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Metabolism of sphingolipids in the gut and its relation to inflammation and cancer development

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Abstract

Sphingolipids are abundant in the microvillar membrane of intestinal epithelial cells where they are essential for structural integrity and may act as receptors for toxins, virus and bacteria. Metabolism of dietary and membrane sphingolipids in the intestine generates ceramide, sphingosine, sphingosine-1-phosphate, and ceramide-1-phosphate, via the action of alkaline sphingomyelinase, neutral ceramidase, sphingosine-1-kinase, and ceramide-1-kinase. These intermediary metabolites act as bioactive lipid messengers, influencing numerous cellular functions including growth, differentiation and apoptosis of both epithelial and immunocompetent cells in the gastrointestinal tract, and also the progress of inflammation and responsiveness of the mucosal cells to pathogens. This review summarizes background and recent progress in the metabolism of dietary and endogenous sphingolipids in the gut and its pathophysiological implications.

Contents

List of abbreviations

Alk-SMase: alkaline sphingomyelinase, BSSL: bile salt stimulated lipase, C1P: ceramide-1-phosphate, CerS: ceramide synthase, COX: cyclooxygenase, CRC: colorectal cancer, GalCer: galactosylceramide, Glyco-SL: glycosphingolipid, IBD: inflammatory bowel disease. JNK: c-JUN N-terminal kinase, LPA: lysophosphatidic acid. LPS: lipopolysaccharide, Lyso-PC: lysophosphatidylcholine, MDR: multidrug resistance protein, N-CDase: neutral ceramidase, NPP: nucleotide pyrophosphatase phosphodiesterase, NSAID: nonsteroidal anti-inflammatory drugs, PAF: platelet activating factor, PC: phosphatidylcholine, PDGF: platelet-derived growth factor, PGE2: prostaglandin E2, PI3K: phosphatidylinositol-3 kinase, PLA2: phospholipase A2, pRb: retinoblastoma protein, S1P: sphingosine-1-phosphate, SM: sphingomyelin, SMase: sphingomyelinase, SphK: sphingosine kinase, TLR: toll-like receptor, TNF-α: tumor necrosis factor. VEGF: vascular endothelial growth factor.

1. Introduction

The interest in sphingolipids is increasing. A number of recent reviews have covered broad perspectives on sphingolipids, including metabolism (1, 2), absorption and transport (3), roles in signaling pathways (4-8), enzymes involved (9-11), and relation to tumorigenesis (12) and inflammation (13). Among the biologically active sphingolipid metabolites, ceramide and sphingosine-1-phosphate (S1P) are considered most important. Ceramide is a major lipid messenger that inhibits cell proliferation and induces apoptosis via dephosphorylation and inactivation of several proliferative and antiapoptotic molecules such as Akt, Bcl-2, PKCα and pRB. It also activates several kinases as Raf kinase and JNK depending on cell types (10, 14, 15). S1P functions as a second messenger inside the cells, and as an extracellular signal via G-protein coupled receptors (5, 16). Increasing evidence indicates important roles of S1P in regulation of cell growth, angiogenesis, immune function and lymphocyte traffic, affecting downstream signaling molecules such as PLC, PI3K, Akt, VEGF, and COX 2 (16-18). Recent studies have indicated that ceramide-1-phosphate (C1P) is also an important lipid signal that affects cell proliferation and inflammation through activation of PLA2 (19, 20) (Fig. 1).

Sphingolipids, in particular glucosylceramide, are abundant in the apical membrane in the absorptive epithelium in the gut, and are considered important for the preservation of structural integrity during exposure to bile salts and enzymes (21). The brush border sphingolipids may also support the insertion of transporters and receptors, necessary for the selective and effective transport of nutrients into the cells, although these aspects are poorly characterized. Sphingolipid composition changes when crypt cells differentiate to mature absorptive cells, reflecting the close connection between sphingolipid synthesis and mucosal regeneration and differentiation.

Hydrolytic ectoenzymes, including those digesting sphingolipids, account for an important part of the proteins of the brush border (21). Sphingolipid metabolites may thus be generated both by intracellular enzymes occurring in most cell types and by ectoenzymes acting on sphingolipids in the diet and in the outer leaflet of the absorptive cells. The generated ceramide, sphingosine, and S1P are intermediates in

the conversion of sphingoid bases to chylomicron palmitic acid or in sphingolipid synthesis; they may reach signaling targets and act as messengers (3).

