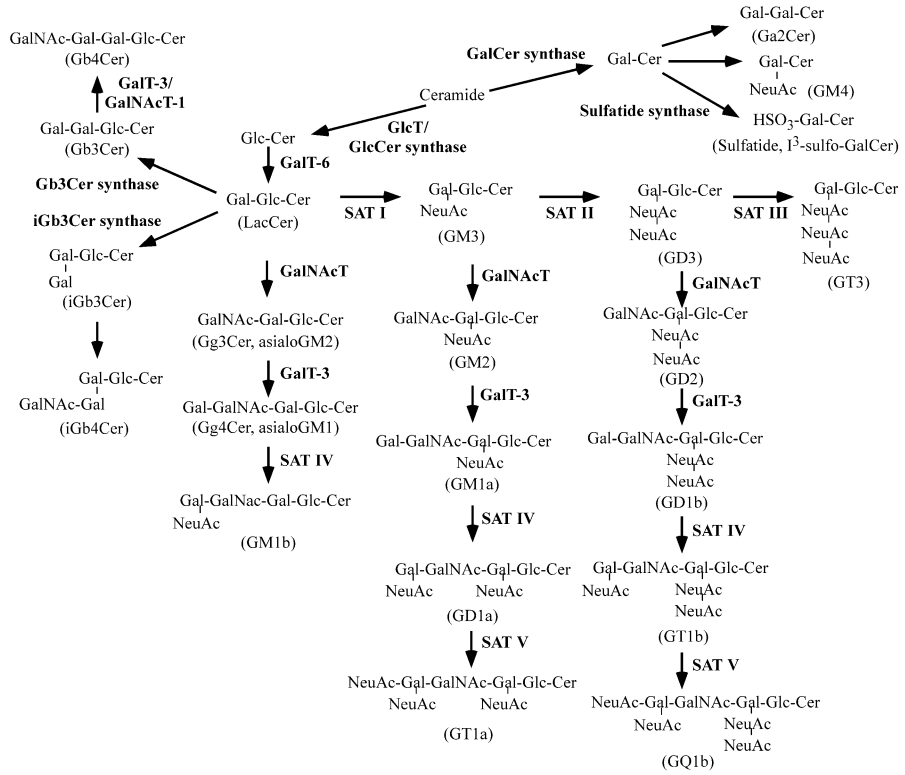

Fig. 3. Biosynthetic pathway of glycosphingolipids in mammalian cells. GlcT, glucosyltransferase; GalT, galactosyltransferase; SAT, sialyltransferase; GalNAcT, *N*-acetylgalactosamine transferase.

as AB_5 toxins. After the binding of the B-subunits to their specific receptors, AB_5 toxins are internalized into host cells, and transported through the Golgi apparatus to the ER (21,22). Then, the A-subunits of the toxins are released from the ER into the cytoplasm, and exert their cytotoxic biochemical reactions. For example, the A-subunits of cholera-like toxins (CLTs) catalyze NAD-dependent ADP-ribosylation of various target proteins. ADP-ribosylation of the α-subunit of the trimeric GTP-binding protein G_s activates adenyl cylase, leading to an increase in cAMP levels that triggers events responsible for enterotoxicity like severe diarrhea. The A-subunits of Shiga-like toxins (SLTs) catalyze de-glycosylation of 28S ribosomal RNA, thereby inhibiting protein synthesis and ultimately killing host cells.

3-1-1-1. CLTs

Cholera toxin and *Escherichia coli* heat-labile enterotoxin are over 80% identical at the amino acid sequence level, and closely resemble each other in overall three-dimensional structure (23-26). The ganglioside GM1 serves as the primary receptor for CLTs (27,28). An artificial high-affinity ligand that antagonizes the recognition of GM1 by CLTs was designed (20).

3-1-1-2. SLTs

Shiga toxin and its close relative Verotoxins cause not only hemorrhagic colitis but also hemolytic uremic syndrome (HUS) (29,30). A high-affinity receptor for these SLTs is the globotriaoside Gb3Cer (31-33). Gb3Cer is the primary receptor for SLTs, because Gb3Cer-less and SLT-insensitive lymphoid cells become SLT-sensitive once exogenous Gb3Cer is incorporated into their membrane (34). Moreover, it has recently been demonstrated that Gb3Cer-less mutant mice because of the disruption of the α 1,4-galactosyltransferase gene for the synthesis of globo-series GSLs, exhibit a complete SLT-resistance (35).

The incidence of HUS after infection with SLT-producing *E. coli* is age-dependent: high in young children, but low in adults (30). It has been suggested that the age-related incidence of HUS is due to the higher level of expression of renal Gb3Cer in the child than adult (30). On the other hand, a recent study showed no significant age-related difference in the Gb3Cer level in the human kidney, suggesting that factors other than basal renal Gb3Cer expression account for age-related HUS (36).

SLT-binding specificity is attributable to the terminal carbohydrate moiety of Gb3Cer (30,31). Nevertheless, the hydrophobic ceramide portion is also important for binding toxin, because replacing the ceramide moiety of Gb3Cer with diacylglycerol results in loss of SLT binding (33), and different molecular species of Gb3Cer with different acyl chains show a different affinity and capacity for binding SLT (37).

Various Gb3Cer-mimicking compounds can bind SLTs. For the ultimate purpose of preventing HUS, artificial Gb3Cer-like compounds (38,39) and recombinant bacteria expressing Gb3Cer-like glycolipids (40) have been developed as absorbers of SLTs released in the gut.

3-1-1-3. Tetanus and botulinum neurotoxins

Clostridium tetani neurotoxin and *C. botulinum* neurotoxin type A and B are generally classified as AB toxins, although these neurotoxins contain an additional domain for translocating them across the membrane, different from CLTs and SLTs. After translocation into the cytosol, the catalytic domain with the activity of Zn^+-dependent protease cleaves specific proteins involved in the exocytosis of neurotransmitters. Several gangliosides act as receptors for the tetanus and botulinum neurotoxins (41-43). Gangliosides are likely the primary receptors, even if they are not essential receptors, for the neurotoxins in vivo, because mice lacking complex gangliosides due to a disrupted GM2/GD2 synthase gene

(GalNAcT gene in Fig. 3) exhibit less sensitivity to tetanus and botulinum toxins, compared with wild-type control mice (44). In contrast, a glycolipid-null mutant cell line is still sensitive to ricin, a plant AB toxin that binds both GSLs and glycoproteins with terminal galactose (45).

3-1-2. Non-AB toxin

Delta-toxin is a hemolytic toxin secreted by *Clostridium perfringens*. The mechanism of delta-toxin-induced hemolysis remains unknown. GM2 or a GM2-like structure acts as a receptor for delta-toxin (46,47).

3-2. Receptors for viruses

3-2-1. Human immunodeficiency virus type 1 (HIV-1)

Although the CD4 molecule is the principal cellular receptor for HIV-1, several CD4-negative cell lines including intestinal and neuronal cell lines can be infected by some HIV-1 strains. Thus, a CD4-independent route(s) of cell infection for HIV-1 must exist. GalCer and/or its sulfated form (sulfatide) act as an alternate receptor for HIV-1 in CD4-negative cells including human U373-MG glioma and SK-N-MC neuroblastoma cells (48), and human colon epithelial HT29 cells (49). The HIV envelope glycoprotein gp120 efficiently binds GalCer/sulfatide, but not other GSL types (48,50).

The GalCer-binding site of gp120 was previously suggested to be located at amino acid 206-275 of the C2 region (50). However, recent studies showed that the GalCer-binding site in HIV-1 is located in the V3 loop of gp120. First, antibodies against the V3 loop inhibit interaction between GalCer and gp120 and block HIV-1 infection of $CD4^-$ and $GalCer^+$ intestinal HT-29 cells (51). Second, synthetic multimeric peptides derived from the V3 loop domain can bind GalCer and block the HIV-1 infection of HT-29 cells (52). Third, synthetic soluble analogs of GalCer bind the V3 loop domain and inhibit the HIV-1 infection of HT-29 cells (53). Notably, one of the soluble GalCer analogs inhibits the HIV-1 infection of $CD4^+$ T-lymphoblastoid C8166 cells, probably by binding to the V3 loop domain, which is also an essential domain for CD4-dependent infectivity of HIV-1 (53). Polysulfated galactose-derivatized dendrimers also inhibit infection by various HIV-1 isolates in vitro (54).

The expression of GalCer is not enough to render $CD4^-$ cell types HIV-1-infectable. HIV-1 infection of HT-29 cells is blocked by both anti-GalCer antibody and anti-CXCR4 antibody (55), suggesting that the CXCR4 co-receptor is also required for the GalCer-dependent pathway of HIV-1 infection in HT-29 cells.

When HIV-1 particles are internalized from the apical surface of epithelial cells via a GalCer-dependent pathway, the particles are often exocytosed from the basolateral side without viral fusion with membranes of host cells (56). To enter the pathway for nondegradative translocation across a cell, so called 'transcytosis', virus particles bound to GalCer use CCR5, not CXCR4, as a co-receptor (57). Primary intestinal jejunal epithelial cells express GalCer and CCR5, but not CXCR4 (57). Thus, in HIV-1 infection across a mucosal surface, $GalCer^+$ and $CCR5^+$ epithelial cells of the mucosa may selectively transmit CCR5-tropic virus. This would explain why HIV-1 isolated from acutely infected people via a gastrointestinal route is predominantly CCR5-tropic, even when both CCR5- and CXCR4-tropic viruses are inoculated onto the mucosa (57). The GalCer-dependent HIV-1 transcytosis is inhibited by depleting cholesterol from host cells, suggesting that it is mediated by lipid-rafts (58) (as for lipid-rafts, see below, Section 5).

3-2-2. Influenza virus and others

Influenza virus enters cells by clathrin-dependent and receptor-mediated endocytosis followed by low pH-triggered fusion between viral and endosomal membranes (59). Influenza virus specifically binds sialoglycoproteins and sialoglycosphingolipids, both of which contain neuraminic acid (its alternative name is sialic acid) as the terminal sugar residue of oligosaccharide chains, on the host cell surface (60,61). Enzymatic removal of cell-surface sialic acids of target cells blocks influenza viral infection, indicating that sialooligosaccharides are required for the infection (62). However, influenza virus was shown to infect a GSL-less mutant cell line as well as its parental cell line, suggesting that the virus uses sialoglycoproteins for infection (63).

Specific gangliosides serve as membrane receptors for murine polyoma virus and simian virus 40 (64,65), both of which are not clinical pathogens of humans. Although many types of viruses infectious to humans exploit host cell glycoconjugates as membrane receptors, the issue of whether GSLs are indeed crucial for infection has been addressed in only a few types of viruses. For more details about carbohydrates as virus receptors, please see other reviews (66,67) and references therein.

3-3. Receptors for bacteria and fungi

3-3-1. Fimbriated *E. coli*

E. coli expresses many types of fimbrial adhesins, which can be divided into different groups by their affinity for specific receptor structures. For example, P fimbrial adhesins bind Galα4Galβ, type I adhesins bind mannosides, S fimbrial adhesins bind NeuAcα3Galβ, and M fimbrial adhesins bind glycophorin A.

3-3-1-1. P-fimbriated *E. coli*

In uncomplicated cystitis and pyelonephritis, the most common pathogen is *E. coli* (~80% of cases) (68,69). The most prevalent and pathogenically important *E. coli* in uncomplicated pyelonephritis is P-fimbriated *E. coli* (68). The P fimbriae bind the globo-series of GSLs such as Gb3Cer and Gb4Cer (70,71). The crystal structure of a binary complex of the PapG subunit (which is the GSL-binding subunit of the P fimbriae [72,73]) and a globoside analog has been reported (74). When mice were fed with the GSL biosynthesis inhibitor *N*-butyldeoxynorijimycin, they showed decreases not only in levels of GSLs but also in the susceptibility to experimental urinary tract infection with P-fimbriated *E. coli* (75), showing that GSLs are indeed functional receptors for P-fimbriated *E. coli* in vivo.

3-3-1-2. F1C-fimbriated *E. coli*

About 10% or more of all uropathogenic *E. coli* strains express F1C-fimbriae (76). The F1C fimbriae, which are members of the S fimbria super family at the amino acid sequence level, also bind various GSLs. A previous study reported that the F1C fimbriae bound various GSLs such as GlcCer, GalCer, LacCer, Gb3Cer, and Gg4Cer (77). But, a more recent study reported that the F1C fimbriae specifically bound GalCer and Gb3Cer with the ceramide portion consisting of phytosphingosine and hydroxy fatty acids (78). The reason for the discrepancy in selectivity between the different studies is unclear.