The relation of the sphingolipids in the gut to intestinal inflammation and colorectal cancer (CRC) is a novel and complex issue. Both CRC and inflammatory bowel diseases (IBD) are common diseases that result from gene - environmental interactions including a dietary influence. Current hypotheses for IBD pathogenesis emphasize a deregulation of the normal inflammatory response to the commensal bacterial flora (22). Studies on gene targeted animals and in patients indicate that deregulation originating from defect barrier integrity, or from innate or specific immunity, may result in similar phenotypes (23). Lipid signaling via eicosanoids, glycerolipid- and sphingolipid messengers is an important feature of IBD (24). Most CRCs involves a stepwise series of mutations resulting in a progression to benign adenomas and eventually CRC, which can long be influenced by diet and drugs (25, 26). The role of lipid messengers is highlighted by the protective effect of cyclooxygenase inhibitors (NSAID, non steroid anti-inflammatory drugs) against CRC development (27) and by the fact that the same types of the drugs make ulcerative colitis worse (28).

This review focuses on the metabolism of sphingolipids in the gastrointestinal tract and the potential relation to mucosal protection, inflammation and carcinogenesis. Since the exposure to exogenous sphingolipids and the enzymes involved are unique features of the gastrointestinal tract, these aspects are covered in some detail.

2. Sphingolipid profile in the intestinal tract

Throughout the gastrointestinal tract sphingolipids are enriched in the apical membrane of the polarized epithelial cells. The sphingolipid profiles have been characterized by TLC, GLC and GLC-MS techniques with regard to sphingoid base, fatty acid and polar head group composition.

The stomach mucosa contains neutral glycolipid species with one, two, three or five sugars (29), acidic glycolipids of the sulphatide and ganglioside classes, and more complex neutral glycolipids with blood group reactivity. It also contains several molecular species of sphingomyelin (SM) (30). The functions of the stomach sphingolipids are only partly known. Upon stimulation the parietal cells undergo a

morphological transformation and membrane rearrangement. The secretory membrane containing the crucial K^+/H^+ ATPase was found to be rich in sphingolipids (31). Glycolipids were later shown to interact with *Helicobacter pylori* (32), which produce a sphingomyelinase (SMase) suggested to be involved in *Helicobacter* induced cell death, gastritis and ulcer development (33). Glycolipid binding proteins were identified in *Helicobacter pylori* (34). The amounts of sulphatides and gangliosides are low, but the proportion of sulphatides is higher in the stomach than in the intestine, and a protective role in the acid environment has been suggested (35).

In the small intestine, about 40% of the lipids in the apical membrane of absorptive villous cells are sphingolipids, which is much more than in the basolateral membrane (21, 36). Bouhours and Glickman (37) analyzed sphingolipids of villous and crypt cells and found that glucosylceramide content increased with differentiation of absorptive villous cells, associated with alterations in fatty acid and sphingoid base composition (38-40). Changes of the sphingolipid pattern also occur during fetal and neonatal development of the intestine to the mature absorptive organ (41).

Glycosphingolipid (Glyco-SL) levels of epithelial cells exceed those of the nonepithelial cells in small intestine and colon (42). For example, epithelial cells of the colon contain three times as much glyco-SLs as the whole organ (29). Gustafsson et al (43) compared the glyco-SL pattern of the gastrointestinal tract in germfree and conventional rats and found rather small differences in mucosal glyco-SLs, indicating little influence of the bacterial flora.

Gangliosides are negatively charged glyco-SLs containing sialic acid. Their presence in the mucosa was shown early (44). Later several classes belonging to the GM, GD and GT types were found in the mucosa (45). GM3 was previously found to be most abundant in the intestine and located primarily to the apical membrane. The levels of GM3 and the key enzyme in ganglioside synthesis, CMP-sialic acid:lactosyl ceramide sialyltransferase, are higher in villous than in crypt cells (46).

There are great similarities in the sphingolipid profiles throughout the gastrointestinal tract, but also some differences. In the rat small intestine, mono- and trihexosylceramide are major neutral glyco-SLs, with monohexosylceramide being high in the proximal third, and trihexosylceramide in the distal segment (47). In monkeys concentration of gangliosides in small intestine is fourfold and that of neutral glyco-SLs twofold higher than in colon (47). Although sphingosine is the major base in neutral glyco-SLs, phytosphingosine is abundant in ganglioside GM3 at

both levels. Generally the high content of phytosphingosine is an important feature of small intestinal glyco-SLs (39).