3-3-1-3. S-fimbriated *E. coli*

S-fimbriated *E. coli* is a major cause of acute bacterial meningitis in neonatal infants (79). The S-fimbrial adhesins consist of four subunits, SfaS, SfaA, SfaG, and SfaH. SfaS has a lectin-like activity to bind NeuAcα3Galβ-containing glycoproteins (80), and this activity is important for specific

binding of the S fimbriae to lumenal surfaces of the vascular endothelium in the neonatal rat brain (81). Curiously, S-fimbriated *E. coli* can also bind GalCer, LacCer and sulfatide, and the binding activity toward these GSLs is attributed to SfaA, not to SfaS (82).

3-3-2. Various pulmonary pathogenic bacteria

Pseudomonas aeruginosa infection in the lung is a leading cause of death of patients with cystic fibrosis (83). Krivan et al. reported that *P. aeruginosa* and *P. cepacia* isolated from cystic fibrosis patients bind specifically Gg3Cer and Gg4Cer (both of which contain the GalNAcβ4Gal sequence) among various GSLs (84). They also found that more bacteria (*Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae*) found in cystic fibrosis can bind GalNAcβ4Gal-containing GSLs (85). However, Feldman et al. have suggested that the flagellin-mediated binding of *P. aeruginosa* to epithelial GSLs plays only a minor role in pulmonary infection (86).

Bordetella pertussis is a human respiratory pathogen. Whereas both virulent and non-virulent *B. pertussis* strains bind Gg3Cer, only the virulent strains bind the sulfatide I^3-sulfo-GalCer in bacteria-lipid overlay assays (87). The attachment of virulent *B. pertussis* cells to cultured human colon adenocarcinoma WiDr cells and hamster trachea cells is partially inhibited by some sulfated compounds, suggesting that sulfatide on the host cell surface acts as a virulence-relevant receptor for *B. pertussis* (87).

Mycoplasma pneumoniae is a small prokaryotic parasite of the human respiratory tract and the etiologic agent of primary atypical pneumonia. *M. pneumoniae* appears to exploit at least two distinct molecules of the host cell surface as receptors: glycoproteins containing terminal NeuAcα3Galβ4GlcNAc-sequences (88), and GSLs containing terminal Gal($3SO_4$) β1-residues (89).

3-3-3. *Helicobacter pylori*

The Gram-negative bacterium *H. pylori* is an etiologic agent of chronic active gastritis, peptic ulcer disease, and gastric adenocarcinima. *H. pylori* has a stringent host range and tissue tropism, and requires human gastric epithelium for colonization. Although *H. pylori* was shown to bind to diverse membrane components including various GSLs in vitro (see [30,90-92] and references therein), it remains controversial whether these interactions are really relevant to the colonization of *H. pylori* in the human stomach. A few specific adhesins of *H. pylori* have been identified to date: BabA protein acts as an adhesin for the Lewis b blood group determinant (expressed on both GSLs and glycoproteins) (93), and SabA protein as an adhesin for sialyl-dimeric Lewis x NeuAcα3Galβ4(Fucα3)GlcNAcβ3Galβ4(Fucα3) GlcNAcβ3Galβ4Glcβ1Cer (94).

3-3-4. *Candida albicans*

C. albicans is an opportunistic fungal pathogen. It is likely that *C. albicans* expresses multiple adhesins with different receptor specificity in adhering to the host cell. Brennan et al. showed, for the first time, evidence that LacCer is a possible receptor for various fungi including *C. albicans* (87). Two other research groups, however, reported that they failed to detect any affinity of *C. albicans* fimbriae for LacCer (95,96). Moreover, one of the latter two research groups reported that the *C. albicans* fimbriae interacted with the asialogangliosides Gg3Cer and Gg4Cer (95), while the other reported that fucose-containing GSLs, rather than asialogangliosides, acted as receptors for *C. albicans* (96). The fimbriae from different *C. albicans* strains may have different glycolipid specificity.

4. Sphingolipids involved in pathogen-induced signaling

4-1. Signaling induced by pathogen-mediated clustering of sphingolipids

Clustering of cell surface sphingolipids often induces intracellular signaling events. Hence, when a pathogen-derived ligand has multivalent binding sites for its sphingolipid receptor, it may trigger signaling responses in host cells. Such responses would play important roles in pathogen-infection and/or host cell-defense.

The receptor binding B subunit of SLTs forms a pentamer. Although the B subunit of SLTs has no direct cytotoxic biochemical activity (see Section 3-1-1), the B subunit alone can induce apoptosis in Burkitt's lymphoma cells, as Gb3Cer ligation with an anti-Gb3Cer antibody can do (97,98). Both anti-Gb3Cer antibody and Shiga toxin can also activate *yes*, a *src*-family kinase in the human renal tubular ACHN cell line (99), although the apoptotic signaling pathway triggered by SLT-binding to Gb3Cer is likely distinct from the pathway triggered by anti-Gb3Cer antibodies (100).

Besides causing watery diarrhea, cholera toxin has the ability to enhance mucosal immunity and acts as a potent adjuvant (101,102). The activation of antigen-presenting cells by cholera toxin requires host cell GM1 ganglioside and also both the B-subunit and the ADP-ribosyltransferase-active A-subunit of the toxin (103), indicating the requirement of internalization of the holo toxin in antigen-presenting cells for exerting its adjuvant activity. Nevertheless, when antigen peptides are covalently linked with the cholera toxin B subunit, the conjugated antigens (even without the A subunit) can effectively promote antigen presentation and increase CD40 and CD86 expression on antigen-presenting cells (104). Molecular mechanisms underlying the modulation of immuno responses by cholera toxin have not been well elucidated.

The Gram-negative bacterium *P. aeruginosa* binds to the host cell receptor Gg4Cer (asialoGM1) through flagellin, a major structural component of the bacterial flagella (86) (see also Section 3-3-2). Ligation of Gg4Cer by the *P. aeruginosa* flagellin triggers the release of ATP from the host cell, followed by autocrine activation of a nucleotide receptor, which is linked to transcriptional stimulation for mucin production (105).

4-2. Ceramide production in response to infection

When infected by several specific pathogens, host cells produce more ceramide. *Neisseria gonorrhoeae* invades non-phagocytic epithelial cells after binding to heparansulfate proteoglycan receptors. At an early stage in the invasion, the acid sphingomyelinase (SM-phospholipase C with its optimal pH in an acidic range; ASMase) of host cells is activated, and ceramide is produced (106). ASMase-deficient mutant fibroblasts are impaired in *N. gonorrhoeae*-induced ceramide production and entry processes (106). The activation of ASMase is also involved in CEACAM-receptor-mediated infection by *N. gonorrhoeae* of human phagocytes (107). These studies indicated that the activation of ASMase in host cells plays a crucial role in *N. gonorrhoeae* infection.

The production of ceramide along with the activation of ASMase also occurs at an early stage after infection by *P. aeruginosa* (which exploits GSLs as membrane receptors as described in 3-3-2). The production of ceramide is important not only for the internalization of *P. aeruginosa* into host cells, but also for host defense, because, when infected with *P. aeruginosa*, ASMase-deficient mutant mice undergo more

severe inflammatory responses (e.g., interleukin [IL]-1β secretion and septic death) than do control mice (108).

Uropathogenic P-fimbriated *E. coli* cells, which employ the globo-series of GSLs as membrane receptors (see also 3-3-1-1), elicit an IL-6 secretion response in mucosal cells. During this response, production of ceramide and ceramide-1-phosphate is induced in the host cells (109). The production of ceramide occurs upstream of the activation of serine/threonine kinase(s) in an IL-6 response cascade (109). The production of ceramide in response to P-fimbriated *E. coli* infection results from enhanced breakdown of the receptor GSLs, not SM (110), different from *N. gonorrhoeae* and *P. aeruginosa* infections, which are accompanied by SM breakdown.

The obligatory intracellular parasite *Leishmania donovani* is the causative agent of visceral leishmaniasis. *L. donovani* down-regulates immune responses of host animals, and this immune down-regulation is likely required for intracellular survival of the parasite. *L. donovani* infection induces the accumulation of de novo synthesized ceramide in invaded macrophages (111). Intracellular survival of *L. donovani* is repressed by treatment of host macrophages with fumonisin B1, an inhibitor of de novo ceramide synthesis, and this survival repression is partly rescued by co-treatment with the ERK inhibitor PD98059 (112). Because ERK activation is important for immune responses such as the activation of AP-1 and NF-κB and the generation of NO, the ceramide-mediated inhibition of the ERK cascade would be the mechanism of down-regulating immune responses in *L. donovani*-infected macrophages (112).

4-3. GSL-mediated signaling with transmembrane co-receptors

Although GSLs are embedded only in the half leaflet of lipid bilayers of membranes, ligand-binding to GSL receptors often triggers intracellular signaling events. To transduce the ligand/GSL association signal occurring at the cell surface into the cytosol, transmembrane co-receptors are postulated to be involved.

In the signaling pathway in response to the endotoxin lipopolysaccharide (LPS), the glycosyl phosphatidylinositol (GPI)-anchored protein CD14 serves as the primary LPS-binding membrane receptor, and toll-like receptor 4 (TLR4) is a transmembrane co-receptor responsible for transducing the LPS stimulus into the cytosol (113-115). P-fimbriated *E. coli*, but not *papG*-deleted *E. coli*, can trigger strong inflammatory responses in CD14-negative epithelial cells in a TLR4-dependent manner (116-118). Therefore, P-fimbriae *E. coli* cells bound to the GSL receptor are likely able to present their own LPS to the TLR4 co-receptor without CD14.

5. Sphingolipids for the formation of detergent-resistant membrane domains, lipid-rafts

In biological membranes, different lipid species are asymmetrically distributed over the exoplasmic and cytoplasmic leaflets of the membrane. For example, complex sphingolipids are preferentially distributed to the exoplasmic leaflet, while acidic glycerophospholipids such as phosphatidylserine and phosphatidylinositol are mainly distributed to the cytoplasmic leaflet. Although lipid bilayers of biological membranes are principally fluid at physiological temperatures, the lipids can also be organized in the lateral dimension. The best-investigated of the lateral lipid organizations are the cholesterol/sphingolipid-enriched membrane domains (13-15,17).

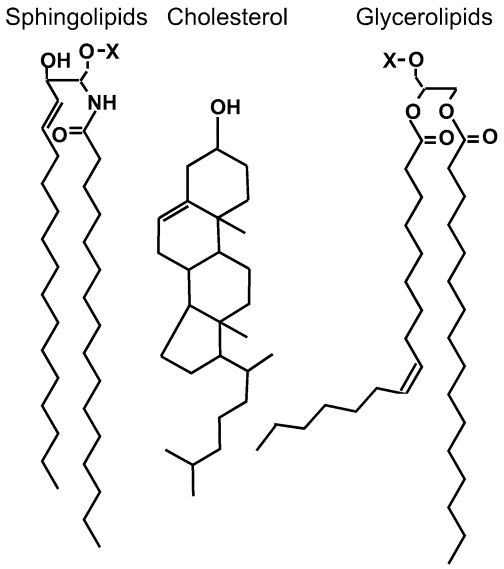

Fig. 4. Structures of sphingolipid, cholesterol, and glycerophospholipid. Cholesterol can be packed more intimately and therefore makes a stronger van der Waals interaction with sphingolipids than with glycerophospholipids. Acyl chains of mammalian glycerophospholipids at the *sn*-2 position are principally unsaturated fatty acids with one or more double bonds of the *cis* configuration. Fatty acids in the phosphatidylinositol moiety of some, if not all, GPI-anchored proteins are remodeled to saturated types during maturation (204), thereby making GPI-anchored proteins interact with cholesterol/sphingolipid-enriched membrane domains.

For reasons of molecular packing, cholesterol interacts more strongly with saturated fatty acids than with unsaturated fatty acids (Fig. 4). Fatty acids of sphingolipids are mainly saturated fatty acids, while most glycerolipids in eukaryotes have unsaturated fatty acids (Fig. 4). Hence, cholesterol, sphingolipids, and proteins linked with saturated fatty acids (e.g., GPI-anchored proteins and palmitoylated proteins) tend to be clustered in membranes. This cluster is assumed to, at least partially, reflect the biochemical observation that, when cells are treated with non-ionic detergents like Triton X-100 at 4°C, these membrane components are selectively recovered as insoluble complexes (15,16). Cholesterol/sphingolipid-enriched membrane domains are thus referred to as detergent-resistant membrane domains (15), and also as lipid-rafts (13). Caveolae (caveolin-coated pits of the plasma membrane) are probably a subset of cholesterol/sphingolipid-enriched detergent-resistant membrane domains (14). These terms have not actually been settled, because of spatio-temporal variations of the lipid membrane domains. Nevertheless, for simplicity, I here use the term lipid-rafts to represent cholesterol/sphingolipid-enriched detergent-resistant membrane domains.

Lipid-rafts are very small (<10 nm in diameter) and transient in resting-state cells (119,120). In response to specific stimuli, each raft gathers to form large and stable lateral domains, which serve as platforms for transmembrane signaling and membrane trafficking (119-122). Lipid-rafts can also be platforms for signaling during infection at the surface of the host cell, and may guide pathogens to their niches in host cells (67,123-125), because many receptors for pathogens are raft components (e.g., GSLs, and GPI-anchored proteins) as described above, and because a raft-dependent and clathrin-independent route of endocytosis does not lead to lysosomal compartments. For more details on lipid rafts in infectious diseases, please see previous reviews (67,123-126) and references therein. In this section, I will

briefly introduce studies showing the functional importance of sphingolipids in raft-mediated infection.

5-1. Conversion of prions to their pathogenic conformers

Transmittable spongiform encephalopathies such as scrapie in sheep, bovine spongiform encephalopathy and Creutzfelt-Jacob disease in humans are caused by prions, protenaceous infectious pathogens (127). Prions have two distinct conformational isoforms: the normal prion protein PrP^{c}, and its pathogenic isoform PrP^{Sc} with a β-sheet-rich abnormal conformation (127). Both forms have a GPI-anchor moiety and are preferentially distributed to lipid-rafts (128). Interestingly, depletion of cellular sphingolipids by using metabolic inhibitors enhances the conversion of PrP^{c} to PrP^{Sc} in cultured neuroblastoma cells (129), while depletion of cellular cholesterol inhibits the conversion (130). We previously showed that sphingolipid deficiency renders GPI-anchored proteins hypersensitive to exogenous phosphatidylinositol-specific phospholipase C, suggesting that sphingolipid deficiency increases the mobility of GPI-anchored proteins at the plasma membrane (131). This would be relevant to the enhancement of PrP^{c} -to-PrP^{Sc} conversion on sphingolipid depletion. Purified infectious prion rods contain host-derived SM and GalCer (132), supporting the possible interaction of prions with sphingolipids.

As described in section 3-2-1, the V3 loop of the HIV-1 gp120 has a GalCer-binding domain. Structural similarity searches suggested the presence of a V3-like sphingolipid-binding domain also in prions and Alzheimer β-amyloid peptide (133). Several studies have shown that sphingolipids affect the biogenesis and accumulation of Alzheimer β-amyloid peptide (134-137).

5-2. Induction of efficient entry of anthrax toxin

Anthrax toxin is one of the two dominant virulence factors produced by *Bacillus anthracis*. After binding to a plasma membrane receptor (a widely expressed type I transmembrane protein termed anthrax toxin receptor), anthrax toxin is internalized and exerts its deadly effects on the host cell. The toxin's oligomerization at the plasma membrane triggers clustering of the membrane receptors within lipid-rafts (138). On clustering, the toxin/receptor complex is efficiently endocytosed via a lipid-raft-mediated clathrin-dependent pathway (138). The clustering of the toxin receptor is inhibited by depleting cholesterol or sphingolipids from host cells (138).

5-3. Invasion and intracellular growth of *Chlamydia*

Chlamydia spp. are obligate intracellular bacterial parasites. *C. trachomatis* is the major cause of infectious blindness worldwide and is the leading cause of sexually transmitted diseases in the western hemisphere (139). *C. trachomatis* can be subdivided into serological variants (serovars). Whereas the entry of some *C. trachomatis* serovars into host cells is independent of lipid-rafts, other *C. trachomatis* serovars, *C. pneumoniae* and *C. psittaci* enter host cells via lipid-rafts (140,141). In the parasitophorus vacuole of infected cells, chlamydiae obtain cholesterol and sphingolipids from host cells (142). Host cell-derived sphingolipids are required for the intracellular growth of the *C. trachomatis* L2 serovar (143) (which enters host cells in a lipid-raft-independent manner [141]).

5-4. Contact and entry of *Shigella*

The enteroinvasive bacterium *Shigella* causes an acute inflammatory bowel disease. One mode of contact of *Shigella flexneri* with the host cell involves interaction between the bacterial invasin protein IpaB and the host transmembrane protein CD44. Not only CD44 but also raft markers (cholesterol and GPI-anchored proteins) of host cells are accumulated at *Shigella* entry loci, and the bacterial entry is inhibited by depleting cholesterol or sphingolipids from the host cells (144). These results indicated that lipid-rafts are involved in *Shigella* binding and entry (140).

To induce invasive engulfment of bacteria in host cells, *Shigella* has to secrete bacterial effectors into the host cell plasma membrane and cytosol though a functional type III secretion system (TTSS). TTSS is activated by contact of *Shigella* with lipid-rafts, and the TTSS activation is dependent on both cholesterol and sphingolipids, but not CD44, of host cells (145).

5-5. Entry of type 1-fimbriated *E. coli* into bladder epithelium

Type 1-fimbriated *E. coli* is the most common human uropathogen, owing much of its virulence to invasion of the uroepithelium. Uroplakin, a membrane receptor for type 1 fimbriae, is located in lipid-rafts (146). Invasion of this bacterium into bladder epithelium likely occurs via caveolin-enriched lipid-rafts, because the bacterial entry is inhibited by reduction of caveolin-1, depletion of cholesterol, and masking of the raft component GM1 ganglioside with the cholera toxin B subunit (146).

5-6. Modulation of translocation of diphtheria toxin across membranes

Diphtheria toxin (DT), which is produced by *Corynebacterium diphtheriae*, belongs to the AB-type toxin (147). The membrane receptor of DT is a heparin-binding EGF-like growth factor precursor. After endocytosed into acidic endosomes, DT exposes a hydrophobic loop of its B-subunit. This loop inserts into the endosome membrane, forms an ion channel, and facilitate translocation of the cytotoxic A-subunit (which catalyzes ADP-ribosylation of elongation factor 2) to the cytosol (147). A recent study showed that depletion of sphingolipids in host cells augments the sensitivity to DT intoxication, and that sphingolipids of host membranes may modulate translocation of DT across the membranes (148). Cholesterol increases the apparent pore size formed by DT in artificial membranes (149). Thus, sphingolipids, in concert with cholesterol, might affect DT translocation across cellular membranes.

6. Sphingolipid/cholesterol-dependent but raft-independent fusion of Semliki Forest virus (SFV) and Sindbis virus (SIN)

The lipid-raft model is a prevailing concept in membrane biology. However, lipid-rafts do not mediate all processes that depend on both cholesterol and sphingolipids. For several types of enveloped viruses such as alphaviruses, membrane fusion occurs when the virions are exposed to mildly acidic pH, which is physiologically attained in endosomes (150). The acidic pH induces conformational changes in the viral envelope protein, and triggers the fusion of the viral and host endosomal membranes (150). Membrane fusion of the alphavirus SFV and SIN requires both cholesterol and sphingolipids in target membranes (151,152). Both the envelope protein E1 and E1* peptide (a soluble ectodomain of E1) of SFV interact with target membranes, dependent on a low pH, cholesterol and sphingolipids, and form highly stable homotrimers for fusion (153,154). Although E1* is selectively associated with detergent-resistant membranes, depending on the proposed fusion peptide region in E1 and cholesterol of target membranes (155), several lines of evidence have

indicated that cholesterol and sphingolipids act as distinct co-factors, rather than as structural constituents of lipid-rafts, to modulate E1 conformational changes. First, the level of sphingolipid required is very low (1-2 mol% of target membrane lipids) (153). Second, the fusion of SFV and SIN with target membranes can occur even when the target membranes do not form detergent-resistant domains (156). Third, a specific E1 mutation enables SFV to fuse with target membranes in a cholesterol-independent manner, although this mutation does not confer a sphingolipid-independence to the virus, suggesting different roles for cholesterol and sphingolipids in viral fusion (157). In addition, whereas the natural C_{16}-D-*erythro* ceramide and its short chain analogs can support the fusion in model membranes, unnatural stereoisomers of C_{16}-ceramide can not (158,159). The sterol androstanol that does not produce detergent-resistant membranes can replace cholesterol for E1*-membrane interaction (155). Collectively, these studies strongly suggest that SFV and SIN do not require lipid-rafts for fusion with target membranes, and that the cholesterol- and sphingolipid-dependence is through direct modulation of E1.

7. Sphingolipids in postinfectious auto-immune diseases

7-1. Guillain-Barré syndrome (GBS)

Since poliomyelitis has been almost completely eliminated, GBS is the most frequent cause of acute neuromuscular paralysis throughout the world. GBS is often (more than 60% of cases) preceded by various infections. The most frequent infectious pathogen associated with GBS is *Campylobacter jejuni*, a major cause of bacterial enteritis (160-162). *C. jejuni* has cell envelope LPSs with glycoconjugate structures identical or similar to those of nerve cell gangliosides (163). Due to this antigenic identity or mimicry, immunization by *C. jejuni* LPSs may produce antibodies capable of attacking host tissues expressing the cross-reacting gangliosides. Actually, antibodies that react with GM1 and closely related gangliosides (such as GM1b and GD1a) are detected in more than the half of patients with *C. jejuni*-associated GBS (160-162). Yuki et al. recently demonstrated that, on sensitization with isolated *C. jejuni* LPS, rabbits developed not only anti-GM1 IgG but also pathological changes virtually identical to GBS (164).

The second most frequent infectious pathogen associated with GBS is cytomegalovirus (CMV) (160-162). Although antibodies reacting with the ganglioside GM2 have been observed in CMV-associated GBS (165,166), their pathogenic relevance remains controversial (167). Because CMV unlikely has any GM2-like epitopes, up-regulation of GM2 expression in host cells after CMV infection might result in the anti-GM2 antibody production (168).

7-2. Miller-Fisher syndrome (MFS)

MFS, a variant of GBS, is characterized by the acute onset of ophthalmoplegia, ataxia, and areflexia (160-162). Various infections also precede MFS. Nearly all MFS patients have antibodies reacting to the ganglioside GQ1b or GT1a in the acute phase (162,169). GQ1b-like epitopes seems to exist in LPSs of *C. jejuni* strains isolated from MFS patients with anti-GQ1b antibody (170). In human tissues, GQ1b is densely localized in the paranodal regions of cranial nerves that innervate the ocular muscles (169,171). This might explain the close association between anti-GQ1b antibodies and ophthalmoplegia in MFS.

8. Self-ligands for CD1d-restricted natural killer T (NKT) cells

NKT cells, a sub-population of T cells that express a rearranged antigen-specific T cell receptor (TCR) and natural killer cell markers such as CD161 simultaneously, are involved in the regulation of various immune responses including autoimmunity, cancer, and infectious diseases (172, 173). A major subset of NKT cells expresses an invariant TCR α-chain rearrangement with a very limited range of TCR β-chains (*Vα14-Jα18* with *Vβ8.2* in mouse, and *Vα24-Jα18* with *Vβ11* in human) (172,173). The major NKT cells with the invariant TCR arrangement (iNKT cells) recognize glycolipid antigens presented by CD1d, a major histocompatibility complex (MHC) I-like molecule, unlike conventional $CD4^+$/$CD8^+$ T cells activated by specific peptide antigens bound to MHC class I or HMC class II molecules.

It has been thought that efficient iNKT cell development requires appropriate self-lipid antigens presented on CD1d though lysosomal compartments (172,173). α-Galactosylceramide (more correctly, α-galactosyl phytoceramide), which was originally derived from a marine sponge, was found to strongly activate CD1d-restricted iNKT cells (174). However, no mammalian-derived α-galactosylceramide has been found to date (mammalian GalCer is β-galctosylceramide, not α-galactosylceramide). Zhou et al. recently revealed that isoglobotriaosylceramide (iGb3Cer) is likely an endogenous primary ligand for maturation of iNKT cells (175). They demonstrated that mutant mice lacking β-hexosamidase, a lysosomal enzyme responsible for degradation of iGb4Cer to iGb3Cer, exhibit severe iNKT cell deficiency (176). In addition, synthetic iGb3Cer, but not other related natural GSLs, can serve as a ligand to potently activate mature CD1d-restricted iNKT cells from both mouse and human (175).

The notion that iGb3Cer is a self-antigen to CD1d-restricted iNKT is also consistent with other recent studies. First, GlcCer synthase deficiency, but not GalCer synthase deficiency, in mouse cells impairs the ability for a CD1d-dependent reaction to iNKT cells, whereas GlcCer itself can not be an activator for CD1d-restricted iNTK cells, indicating that β-GlcCer derivatives, but not β-GalCer derivatives, are likely endogenous ligands for CD1d-restricted iNKT cells (177). Second, disruption of the prosaposin gene (which encodes four saposins, lipid-transfer proteins, crucial for de-glycosylation of various GSLs in the acidic compartments) causes iNKT deficiency in mice, indicating that saposins are involved in the generation and/or loading of a natural thymic antigen(s) for maturation of CD1d-restricted iNKT cells (176).