In conclusion sphingolipid biosynthesis and cellular location are intimately linked to mucosal differentiation and maturation in the small intestine, and exhibit both specific and common features at different levels of the gastrointestinal tract. The pattern is determined developmentally rather than by bacterial influence.

3. Metabolism of sphingolipids in the intestinal tract.

3-1. Synthesis and degradation of sphingolipids in the gut

The differentiation and turnover of mucosal cells in the gastrointestinal tract are rapid. During the process, glyco-SLs and SM must be synthesized and degraded accordingly. Furthermore 6-10% of the polar lipids in chylomicrons secreted into chyle are SM. The need of sphingolipids for mucosal renewal and lipoprotein secretion is difficult to estimate precisely, but may be of the order 1.5 g per day in humans (3). Since there is no evidence for any substantial uptake of plasma lipoprotein sphingolipids from blood to the mucosa, this amount must be supplied primarily by local de novo synthesis.

Synthesis of sphingolipids begins with the condensation of serine and palmitoyl-CoA, catalyzed by serine palmitoyltransferase, which is a ubiquitous enzyme and expressed also in the intestine (48). The product, 3-ketosphinganine is reduced to sphinganine (dihydrosphingosine), which is subsequently acylated to dihydroceramide. Dihydroceramide is converted to ceramide by a desaturase that introduces a double bond at position 4 of the sphingoid base. Ceramide/dihydroceramide is synthesized by ceramide synthases that require ATP and formation of CoA derivatives of the fatty acids. These enzymes are located to the endoplasmic reticulum and use newly synthesized sphinganine or sphingoid bases from degraded sphingolipids (49). The genes coding for different ceramide synthases (*CerS 1-6*) have varying fatty acid and sphingoid base specificities and all except *CerS-3* are expressed in the intestine according to Unigene expression profile. The formed ceramides are located in the endoplasmic reticulum and transferred to the Golgi structures where glyco-SLs and SM are formed (50-52) facilitated by ceramide transfer protein (53). Two types of SM synthase have been identified and cloned. The

type 1 is located to the Golgi and the type 2 resides primarily at the plasma membrane (54).

A number of glucosyltransferases catalyze the sequential addition of carbohydrate moieties to the 1 hydroxyl group of ceramide (55). In the intestine the glucosylceramide is a major glycolipid. The extended carbohydrate chain of the other glycolipids consists of galactose, N-acetyl-glucosamine, fucose and sialic acid derivatives. The glucosylceramide synthase is highly expressed in the stomach, small intestine and colon (Unigene profile). Additional carbohydrates are then added by a series of glycosyltransferases usually catalyzing the reaction with a UDP derivative of the respective sugar. Regarding synthesis of gangliosides (56), sialyltransferases have been identified in intestine. The targeting of sphingolipids to the brush border membranes results from the localization of synthases as well as transfer proteins (51, 57). Current views on glycolipid synthesis and targeting have been recently summarized (57). Mechanisms behind the differential compartmentalization of SM to chyle lipoproteins and of glycolipids to the brush border are poorly known. The presence of the enzymes responsible for sphingolipid metabolism in the intestine is summarized in Table 1.

3-2. Digestion and absorption of sphingolipids in the gut

Calculations based on available analytical data indicate that adult humans on an ordinary Western diet ingest about 0.3-0.4 g sphingolipids per day (58) and the suckling baby ingesting milk consumes 50-150 mg SM per day (59-61) as well as milk lactosylceramide and gangliosides.