It should also be noted that CD1d-restricted NKT cells might respond to more self-ligands, because a subset of NKT cells proliferated when stimulated with the ganglioside GD3 (178). CD1d can bind a variety of mammalian lipids including GSLs (179), phospholipids (179,180), and GPI (181, 182), although most of these endogenous lipids are unable to activate CD1d-restricted NKT cells. See also 'Additions in proofs' for microbial ligands that can activate CD1d-restricted iNKT cells.

9. Sphingolipids in microbes as anti-infectious disease targets

The enzymes involved in the early steps of sphingolipid biosynthesis are well conserved. Such conserved enzymes may not be good candidates for anti-microbial targets, because

of poor selectivity between pathogens and humans. However, evolutionarily separated organisms produce different types of complex sphingolipids, and thus, express different types of enzymes, which can be rational anti-microbial targets.

9-1. Anti-fungal targets

Polar head groups of complex sphingolipids differ quite extensively between mammals and fungi. In fungi, the predominant complex sphingolipids are inositol phosphoceramide (IPC) and its mannosylated derivatives (4), which are absent in mammalian cells. A key enzyme for the synthesis of fungal sphingolipids is IPC synthase (IPCS), which catalyzes the transfer of a phosphoinositol group from phosphatidylinositol to ceramide or phytoceramide (4). No homologs of IPCS are encoded by the human genome. The indispensability of the IPCS gene for fungal growth was initially demonstrated in the budding yeast *Saccharomyces cerevisiae* (183). The fungal toxicity that results from the blocking of IPCS is largely attributed to accumulation of the precursor ceramide, because conversion of IPC to more complex glycolipids is not essential for the growth of *S. cerevisiae* (184,185), and partial inhibition of the de novo synthesis of ceramide can reduce the cytotoxic effects on fungi of inhibiting IPCS (183,186).

Three types of natural inhibitors of IPCS with quite different structures have been discovered to date: they are aureobasidin A (183), khafrefungin (187) and rustmicin (188), all of which show anti-fungal activity toward not only *S. cerevisiae* but also various pathogenic fungi including *C. albicans* and *Cryptococcus neoformans* in vitro (183,187-191). Of note, whereas the IPCS activity in lysate of *Aspergillus fumigatus* cells is sensitive to these IPCS inhibitors, the growth of *A. fumigatus* in culture is resistant to them (183,187,188, 192). This is likely due to efficient efflux of the drugs from *A. fumigatus*, because inhibitors of a multidrug efflux pump rendered *A. fumigatus* sensitive to aureobasidin A (192).

Compulsory down-regulation of IPCS in *C. neoformans* markedly impaired the pathogenecity of *C. neoformans* in a rabbit model, and also decreased the intracellular growth of *C. neoformans* in J774.1 murine macrophage-like cells (193). In spite of these results, no IPCS inhibitors have been developed into actual clinical drugs to date, implying that they have crucial drawbacks. New types of IPCS inhibitors are desired.

9-2. Anti-protozoan targets

Plasmodium falciparum is responsible for the most virulent form of malaria, which remains a major cause of human deaths in tropical and subtropical areas. Lipid metabolism has gathered attention as an anti-malarial target (194, 195). Pharmacological analysis of plasmodial SM synthase (SMS) suggested that *P. falciparum* has two types of the enzyme: one is similar to mammalian SMS, the other is not (196). The latter type of SMS seems to be essential for the intraerythrocytic growth of the parasite in culture (196), suggesting that its specific inhibitors may be potential anti-malarial drugs. A plasmodial phospholipase C that hydrolyzes SM and choline-containing lyso-phospholipids (including sphingosylphosphocholine, lyso-phosphatidylcholine, and lyso-platelet activating factor) may also be a target for anti-malarial chemotherapy (197), although the structural similarity between the plasmodial phospholipase C and its mammalian counterparts is a drawback.

The kinetoplastid parasitic protozoa are the etiologic agents of the leishmaniases (*Leishmania* spp.), Chaga's dieases (*Trypanosoma cruzi*), and African sleeping sickness (*Trypanosoma brucei*). Various members of the kinetoplastid spp. synthesize IPC as a membrane-bound linker to some cell-surface proteins, although it remains unknown if the kinetoplastid spp. have homologs of fungal IPCS. Kinetoplastid gene homologs of LCB1 and LCB2 subunits of serine palmitoyltransfease (which is the enzyme responsible for the initial step of de novo sphingolipid synthesis [3]) have been identified (198,199). Interestingly, the *L. major* LCB2 subunit is highly expressed at the non-infective and proliferative procyclic stage of the extracellular promastigotes, but is strongly down-regulated at the infective and non-proliferative metacyclic stage of the promastigotes and in the intramacrophage amastigotes (198,199). *LCB2* gene-*disrupted L. major* cells remain viable even without discernible sphingolipid metabolites at the procyclic stage (198,199). However, the *LCB2* gene-disrupted *Leishmania* can not differentiate into the infective metacyclic form: the mutant metacyclic form retains the ability to enter macrophages, but does not survive in macrophages (198,199). In the mutant *Leishmania* cells, glycoconjugates and membrane vesicles are abnormally accumulated, suggesting that membrane trafficking in *Leishmania* is affected by depletion of sphingolipids (198-200). The predominant sphingolipid metabolite in *Leishmania* is IPC, unlike in vertebrates (198,199). It has been reported that aureobasidin A, a natural inhibitor for fungal IPCS (see above), inhibited the synthesis of IPC in *T. cruzi* and impaired the in vitro differentiation of trypomastigotes (corresponding to the bloodstream form) to amastigotes (corresponding to the intracellular parasitic form) induced by acidic pH (201). The metabolism of IPC may be a novel target for anti-kinetoplastid protozoa treatment.

10. Conclusions and perspectives

Both sphingolipids and glycerolipids are ubiquitous constituents of membranes in eukaryotes. Nevertheless, besides their hydrophobic backbones, by which these two lipid groups are defined, there are various differences between them. Sphingolipids have a high level of diversity in their polar head groups and have more saturated fatty acyl chains, compared with glycerolipids. The subcellular localization of complex sphingolipids (preferentially distributed to the exoplasmic leaflet of the plasma membrane and the lysosomes) differs from that of glycerophospholipids, which are the predominant lipids in all organelles. In addition, expression patterns of GSLs are highly dependent on types and developmental stages of tissues in mammals. These distinct features of sphingolipids appear to make this lipid group relevant to various infectious diseases, as described in this review.

Various anti-bacterial drugs have been developed to date, based on the different mechanisms of DNA replication, RNA transcription, protein translation, and cell surface construction between prokaryotes and eukaryotes. By contrast, the repertoire of clinical drugs against eukaryotic pathogens remains poor, and more targets for anti-eukaryotic pathogens have been sought. The metabolism of lipids is attractive in this regard. Various lower eukaryotes produce various lipid types that are essential for their own survival but absent in mammals. Even if humans produce the same essential lipid, the metabolic pathways for this lipid sometimes differ from those in microbes.

Because various pathogens require sphingolipids of host cells for infection, modulating sphingolipids in host cells affects infection by the pathogens as summarized above. Thus, the modulation of sphingolipids of host cells would be an anti-

infectious disease treatment, if it does not have significant toxicity to humans. In this regard, it should also be noted that treatment of various human $CD4^+$ cell lines with exogenous long-chain ceramide or some pharmacological drugs, which up-regulate the level of endogenous ceramide, inhibits infection by various HIV-1 strains without severe cytotoxity in vitro (202). In addition, a previous study showed that treatment of mouse macrophage-like J774A.1 cells with various sphingolipids including ceramide, sphingosine, and SM resulted in a significant decrease in intracellular growth of infected *Mycobacterium tuberculosis* (203).

Finally, I would like to state that the importance of metabolite-oriented research should be re-evaluated especially in the field of infectious diseases. In the last decade, gene-oriented research has prevailed among various fields of biology, while the importance of the basic biochemistry of metabolism has tended to be underestimated. Although there is no doubt that genetics and genomics are powerful and productive tools, they will not provide us with any conclusive answers to questions about yet-uncharacterized metabolic reactions and metabolites. The sound development of molecular biology therefore will continue to require both genetic and biochemical tools, and probably other novel tools.

Additions in proofs

During preparing proofs of this review article, two papers that identified microbial ligands for CD1d-restricted iNKT cells appeared (208, 209). The Gram-negative bacteria *Sphingomonas* spp. have GSLs as cell envelope components in place of LPS (210,211). α-Linked glucuronosyl and galacturonosyl ceramides from *Sphingomonas* spp. were found to activate CD1d-restricted iNKT cells (208,209).

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REFERENCES

1. Karlsson, K. -A. (1970): On the chemistry and occurence of sphingolipid long-chain bases. Chem. Phys. Lipids, 5, 6-43.
2. Hakomori, S. (1983): Chemistry of glycosphingolipids. p. 1-165. *In* J. D. Kanfer and S. Hakomori (eds.), Sphingolipid Biochemistry. Plenum Press, New York, N.Y.
3. Hanada, K. (2003): Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. Biochim. Biophys. Acta, 1632, 16-30.
4. Dickson, R. C. (1998): Sphingolipid functions in *Saccharomyces cerevisiae*: comparison to mammalians. Annu. Rev. Biochem., 67, 27-48.
5. Acharya, U. and Acharya, J. K. (2005): Enzymes of sphingolipid metabolism in *Drosophila melanogaster*. Cell. Mol. Life Sci., 62, 128-142.
6. Hanada, K., Nishijima, M., Kiso, M., Hasegawa, A., Fujita, S., Ogawa, T. and Akamatsu, Y. (1992): Sphingolipids are essential for the growth of Chinese hamster ovary cells. Restoration of the growth of a mutant defective in sphingoid base biosynthesis by exogenous sphingolipids. J. Biol. Chem., 267, 23527-23533.
7. Adachi-Yamada, T., Gotoh, T., Sugimura, I., Tateno, M., Nishida, Y., Onuki, T. and Date, H. (1999): De novo synthesis of sphingolipids is required for cell survival by down-regulating c-Jun N-terminal kinase in *Drosophila* imaginal discs. Mol. Cell. Biol., 19, 7276-7286.
8. Pinto, W. J., Wells, G. W. and Lester, R. L. (1992): Characterization of enzymatic synthesis of sphingolipid long-chain bases in *Saccharomyces cerevisiae*: mutant strains exhibiting long-chain-base auxotrophy are deficient in serine palmitoyltransferase activity. J. Bacteriol., 174, 2575-2581.
9. Pettus, B. J., Chalfant, C. E. and Hannun, Y. A. (2002): Ceramide in apoptosis: an overview and current perspectives. Biochim. Biophys. Acta, 1585, 114-125.
10. Watterson, K. R., Ratz, P. H. and Spiegel, S. (2005): The role of sphingosine-1-phosphate in smooth muscle contraction. Cell Signal., 17, 289-298.
11. Proia, R. L. (2003): Glycosphingolipid functions: insights from engineered mouse models. Philos. Trans. R. Soc. Lond. B, 358, 879-883.
12. Furukawa, K., Tokuda, N., Okuda, T. and Tajima, O. (2004): Glycosphingolipids in engineered mice: insights into function. Semin. Cell Dev. Biol., 15, 389-396.
13. Simons, K. and Ikonen, E. (1997): Functional rafts in cell membranes. Nature, 387, 569-572.
14. Anderson, R. G. (1998): The caveolae membrane system. Ann. Rev. Biochem., 67, 199-225.
15. London, E. and Brown, D. A. (2000): Insolubility of lipids in Triton X-100: physical origin and relationship to sphingolipid/cholesterol membrane domains (rafts). Biochim. Biophys. Acta, 1508, 182-195.
16. Lucero, H. A. and Robbins, P. W. (2004): Lipid rafts-protein association and the regulation of protein activity. Arch. Biochem. Biophys., 426, 208-224.
17. Barenholz, Y. (2004): Sphingomyelin and cholesterol: from membrane biophysics and rafts to potential medical applications. Subcell. Biochem., 37, 167-215.
18. Kolter, T. and Sandhoff, K. (1999): Sphingolipids: their metabolic pathways and the pathobiochemistry of neurodegenerative diseases. Angew. Chem. Int. Ed., 38, 1532-1568.
19. Futerman, A. H. and van Meer, G. (2004): The cell biology of lysosomal storage disorders. Nat. Rev. Mol. Cell Biol., 5, 554-565.
20. Merritt, E. A., Zhang, Z., Pickens, J. C., Ahn, M., Hol, W. G. and Fan, E. (2002): Characterization and crystal structure of a high-affinity pentavalent receptor-binding inhibitor for cholera toxin and *E. coli* heat-labile enterotoxin. J. Am. Chem. Soc., 124, 8818-8824.
21. Lord, J. M. and Roberts, L. M. (1998): Toxin entry: retrograde transport through the secretory pathway. J. Cell Biol., 140, 733-736.
22. Sandvig, K. and van Deurs, B. (2000): Entry of ricin and Shiga toxin into cells: molecular mechanisms and medical perspectives. EMBO J., 19, 5943-5950.
23. Merritt, E. A., Sarfaty, S., van den Akker, F., L'Hoir, C., Martial, J. A. and Hol, W. G. (1994): Crystal structure of cholera toxin B-pentamer bound to receptor GM1 pentasaccharide. Protein Sci., 3, 166-175.
24. Zhang, R. G., Scott, D. L., Westbrook, M. L., Nance,

S., Spangler, B. D., Shipley, G. G. and Westbrook, E. M. (1995): The three-dimensional crystal structure of cholera toxin. J. Mol. Biol., 251, 563-573.
25. Zhang, R. G., Westbrook, M. L., Westbrook, E. M., Scott, D. L., Otwinowski, Z., Maulik, P. R., Reed, R. A. and Shipley, G. G. (1995): The 2.4 Å crystal structure of cholera toxin B subunit pentamer: choleragenoid. J. Mol. Biol., 251, 550-562.
26. Sixma, T. K., Kalk, K. H., van Zanten, B. A., Dauter, Z., Kingma, J., Witholt, B. and Hol, W. G. (1993): Refined structure of *Escherichia coli* heat-labile enterotoxin, a close relative of cholera toxin. J. Mol. Biol., 230, 890-918.
27. Van Heyningen, S. (1974): Cholera toxin: interaction of subunits with ganglioside GM1. Science, 183, 656-657.
28. Hirst, T. R., Fraser, S., Soriani, M., Aman, A. T., de, H. L., Hearn, A. and Merritt, E. (2002): New insights into the structure-function relationships and therapeutic applications of cholera-like enterotoxins. Int. J. Med. Microbiol., 291, 531-535.
29. Arduino, R. C. and DuPont, H. L. (1999): Entertis, enterocolitis and infectious diarrhea syndromes. p. 2.35.1-2.35.10. *In* D. Armstrong and J. Cohen (eds.), Infectious Diseases, Mosby, London, UK,
30. Lingwood, C. A. (1999): Glycolipid receptors for verotoxin and *Helicobacter pylori*: role in pathology. Biochim. Biophys. Acta, 1455, 375-386.
31. Jacewicz, M., Clausen, H., Nudelman, E., Donohue-Rolfe, A. and Keusch, G. T. (1986): Pathogenesis of shigella diarrhea. XI. Isolation of a shigella toxin-binding glycolipid from rabbit jejunum and HeLa cells and its identification as globotriaosylceramide. J Exp. Med., 163, 1391-1404.
32. Lingwood, C. A., Law, H., Richardson, S., Petric, M., Brunton, J. L., De Grandis, S. and Karmali, M. (1987): Glycolipid binding of purified and recombinant *Escherichia coli* produced verotoxin in vitro. J. Biol. Chem., 262, 8834-8839.
33. Waddell, T., Head, S., Petric, M., Cohen, A. and Lingwood, C. (1988): Globotriosyl ceramide is specifically recognized by the *Escherichia coli* verocytotoxin 2. Biochem. Biophys. Res. Commun., 152, 674-679.
34. Waddell, T., Cohen, A. and Lingwood, C. A. (1990): Induction of verotoxin sensitivity in receptor-deficient cell lines using the receptor glycolipid globotriosylceramide. Proc. Natl. Acad. Sci. USA, 87, 7898-7901.
35. Okuda, T., Tokuda, N., Ito, M., Ohta, M. and Furukawa, K. (2004): Alfa 1,4-galactosyltransferase gene knockout mice lack globo-series glycolipids and sensitivity to verotoxins. Seikagaku (Japan), 76, 1002 (Meeting Abstract).
36. Ergonul, Z., Clayton, F., Fogo, A. B. and Kohan, D. E. (2003): Shigatoxin-1 binding and receptor expression in human kidneys do not change with age. Pediatr. Nephrol., 18, 246-253.
37. Kiarash, A., Boyd, B. and Lingwood, C. A. (1994): Glycosphingolipid receptor function is modified by fatty acid content. Verotoxin 1 and verotoxin 2c preferentially recognize different globotriaosyl ceramide fatty acid homologues. J. Biol. Chem., 269, 11138-11146.
38. Kitov, P. I., Sadowska, J. M., Mulvey, G., Armstrong, G. D., Ling, H., Pannu, N. S., Read, R. J. and Bundle, D. R. (2000): Shiga-like toxins are neutralized by tailored multivalent carbohydrate ligands. Nature, 403, 669-672.
39. Nishikawa, K., Matsuoka, K., Kita, E., Okabe, N., Mizuguchi, M., Hino, K., Miyazawa, S., Yamasaki, C., Aoki, J., Takashima, S., Yamakawa, Y., Nishijima, M., Terunuma, D., Kuzuhara, H. and Natori, Y. (2002): A therapeutic agent with oriented carbohydrates for treatment of infections by Shiga toxin-producing *Escherichia coli* O157:H7. Proc. Natl. Acad. Sci. USA, 99, 7669-7674.
40. Pinyon, R. A., Paton, J. C., Paton, A. W., Botten, J. A. and Morona, R. (2004): Refinement of a therapeutic Shiga toxin-binding probiotic for human trials. J. Infect. Dis., 189, 1547-1555.
41. Simpson, L. L. and Rapport, M. M. (1971): The binding of botulinum toxin to membrane lipids: sphingolipids, steroids and fatty acids. J. Neurochem., 18, 1751-1759.
42. Kitamura, M., Iwamori, M. and Nagai, Y. (1980): Interaction between *Clostridium botulinum* neurotoxin and gangliosides. Biochim. Biophys. Acta, 628, 328-335.
43. Swaminathan, S. and Eswaramoorthy, S. (2000): Structural analysis of the catalytic and binding sites of *Clostridium botulinum* neurotoxin B. Nat. Struct. Biol., 7, 693-699.
44. Kitamura, M., Takamiya, K., Aizawa, S. and Furukawa, K. (1999): Gangliosides are the binding substances in neural cells for tetanus and botulinum toxins in mice. Biochim. Biophys. Acta, 1441, 1-3.
45. Spilsberg, B., Van Meer, G. and Sandvig, K. (2003): Role of lipids in the retrograde pathway of ricin intoxication. Traffic, 4, 544-552.
46. Alouf, J. E. and Jolivet-Reynaud, C. (1981): Purification and characterization of *Clostridium perfringens* delta-toxin. Infect. Immun., 31, 536-546.
47. Jolivet-Reynaud, C., Hauttecoeur, B. and Alouf, J. E. (1989): Interaction of *Clostridium perfringens* delta toxin with erythrocyte and liposome membranes and relation with the specific binding to the ganglioside GM2. Toxicon, 27, 1113-1126.
48. Harouse, J. M., Bhat, S., Spitalnik, S. L., Laughlin, M., Stefano, K., Silberberg, D. H. and Gonzalez-Scarano, F. (1991): Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide. Science, 253, 320-323.
49. Yahi, N., Baghdiguuian, S., Moreau, H. and Fantini, J. (1992): Galactosyl ceramide (or a closely related molecule) is the receptor for human immunodeficiency virus type 1 on human colon epithelial HT29 cells. J. Virol., 66, 4848-4854.
50. Bhat, S., Spitalnik, S. L., Gonzalez-Scarano, F. and Silberberg, D. H. (1991): Galactosyl ceramide or a derivative is an essential component of the neural receptor for human immunodeficiency virus type 1 envelope glycoprotein gp120. Proc. Natl. Acad. Sci. USA, 88, 7131-7134.
51. Cook, D. G., Fantini, J., L., S. S. and Gonzalez-Scarano, F. (1994): Binding of human immunodeficiency virus type I (HIV-1) gp120 to galactosylceramide (GalCer): relationship to the V3 loop. Virology, 201, 206-214.
52. Yahi, N., Sabatier, J. M., Baghdiguian, S., Gonzalez-Scarano, F. and Fantini, J. (1995): Synthetic multimeric peptides derived from the principal neutralization domain (V3 loop) of human immunodeficiency virus type 1 (HIV-1) gp120 bind to galactosylceramide and

block HIV-1 infection in a human CD4-negative mucosal epithelial cell line. J. Virol., 69, 320-325.
53. Fantini, J., Hammache, D., Delézay, O., Yahi, N., André-Barrés, C., Rico-Lattes, I. and Lattes, A. (1997): Synthetic soluble analogs of galactosylceramide (GalCer) bind to the V3 domain of HIV-1 gp120 and inhibit HIV-1-induced fusion and entry. J. Biol. Chem., 272, 7245-7252.
54. Kensinger, R. D., Catalone, B. J., Krebs, F. C., Wigdahl, B. and Schengrund, C. L. (2004): Novel polysulfated galactose-derivatized dendrimers as binding antagonists of human immunodeficiency virus type 1 infection. Antimicrob. Agents Chemother., 48, 1614-1623.
55. Delézay, O., Koch, N., Yahi, N., Hammache, D., Tourres, C., Tamalet, C. and Fantini, J. (1997): Co-expression of CXCR4/fusin and galactosylceramide in the human intestinal epithelial cell line HT-29. AIDS, 11, 1311-1318.
56. Bomsel, M. (1997): Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. Nat. Med., 3, 42-47.
57. Meng, G., Wei, X., Wu, X., Sellers, M. T., Decker, J. M., Moldoveanu, Z., Orenstein, J. M., Graham, M. F., Kappes, J. C., Mestecky, J., Shaw, G. M. and Smith, P. D. (2002): Primary intestinal epithelial cells selectively transfer R5 HIV-1 to CCR5+ cells. Nat. Med., 8, 150-156.
58. Alfsen, A., Iniguez, P., Bouguyon, E. and Bomsel, M. (2001): Secretory IgA specific for a conserved epitope on gp41 envelope glycoprotein inhibits epithelial transcytosis of HIV-1. J. Immunol., 166, 6257-6265.
59. Lakadamyali, M., Rust, M. J. and Zhuang, X. (2004): Endocytosis of influenza viruses. Microbes Infect., 6, 929-936.
60. Suzuki, Y. (1994): Gangliosides as influenza virus receptors. Variation of influenza viruses and their recognition of the receptor sialo-sugar chains. Prog. Lipid Res., 33, 429-457.
61. Weis, W., Brown, J. H., Cusack, S., Paulson, J. C., Skehel, J. J. and Wiley, D. C. (1988): Structure of the influenza virus haemagglutinin complexed with its receptor, sialic acid. Nature, 333, 426-431.
62. Bergelson, L. D., Bukrinskaya, A. G., Prokazova, N. V., Shaposhnikova, G. I., Kocharov, S. L., Shevchenko, V. P., Kornilaeva, G. V. and Fomina-Ageeva, E. V. (1982): Role of gangliosides in reception of influenza virus. Eur. J. Biochem., 128, 467-474.
63. Ablan, S., Rawat, S. S., Blumenthal, R. and Puri, A. (2001): Entry of influenza virus into a glycosphingolipid-deficient mouse skin fibroblast cell line. Arch. Virol., 146, 2227-2238.
64. Tsai, B., Gilbert, J. M., Stehle, T., Lencer, W., Benjamin, T. L. and Rapoport, T. A. (2003): Gangliosides are receptors for murine polyoma virus and SV40. EMBO J., 22, 4346-4355.
65. Smith, A. E., Lilie, H. and Helenius, A. (2003): Ganglioside-dependent cell attachment and endocytosis of murine polyomavirus-like particles. FEBS Lett., 555, 199-203.
66. Bomsel, M. and Alfsen, A. (2003): Entry of viruses through the epithelial barrier: pathogenic trickery. Nat. Rev. Mol. Cell. Biol., 4, 57-68.
67. Smith, A. E. and Helenius, A. (2004): How viruses enter animal cells. Science, 304, 237-242.
68. Chambers, S. T. (1999): Cystitis and urethral syndromes. p. 2.57.1-2.57.8. *In* D. Armstrong and J. Cohen (eds.), Infectious Diseases, Mosby, London, UK .
69. Johnson, J. R. (1999): Pyelonephritis and abscess of the kidney. p. 2.59.1-2.59.8. *In* D. Armstrong and J. Cohen (eds.), Infectious Diseases, Mosby, London, UK.
70. Kallenius, G., Mollby, R., Svenson, S. B., Winberg, J. and Hultberg, H. (1980): Identification of a carbohydrate receptor recognized by uropathogenic *Escherichia coli*. Infection, 8 Suppl. 3, 288-293.
71. Leffler, H. and Svanborg-Edén, C. (1981): Glycolipid receptors for uropathogenic *Escherichia coli* on human erythrocytes and uroepithelial cells. Infect. Immun., 34, 920-929.
72. Hultgren, S. J., Lindberg, F., Magnusson, G., Kihlberg, J., Tennent, J. M. and Normark, S. (1989): The PapG adhesin of uropathogenic *Escherichia coli* contains separate regions for receptor binding and for the incorporation into the pilus. Proc. Natl. Acad. Sci. USA, 86, 4357-4361.
73. Hoschützky, H., Lottspeich, F. and Jann, K. (1989): Isolation and characterization of the α-galactosyl-1,4-β-galactosyl-specific adhesin (P adhesin) from fimbriated *Escherichia coli*. Infect. Immun., 57, 76-81.
74. Dodson, K. W., Pinkner, J. S., Rose, T., Magnusson, G., Hultgren, S. J. and Waksman, G. (2001): Structural basis of the interaction of the pyelonephritic *E. coli* adhesin to its human kidney receptor. Cell, 105, 733-743.
75. Svensson, M., Frendeus, B., Butters, T., Platt, F., Dwek, R. and Svanborg, C. (2003): Glycolipid depletion in antimicrobial therapy. Mol. Microbiol., 47, 453-461.
76. Siitonen, A., Martikainen, R., Ikaheimo, R., Palmgren, J. and Makela, P. H. (1993): Virulence-associated characteristics of *Escherichia coli* in urinary tract infection: a statistical analysis with special attention to type 1C fimbriation. Microb. Pathog., 15, 65-75.
77. Khan, A. S., Kniep, B., Oelschlaeger, T. A., Van Die, I., Korhonen, T. and Hacker, J. (2000): Receptor structure for F1C fimbriae of uropathogenic *Escherichia coli*. Infect. Immun., 68, 3541-3547.
78. Backhed, F., Alsen, B., Roche, N., Angstrom, J., von Euler, A., Breimer, M. E., Westerlund-Wikstrom, B., Teneberg, S. and Richter-Dahlfors, A. (2002): Identification of target tissue glycosphingolipid receptors for uropathogenic, F1C-fimbriated *Escherichia coli* and its role in mucosal inflammation. J. Biol. Chem., 277, 18198-18205.
79. Leib, S. L. and Tauber, M. G. (1999): Acute and choroic meningitis. p. 15.1-2.15.12. *In* D. Armstrong and J. Cohen (eds.), Infectious Diseases, Mosby, London, UK.
80. Moch, T., Hoschützky, H., Hacker, J., Kröncke, K. D. and Jann, K. (1987): Isolation and characterization of the α-sialyl-β-2,3-galactosyl-specific adhesin from fimbriated *Escherichia coli*. Proc. Natl. Acad. Sci. USA, 84, 3462-3466.
81. Parkkinen, J., Korhonen, T. K., Pere, A., Hacker, J. and Soinila, S. (1988): Binding sites in the rat brain for *Escherichia coli* S fimbriae associated with neonatal meningitis. J. Clin. Invest., 81, 860-865.
82. Prasadarao, N. V., Wass, C. A., Hacker, J., Jann, K. and Kim, K. S. (1993): Adhesion of S-fimbriated *Escherichia coli* to brain glycolipids mediated by sfaA gene-encoded protein of S-fimbriae. J. Biol. Chem., 268, 10356-10363.

83. Yankaskas, J. R. (1999): Bronchitis, bronchiectasis and cystic fibrosis. p. 2.26.1-26.8. *In* D. Armstrong and J. Cohen (eds.), Infectious Diseases, Mosby, London, UK.
84. Krivan, H. C., Ginsburg, V. and Roberts, D. D. (1988): *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from cystic fibrosis patients bind specifically to gangliotetraosylceramide (asialo GM1) and gangliotriaosylceramide (asialo GM2). Arch. Biochem. Biophys., 260, 493-496.
85. Krivan, H. C., Roberts, D. D. and Ginsburg, V. (1988): Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNAc beta 1-4Gal found in some glycolipids. Proc. Natl. Acad. Sci. USA, 85, 6157-6161.
86. Feldman, M., Bryan, R., Rajan, S., Scheffler, L., Brunnert, S., Tang, H. and Prince, A. (1998): Role of flagella in pathogenesis of *Pseudomonas aeruginosa* pulmonary infection. Infect. Immun., 66, 43-51.
87. Brennan, M. J., Hannah, J. H. and Leininger, E. (1991): Adhesion of *Bordetella pertussis* to sulfatides and to the GalNAc beta 4Gal sequence found in glycosphingolipids. J. Biol. Chem., 266, 18827-18831.
88. Roberts, D. D., Olson, L. D., Barile, M. F., Ginsburg, V. and Krivan, H. C. (1989): Sialic acid-dependent adhesion of *Mycoplasma pneumoniae* to purified glycoproteins. J. Biol. Chem., 264, 9289-9293.
89. Krivan, H. C., Olson, L. D., Barile, M. F., Ginsburg, V. and Roberts, D. D. (1989): Adhesion of *Mycoplasma pneumoniae* to sulfated glycolipids and inhibition by dextran sulfate. J. Biol. Chem., 264, 9283-9288.
90. Tang, W., Seino, K., Ito, M., Konishi, T., Senda, H., Makuuchi, M., Kojima, N. and Mizuochi, T. (2001): Requirement of ceramide for adhesion of *Helicobacter pylori* to glycosphingolipids. FEBS Lett., 504, 31-35.
91. Teneberg, S., Leonardsson, I., Karlsson, H., Jovall, P. A., Angstrom, J., Danielsson, D., Naslund, I., Ljungh, A., Wadstrom, T. and Karlsson, K. A. (2002): Lactotetraosylceramide, a novel glycosphingolipid receptor for *Helicobacter pylori*, present in human gastric epithelium. J. Biol. Chem., 277, 19709-19719.
92. Roche, N., Angstrom, J., Hurtig, M., Larsson, T., Boren, T. and Teneberg, S. (2004): *Helicobacter pylori* and complex gangliosides. Infect. Immun., 72, 1519-1529.
93. Boren, T., Falk, P., Roth, K. A., Larson, G. and Normark, S. (1993): Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science, 262, 1892-1895.
94. Mahdavi, J., Sonden, B., Hurtig, M., Olfat, F. O., Forsberg, L., Roche, N., Angstrom, J., Larsson, T., Teneberg, S., Karlsson, K. A., Altraja, S., Wadstrom, T., Kersulyte, D., Berg, D. E., Dubois, A., Petersson, C., Magnusson, K. E., Norberg, T., Lindh, F., Lundskog, B. B., Arnqvist, A., Hammarstrom, L. and Boren, T. (2002): *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. Science, 297, 573-578.
95. Yu, L., Lee, K. K., Sheth, H. B., Lane-Bell, P., Srivastava, G., Hindsgaul, O., Paranchych, W., Hodges, R. S. and Irvin, R. T. (1994): Fimbria-mediated adherence of *Candida albicans* to glycosphingolipid receptors on human buccal epithelial cells. Infect. Immun., 62, 2843-2848.
96. Cameron, B. J. and Douglas, L. J. (1996): Blood group glycolipids as epithelial cell receptors for *Candida albicans*. Infect. Immun., 64, 891-896.
97. Mangeney, M., Lingwood, C. A., Taga, S., Caillou, B., Tursz, T. and Wiels, J. (1993): Apoptosis induced in Burkitt's lymphoma cells via Gb3/CD77, a glycolipid antigen. Cancer Res., 53, 5314-5319.
98. Taga, S., Carlier, K., Mishal, Z., Capoulade, C., Mangeney, M., Lécluse, Y., Coulaud, D., Tétaud, C., Pritchard, L. L., Tursz, T. and Wiels, J. (1997): Intracellular signaling events in CD77-mediated apoptosis of Burkitt's lymphoma cells. Blood, 90, 2757-2767.
99. Katagiri, Y. U., Mori, T., Nakajima, H., Katagiri, C., Taguchi, T., Takeda, T., Kiyokawa, N. and Fujimoto, J. (1999): Activation of Src family kinase yes induced by Shiga toxin binding to globotriaosyl ceramide (Gb3/CD77) in low density, detergent-insoluble microdomains. J. Biol. Chem., 274, 35278-35282.
100. Tétaud, C., Falguiéres, T., Carlier, K., Lécluse, Y., Garibal, J., Coulaud, D., Busson, P., Steffensen, R., Clausen, H., Johannes, L. and Wiels, J. (2003): Two distinct Gb3/CD77 signaling pathways leading to apoptosis are triggered by anti-Gb3/CD77 mAb and verotoxin-1. J. Biol. Chem., 278, 45200-45208.
101. Spangler, B. D. (1992): Structure and function of cholera toxin and the related *Escherichia coli* heat-labile enterotoxin. Microbiol. Rev., 56, 622-647.
102. Plant, A. and Williams, N. A. (2004): Modulation of the immune response by the cholera-like enterotoxins. Curr. Top. Med. Chem., 4, 509-519.
103. Kawamura, Y. I., Kawashima, R., Shirai, Y., Kato, R., Hamabata, T., Yamamoto, M., Furukawa, K., Fujihashi, K., McGhee, J. R., Hayashi, H. and Dohi, T. (2003): Cholera toxin activates dendritic cells through dependence on GM1-ganglioside which is mediated by NF-kappaB translocation. Eur. J. Immunol., 33, 3205-3212.
104. George-Chandy, A., Eriksson, K., Lebens, M., Nordström, I., Schön, E. and Holmgren, J. (2001): Cholera toxin B subunit as a carrier molecule promotes antigen presentation and increases CD40 and CD86 expression on antigen-presenting cells. Infect. Immun., 69, 5716-5725.
105. McNamara, N., Khong, A., McKemy, D., Caterina, M., Boyer, J., Julius, D. and Basbaum, C. (2001): ATP transduces signals from ASGM1, a glycolipid that functions as a bacterial receptor. Proc. Natl. Acad. Sci. USA, 98, 9086-9091.
106. Grassme, H., Gulbins, E., Brenner, B., Ferlinz, K., Sandhoff, K., Harzer, K., Lang, F. and Meyer, T. F. (1997): Acidic sphingomyelinase mediates entry of *N. gonorrhoeae* into nonphagocytic cells. Cell, 91, 605-615.
107. Hauck, C. R., Grassme, H., Bock, J., Jendrossek, V., Ferlinz, K., Meyer, T. F. and Gulbins, E. (2000): Acid sphingomyelinase is involved in CEACAM receptor-mediated phagocytosis of *Neisseria gonorrhoeae*. FEBS Lett., 478, 260-266.
108. Grassmé, H., Jendrossek, V., Riehle, A., Von Kürthy, G., Berger, J., Schwarz, H., Weller, M., Kolesnick, R. and Gulbins, E. (2003): Host defense against *Pseudomonas aeruginosa* requires ceramide-rich membrane rafts. Nat. Med., 9, 322-330.
109. Hedlund, M., Svensson, M., Nilsson, A., Duan, R. D. and Svanborg, C. (1996): Role of the ceramide-signaling pathway in cytokine responses to P-fimbriated *Escherichia coli*. J. Exp. Med., 183, 1037-1044.
110. Hedlund, M., Duan, R. D., Nilsson, A. and Svanborg,

C. (1998): Sphingomyelin, glycosphingolipids and ceramide signalling in cells exposed to P-fimbriated *Escherichia coli*. Mol. Microbiol., 29, 1297-1306.
111. Ghosh, S., Bhattacharyya, S., Das, S., Raha, S., Maulik, N., Das, D. K., Roy, S. and Majumdar, S. (2001): Generation of ceramide in murine macrophages infected with *Leishmania donovani* alters macrophage signaling events and aids intracellular parasitic survival. Mol. Cell. Biochem., 223, 47-60.
112. Ghosh, S., Bhattacharyya, S., Sirkar, M., Sa, G. S., Das, T., Majumdar, D., Roy, S. and Majumdar, S. (2002): *Leishmania donovani* suppresses activated protein 1 and NF-κB activation in host macrophages via ceramide generation: involvement of extracellular signal-regulated kinase. Infect. Immun., 70, 6828-6838.
113. Palsson-McDermott, E. M. and O'Neill, L. A. (2004): Signal transduction by the lipopolysaccharide receptor, Toll-like receptor-4. Immunology, 113, 153-162.
114. Miyake, K. (2004): Endotoxin recognition molecules, Toll-like receptor 4-MD-2. Semin. Immunol., 16, 11-16.
115. Beutler, B. (2002): TLR4 as the mammalian endotoxin sensor. Curr. Top. Microbiol. Immunol., 270, 109-120.
116. Frendeus, B., Wachtler, C., Hedlund, M., Fischer, H., Samuelsson, P., Svensson, M. and Svanborg, C. (2001): *Escherichia coli P* fimbriae utilize the Toll-like receptor 4 pathway for cell activation. Mol. Microbiol., 40, 37-51.
117. Hedlund, M., Frendeus, B., Wachtler, C., Hang, L., Fischer, H. and Svanborg, C. (2001): Type 1 fimbriae deliver an LPS- and TLR4-dependent activation signal to CD14-negative cells. Mol. Microbiol., 39, 542-552.
118. Bergsten, G., Samuelsson, M., Wullt, B., Leijonhufvud, I., Fischer, H. and Svanborg, C. (2004): PapG-dependent adherence breaks mucosal inertia and triggers the innate host response. J. Infect. Dis., 189, 1734-1742.
119. Kusumi, A., Koyama-Honda, I. and Suzuki, K. (2004): Molecular dynamics and interactions for creation of stimulation-induced stabilized rafts from small unstable steady-state rafts. Traffic, 5, 213-230.
120. Mayor, S. and Rao, M. (2004): Rafts: scale-dependent, active lipid organization at the cell surface. Traffic, 5, 231-240.
121. Simons, K. and Toomre, D. (2000): Lipid rafts and signal transduction. Nat. Rev. Mol. Cell Biol., 1, 31-39.
122. Cohen, A. W., Hnasko, R., Schubert, W. and Lisanti, M. P. (2004): Role of caveolae and caveolins in health and disease. Physiol. Rev., 84, 1341-1379.
123. Duncan, M. J., Shin, J. S. and Abraham, S. N. (2002): Microbial entry through caveolae: variations on a theme. Cell. Microbiol., 4, 783-791.
124. Lafont, F., Abrami, L. and van der Goot, F. G. (2004): Bacterial subversion of lipid rafts. Curr. Opin. Microbiol., 7, 4-10.
125. Rawat, S. S., Viard, M., Gallo, S. A., Rein, A., Blumenthal, R. and Puri, A. (2003): Modulation of entry of enveloped viruses by cholesterol and sphingolipids. Mol. Membr. Biol., 20, 243-254.
126. Palmer, M. (2004): Cholesterol and the activity of bacterial toxins. FEMS Microbiol. Lett., 238, 281-289.
127. Prusiner, S. B. (2001): Shattuck lecture–neurodegenerative diseases and prions. N. Engl. J. Med., 344, 1516-1526.
128. Naslavsky, N., Stein, R., Yanai, A., Friedlander, G. and Taraboulos, A. (1997): Characterization of detergent-insoluble complexes containing the cellular prion protein and its scrapie isoform. J. Biol. Chem., 272, 6324-6331.
129. Naslavsky, N., Shmeeda, H., Friedlander, G., Yanai, A., Futerman, A. H., Barenholz, Y. and Taraboulos, A. (1999): Sphingolipid depletion increases formation of the scrapie prion protein in neuroblastoma cells infected with prions. J. Biol. Chem., 274, 20763-20771.
130. Taraboulos, A., Scott, M., Semenov, A., Avrahami, D., Laszlo, L. and Prusiner, S. B. (1995): Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform. J. Cell Biol., 129, 121-132.
131. Hanada, K., Izawa, K., Nishijima, M. and Akamatsu, Y. (1993): Sphingolipid deficiency induces hypersensitivity of CD14, a glycosyl phosphatidylinositol-anchored protein, to phosphatidylinositol-specific phospholipase C. J. Biol. Chem., 268, 13820-13823.
132. Klein, T. R., Kirsch, D., Kaufmann, R. and Riesner, D. (1998): Prion rods contain small amounts of two host sphingolipids as revealed by thin-layer chromatography and mass spectrometry. Biol. Chem., 379, 655-666.
133. Mahfoud, R., Garmy, N., Maresca, M., Yahi, N., Puigserver, A. and Fantini, J. (2002): Identification of a common sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins. J. Biol. Chem., 277, 11292-11296.
134. Yanagisawa, K., Odaka, A., Suzuki, N. and Ihara, Y. (1995): GM1 ganglioside-bound amyloid β-protein (Aβ): a possible form of preamyloid in Alzheimer's disease. Nat. Med., 1, 1062-1066.
135. Kakio, A., Nishimoto, S., Yanagisawa, K., Kozutsumi, Y. and Matsuzaki, K. (2002): Interactions of amyloid β-protein with various gangliosides in raft-like membranes: importance of GM1 ganglioside-bound form as an endogenous seed for Alzheimer amyloid. Biochemistry, 41, 7385-7390.
136. Puglielli, L., Ellis, B. C., Saunders, A. J. and Kovacs, D. M. (2003): Ceramide stabilizes β-site amyloid precursor protein-cleaving enzyme 1 and promotes amyloid β-peptide biogenesis. J. Biol. Chem., 278, 19777-19783.
137. Sawamura, N., Ko, M., Yu, W., Zou, K., Hanada, K., Suzuki, T., Gong, J. S., Yanagisawa, K. and Michikawa, M. (2004): Modulation of amyloid precursor protein cleavage by cellular sphingolipids. J. Biol. Chem., 279, 11984-11991.
138. Abrami, L., Liu, S., Cosson, P., Leppla, S. H. and van der Goot, F. G. (2003): Anthrax toxin triggers endocytosis of its receptor via a lipid raft-mediated clathrin-dependent process. J. Cell Biol., 160, 321-328.
139. Saikku, P. A. (1999): *Chlamydia*. p. 25.1-25.8. *In* D. Armstrong and J. Cohen (eds.), Infectious Diseases, Mosby, London, UK.
140. Norkin, L. C., Wolfrom, S. A. and Stuart, E. S. (2001): Association of caveolin with *Chlamydia trachomatis* inclusions at early and late stages of infection. Exp. Cell Res., 266, 229-238.
141. Stuart, E. S., Webley, W. C. and Norkin, L. C. (2003): Lipid rafts, caveolae, caveolin-1, and entry by *Chlamydiae* into host cells. Exp. Cell Res., 287, 67-78.
142. Carabeo, R. A., Mead, D. J. and Hackstadt, T. (2003): Golgi-dependent transport of cholesterol to the *Chlamydia trachomatis* inclusion. Proc. Natl. Acad. Sci. USA,

100, 6771-6776.
143. van Ooij, C., Kalman, L., van, I., Nishijima, M., Hanada, K., Mostov, K. and Engel, J. N. (2000): Host cell-derived sphingolipids are required for the intracellular growth of *Chlamydia trachomatis*. Cell. Microbiol., 2, 627-637.
144. Lafont, F., Tran Van Nhieu, G., Hanada, K., Sansonetti, P. and van der Goot, F. G. (2002): Initial steps of *Shigella* infection depend on the cholesterol/sphingolipid raft-mediated CD44-IpaB interaction. EMBO J., 21, 4449-4457.
145. van der Goot, F. G., Tran van Nhieu, G., Allaoui, A., Sansonetti, P. and Lafont, F. (2004): Rafts can trigger contact-mediated secretion of bacterial effectors via a lipid-based mechanism. J. Biol. Chem., 279, 47792-47798.
146. Duncan, M. J., Li, G., Shin, J. S., Carson, J. L. and Abraham, S. N. (2004): Bacterial penetration of bladder epithelium through lipid rafts. J. Biol. Chem., 279, 18944-18951.
147. Collier, R. J. (2001): Understanding the mode of action of diphtheria toxin: a perspective on progress during the 20th century. Toxicon, 39, 1793-1803.
148. Spilsberg, B., Hanada, K. and Sandvig, K. (2005): Diphtheria toxin translocation across cellular membranes is regulated by sphingolipids. Biochem. Biophys. Res. Commun., 329, 465-473.
149. Sharpe, J. C. and London, E. (1999): Diphtheria toxin forms pores of different sizes depending on its concentration in membranes: probable relationship to oligomerization. J. Membr. Biol, 171, 209-221.
150. Strauss, J. H. and Strauss, E. G. (1994): The alphaviruses: gene expression, replication, and evolution. Microbiol. Rev., 58, 491-562.
151. Kielian, M. C. and Helenius, A. (1984): Role of cholesterol in fusion of Semliki Forest virus with membranes. J. Virol., 52, 281-283.
152. Phalen, T. and Kielian, M. (1991): Cholesterol is required for infection by Semliki Forest virus. J. Cell Biol., 112, 615-623.
153. Nieva, J. L., Bron, R., Corver, J. and Wilschut, J. (1994): Membrane fusion of Semliki Forest virus requires sphingolipids in the target membrane. EMBO J., 13, 2797-2804.
154. Klimjack, M. R., Jeffrey, S. and Kielian, M. (1994): Membrane and protein interactions of a soluble form of the Semliki Forest virus fusion protein. J. Virol., 68, 6940-6946.
155. Ahn, A., Gibbons, D. L. and Kielian, M. (2002): The fusion peptide of Semliki Forest virus associates with sterol-rich membrane domains. J Virol., 76, 3267-3275.
156. Waarts, B. L., Bittman, R. and Wilschut, J. (2002): Sphingolipid and cholesterol dependence of alphavirus membrane fusion. Lack of correlation with lipid raft formation in target liposomes. J. Biol. Chem., 277, 38141-38147.
157. Chatterjee, P. K., Eng, C. H. and Kielian, M. (2002): Novel mutations that control the sphingolipid and cholesterol dependence of the Semliki Forest virus fusion protein. J. Virol., 76, 12712-12722.
158. Moesby, L., Corver, J., Erukulla, R. K., Bittman, R. and Wilschut, J. (1995): Sphingolipids activate membrane fusion of Semliki Forest virus in a stereospecific manner. Biochemistry, 34, 10319-10324.
159. Samsonov, A. V., Chatterjee, P. K., Razinkov, V. I., Eng, C. H., Kielian, M. and Cohen, F. S. (2002): Effects of membrane potential and sphingolipid structures on fusion of Semliki Forest virus. J. Virol., 76, 12691-12702.
160. Schwerer, B. (2002): Antibodies against gangliosides: a link between preceding infection and immunopathogenesis of Guillain-Barré syndrome. Microbes Infect., 4, 373-384.
161. Yuki, N. (2001): Infectious origins of, and molecular mimicry in, Guillain-Barré and Fisher syndromes. Lancet Infect. Dis., 1, 29-37.
162. Willison, H. J. and O'Hanlon, G. M. (1999): The immunopathogenesis of Miller Fisher syndrome. J. Neuroimmunol., 100, 3-12.
163. Yuki, N., Taki, T., Inagaki, F., Kasama, T., Takahashi, M., Saito, K., Handa, S. and Miyatake, T. (1993): A bacterium lipopolysaccharide that elicits Guillain-Barré syndrome has a GM1 ganglioside-like structure. J. Exp. Med., 178, 1771-1775.
164. Yuki, N., Susuki, K., Koga, M., Nishimoto, Y., Odaka, M., Hirata, K., Taguchi, K., Miyatake, T., Furukawa, K., Kobata, T. and Yamada, M. (2004): Carbohydrate mimicry between human ganglioside GM1 and *Campylobacter jejuni* lipooligosaccharide causes Guillain-Barré syndrome. Proc. Natl. Acad. Sci. USA, 101, 11404-11409.
165. Irie, S., Saito, T., Nakamura, K., Kanazawa, N., Ogino, M., Nukazawa, T., Ito, H., Tamai, Y. and Kowa, H. (1996): Association of anti-GM2 antibodies in Guillain-Barré syndrome with acute cytomegalovirus infection. J. Neuroimmunol., 68, 19-26.
166. Yuki, N. and Tagawa, Y. (1998): Acute cytomegalovirus infection and IgM anti-GM2 antibody. J. Neurol. Sci., 154, 14-17.
167. Khalili-Shirazi, A., Gregson, N., Gray, I., Rees, J., Winer, J. and Hughes, R. (1999): Antiganglioside antibodies in Guillain-Barre syndrome after a recent cytomegalovirus infection. J. Neurol. Neurosurg. Psychiatry, 66, 376-379.
168. Ang, C. W., Jacobs, B. C., Brandenburg, A. H., Laman, J. D., van der Meche, F. G., Osterhaus, A. D. and van Doorn, P. A. (2000): Cross-reactive antibodies against GM2 and CMV-infected fibroblasts in Guillain-Barre syndrome. Neurology, 54, 1453-1458.
169. Chiba, A., Kusunoki, S., Obata, H., Machinami, R. and Kanazawa, I. (1993): Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barre syndrome: clinical and immunohistochemical studies. Neurology, 43, 1911-1917.
170. Yuki, N., Taki, T., Takahashi, M., Saito, K., Yoshino, H., Tai, T., Handa, S. and Miyatake, T. (1994): Molecular mimicry between GQ1b ganglioside and lipopolysaccharides of *Campylobacter jejuni* isolated from patients with Fisher's syndrome. Ann. Neurol., 36, 791-793.
171. Chiba, A., Kusunoki, S., Obata, H., Machinami, R. and Kanazawa, I. (1997): Ganglioside composition of the human cranial nerves, with special reference to pathophysiology of Miller Fisher syndrome. Brain Res., 745, 32-36.
172. Brigl, M. and Brenner, M. B. (2004): CD1: antigen presentation and T cell function. Annu. Rev. Immunol.,

22, 817-890.
173. Godfrey, D. I. and Kronenberg, M. (2004): Going both ways: immune regulation via CD1d-dependent NKT cells. J. Clin. Invest., 114, 1379-1388.
174. Kawano, T., Cui, J., Koezuka, Y., Toura, I., Kaneko, Y., Motoki, K., Ueno, H., Nakagawa, R., Sato, H., Kondo, E., Koseki, H. and Taniguchi, M. (1997): CD1d-restricted and TCR-mediated activation of Vα14 NKT cells by glycosylceramides. Science, 278, 1626-1629.
175. Zhou, D., Mattner, J., Cantu, C., 3rd, Schrantz, N., Yin, N., Gao, Y., Sagiv, Y., Hudspeth, K., Wu, Y. P., Yamashita, T., Teneberg, S., Wang, D., Proia, R. L., Levery, S. B., Savage, P. B., Teyton, L. and Bendelac, A. (2004): Lysosomal glycosphingolipid recognition by NKT cells. Science, 306, 1786-1789.
176. Zhou, D., Cantu, C., 3rd, Sagiv, Y., Schrantz, N., Kulkarni, A. B., Qi, X., Mahuran, D. J., Morales, C. R., Grabowski, G. A., Benlagha, K., Savage, P., Bendelac, A. and Teyton, L. (2004): Editing of CD1d-bound lipid antigens by endosomal lipid transfer proteins. Science, 303, 523-527.
177. Stanic, A. K., De Silva, A. D., Park, J. J., Sriram, V., Ichikawa, S., Hirabyashi, Y., Hayakawa, K., Van Kaer, L., Brutkiewicz, R. R. and Joyce, S. (2003): Defective presentation of the CD1d1-restricted natural Va14Ja18 NKT lymphocyte antigen caused by β-D-glucosylceramide synthase deficiency. Proc. Natl. Acad. Sci. USA, 100, 1849-1854.
178. Wu, D. Y., Segal, N. H., Sidobre, S., Kronenberg, M. and Chapman, P. B. (2003): Cross-presentation of disialoganglioside GD3 to natural killer T cells. J. Exp. Med., 198, 173-181.
179. Naidenko, O. V., Maher, J. K., Ernst, W. A., Sakai, T., Modlin, R. L. and Kronenberg, M. (1999): Binding and antigen presentation of ceramide-containing glycolipids by soluble mouse and human CD1d molecules. J. Exp. Med., 190, 1069-1080.
180. Gumperz, J. E., Roy, C., Makowska, A., Lum, D., Sugita, M., Podrebarac, T., Koezuka, Y., Porcelli, S. A., Cardell, S., Brenner, M. B. and Behar, S. M. (2000): Murine CD1d-restricted T cell recognition of cellular lipids. Immunity, 12, 211-221.
181. Joyce, S., Woods, A. S., Yewdell, J. W., Bennink, J. R., De Silva, A. D., Boesteanu, A., Balk, S. P., Cotter, R. J. and Brutkiewicz, R. R. (1998): Natural ligand of mouse CD1d1: cellular glycosylphosphatidylinositol. Science, 279, 1541-1544.
182. Schofield, L., McConville, M. J., Hansen, D., Campbell, A. S., Fraser-Reid, B., Grusby, M. J. and Tachado, S. D. (1999): CD1d-restricted immunoglobulin G formation to GPI-anchored antigens mediated by NKT cells. Science, 283, 225-229.
183. Nagiec, M. M., Nagiec, E. E., Baltisberger, J. A., Wells, G. B., Lester, R. L. and Dickson, R. C. (1997): Sphingolipid synthesis as a target for antifungal drugs. Complementation of the inositol phosphorylceramide synthase defect in a mutant strain of *Saccharomyces cerevisiae* by the *AUR1* gene. J. Biol. Chem., 272, 9809-9817.
184. Beeler, T. J., Fu, D., Rivera, J., Monaghan, E., Gable, K. and Dunn, T. M. (1997): *SUR1* (*CSG1*/*BCL21*), a gene necessary for growth of *Saccharomyces cerevisiae* in the presence of high Ca^{2+} concentrations at 37°C, is required for mannosylation of inositolphosphorylceramide. Mol. Gen. Genet., 255, 570-579.
185. Dickson, R. C., Nagiec, E. E., Wells, G. B., Nagiec, M. M. and Lester, R. L. (1997): Synthesis of mannose-(inositol-P)2-ceramide, the major sphingolipid in *Saccharomyces cerevisiae*, requires the *IPT1* (*YDR072c*) gene. J. Biol. Chem., 272, 29620-29625.
186. Cheng, J., Park, T. S., Fischl, A. S. and Ye, X. S. (2001): Cell cycle progression and cell polarity require sphingolipid biosynthesis in *Aspergillus nidulans*. Mol. Cell. Biol., 21, 6198-6209.
187. Mandala, S. M., Thornton, R. A., Rosenbach, M., Milligan, J., Garcia-Calvo, M., Bull, H. G. and Kurtz, M. B. (1997): Khafrefungin, a novel inhibitor of sphingolipid synthesis. J. Biol. Chem., 272, 32709-32714.
188. Mandala, S. M., Thornton, R. A., Milligan, J., Rosenbach, M., Garcia-Calvo, M., Bull, H. G., Harris, G., Abruzzo, G. K., Flattery, A. M., Gill, C. J., Bartizal, K., Dreikorn, S. and Kurtz, M. B. (1998): Rustmicin, a potent antifungal agent, inhibits sphingolipid synthesis at inositol phosphoceramide synthase. J. Biol. Chem., 273, 14942-14949.
189. Takesako, K., Kuroda, H., Inoue, T., Haruna, F., Yoshikawa, Y., Kato, I., Uchida, K., Hiratani, T. and Yamaguchi, H. (1993): Biological properties of aureobasidin A, a cyclic depsipeptide antifungal antibiotic. J. Antibiot. (Tokyo), 46, 1414-1420.
190. Takatsu, T., Nakayama, H., Shimazu, A., Furihata, K., Ikeda, K., Seto, H. and Otake, N. (1985): Rustmicin, a new macrolide antibiotic active against wheat stem rust fungus. J. Antibiot. (Tokyo), 38, 1806-1809.
191. Sugimoto, Y., Sakoh, H. and Yamada, K. (2004): IPC synthase as a useful target for antifungal drugs. Curr. Drug Targets Infect. Disord., 4, 311-322.
192. Zhong, W., Jeffries, M. W. and Georgopapadakou, N. H. (2000): Inhibition of inositol phosphorylceramide synthase by aureobasidin A in *Candida* and *Aspergillus* species. Antimicrob. Agents. Chemother., 44, 651-653.
193. Luberto, C., Toffaletti, D. L., Wills, E. A., Tucker, S. C., Casadevall, A., Perfect, J. R., Hannun, Y. A. and Del Poeta, M. M. (2001): Roles for inositol-phosphoryl ceramide synthase 1 (IPC1) in pathogenesis of *C. neoformans*. Genes Dev., 15, 201-212.
194. Jomaa, H., Wiesner, J., Sanderbrand, S., Altincicek, B., Weidemeyer, C., Hintz, M., Turbachova, I., Eberl, M., Zeidler, J., Lichtenthaler, H. K., Soldati, D. and Beck, E. (1999): Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. Science, 285, 1573-1576.
195. Wengelnik, K., Vidal, V., Ancelin, M. L., Cathiard, A. M., Morgat, J. L., Kocken, C. H., Calas, M., Herrera, S., Thomas, A. W. and Vial, H. J. (2002): A class of potent antimalarials and their specific accumulation in infected erythrocytes. Science, 295, 1311-1314.
196. Lauer, S. A., Ghori, N. and Haldar, K. (1995): Sphingolipid synthesis as a target for chemotherapy against malaria parasites. Proc. Natl. Acad. Sci. USA, 92, 9181-9185.
197. Hanada, K., Palacpac, N. M., Magistrado, P. A., Kurokawa, K., Rai, G., Sakata, D., Hara, T., Horii, T., Nishijima, M. and Mitamura, T. (2002): *Plasmodium falciparum* phospholipase C hydrolyzing sphingomyelin and lysocholinephospholipids is a possible target for malaria chemotherapy. J. Exp. Med., 195, 23-34.

198. Zhang, K., Showalter, M., Revollo, J., Hsu, F. F., Turk, J. and Beverley, S. M. (2003): Sphingolipids are essential for differentiation but not growth in *Leishmania*. EMBO J., 22, 6016-6026.
199. Denny, P. W., Goulding, D., Ferguson, M. A. and Smith, D. F. (2004): Sphingolipid-free *Leishmania* are defective in membrane trafficking, differentiation and infectivity. Mol. Microbiol., 52, 313-327.
200. Zhang, K., Hsu, F. F., Scott, D. A., Docampo, R., Turk, J. and Beverley, S. M. (2005): *Leishmania* salvage and remodelling of host sphingolipids in amastigote survival and acidocalcisome biogenesis. Mol. Microbiol., 55, 1566-1578.
201. Salto, M. L., Bertello, L. E., Vieira, M., Docampo, R., Moreno, S. N. and de Lederkremer, R. M. (2003): Formation and remodeling of inositolphosphoceramide during differentiation of *Trypanosoma cruzi* from trypomastigote to amastigote. Eukaryot. Cell, 2, 756-768.
202. Finnegan, C. M., Rawat, S. S., Puri, A., Wang, J. M., Ruscetti, F. W. and Blumenthal, R. (2004): Ceramide, a target for antiretroviral therapy. Proc. Natl. Acad. Sci. USA, 101, 15452-15457.
203. Anes, E., Kühnel, M. P., Bos, E., Moniz-Pereira, J., Habermann, A. and Griffiths, G. (2003): Selected lipids activate phagosome actin assembly and maturation resulting in killing of pathogenic mycobacteria. Nat. Cell Biol., 5, 793-802.
204. McConville, M. J. and Ferguson, M. A. (1993): The structure, biosynthesis and function of glycosylated phosphatidylinositols in the parasitic protozoa and higher eukaryotes. Biochem. J., 294, 305-324.
205. IUPAC-IUB Commission on Biochemical Nomenclature (1978): The nomenctature of lipids. Biochem. J., 171, 21-35.
206. IUPAC-IUB Joint Commission on Biochemical Nomenclature (1998): Nomenclature of glycolipids. Eur. J. Biochem., 257, 293-298.
207. Svennerholm, L. (1963): Chromatographic separation of human brain gangliosides. J. Neurochem., 10, 613-623.
208. Kinjo, Y., Wu, D., Kim, G., Xing, G. W., Poles, M. A., Ho, D. D., Tsuji, M., Kawahara, K., Wong, C. H. and Kronenberg, M. (2005): Recognition of bacterial glycosphingolipids by natural killer T cells. Nature, 434, 520-525.
209. Mattner, J., Debord, K. L., Ismail, N., Goff, R. D., Cantu, C., 3rd, Zhou, D., Saint-Mezard, P., Wang, V., Gao, Y., Yin, N., Hoebe, K., Schneewind, O., Walker, D., Beutler, B., Teyton, L., Savage, P. B. and Bendelac, A. (2005): Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. Nature, 434, 525-529.
210. Kawahara, K., Moll, H., Knirel, Y. A., Seydel, U. and Zahringer, U. (2000): Structural analysis of two glycosphingolipids from the lipopolysaccharide-lacking bacterium *Sphingomonas capsulata*. Eur. J. Biochem., 267, 1837-1846.
211. Kawahara, K., Seydel, U., Matsuura, M., Danbara, H., Rietschel, E. T. and Zahringer, U. (1991): Chemical structure of glycosphingolipids isolated from *Sphingomonas paucimobilis*. FEBS Lett., 292, 107-110.