Early studies raised the question whether dietary SM could directly contribute to the SM pools in atherogenic cholesterol- and triglyceride rich plasma lipoproteins and in chylomicrons (62, 63). The answer was no. When radioactive SM, ceramide and glucosylceramide were fed to lymphatic duct cannulated and intact animals, little or no labeled SM, ceramide or glucosylceramide was absorbed intact into the chyle (64). Although SM and glycosylceramides were found to be resistant to digestion by pancreatic enzymes, both substrates were sequentially hydrolyzed to ceramide, sphingosine and free fatty acids in vivo (65). Free sphingosine and dihydrosphingosine were rapidly absorbed and metabolized in the mucosal cells to chylomicron palmitic acid (64). A smaller portion of the sphingoid bases is reincorporated into mucosal ceramide and more complex sphingolipids (64, 66) (Fig

2). Interestingly plant glucosylceramide containing the sphinga-4,8-diene as the major sphingoid bases are digested as effectively as glucosylceramide containing sphingosine and both sphingoid bases were taken up as effectively by CaCo2 cells (67). Inhibition of P-glycoprotein (mdr1) with verapamil increased, however, accumulation of sphinga-4,8-diene but not of sphingosine in the cells, suggesting that this sphingoid base may be more effectively expelled (68).

The rapid conversion of absorbed sphingosine and sphinganine to palmitic acid undoubtedly show the expression of two key enzymes in this reaction, i.e. sphingosine kinase (Sph K) catalyzing the formation of S1P from sphingosine and S1P-lyase catalyzing the conversion of S1P to hexadecenal and ethanolamine phosphate (64) (69). Yatomi et al. (70) analyzed S1P in different tissues and found high levels in testes and intestine. Two isoforms of Sph K, Sph K1 and K2, have been cloned. Fukuda et al. (71) studied the expression of the different isoforms and found that Sph K1 contributed 40-70% of the activity in the small intestine and the major part of the activity in the colon, indicating that another Sph K contributed a substantial part of the activity in the small intestine. Sph K, S1P lyase and the aldehyde oxidase that converts hexadecenal to palmitic acid are all expressed at high levels in the intestinal mucosa (72). However, the longitudinal distribution of the enzymes in the gastrointestinal tract has not been studied. Furthermore the expression of S1P phosphatases that specifically hydrolyze S1P to sphingosine and phosphate (73), thereby inactivating the important messenger S1P and channeling sphingosine to reutilization for ceramide and sphingolipid synthesis, has not been studied specifically in the gastrointestinal tract, although S1P phosphatase 1 and 2 have both been cloned (74-76).

Interestingly Sph K2 but not 1 acted in concerted action with sphingosine phosphatase 1 to regulate recycling of sphingosine into ceramide, which was considered an evolutionary conserved pathway for sphingosine salvage (77). Thus compartmentalization as well as differential action of Sph K isoforms may influence partitioning of sphingosine for irreversible metabolism to palmitic acid or reutilization for ceramide formation.

In conclusion the gut expresses high levels of enzymes that catalyze the irreversible conversion of sphingosine to palmitic acid. Salvage of some sphingosine for ceramide and sphingolipid formation does, however, occur. The expression of Sph K, S1P lyase and S1P phosphatases, as well as the intracellular compartmentalization

of the metabolites formed and thereby their access to signaling targets are expected to determine the biological effects of sphingolipids in the gut.

3-3. Alkaline sphingomyelinase and neutral ceramidase in the intestine.

The major enzymes that are responsible for SM degradation in the intestinal lumen and mucosa are alkaline SMase (Alk-SMase) and neutral ceramidase (N-CDase) (78). Both alk-SMase and N-CDase are ectoenzymes that bind to the surface of mucosal membrane with a transmembrane domain and the catalytic domains are located outside the cells (79). A glycosylceramidase activity in the gut, which could not be separated from the lactase-phlorizine hydrolase, was also reported. Later studies confirmed that lactase-phlorizine hydrolase converts both glucosyl- and lactosylceramide to ceramide (80). Thus both SM and the glycosylceramides can be converted to ceramide.

Alk-SMase is the major enzyme catalyzing the first step in the digestion of SM and is present in all species examined except guinea pig (81). It is also found in human bile but not in bile of many other species (81, 82). Its longitudinal distribution shows a maximum in the jejunum and a low level in colon. Purification and cloning of the rat and human enzyme demonstrated a lack of homology to known neutral and acid SMases but a relation to the nucleotide-pyrophosphatase/phosphodiesterase (NPP) family (79, 83). Accordingly the enzyme is now also designated NPP7. It does, however, not hydrolyze nucleotides but only choline phospholipids including SM, lysophosphatidylcholine, phosphatidylcholine and platelet-activating factor, preferring SM. The enzyme is bile salt dependent, taurocholate and taurochenodeoxycholate being the most effective stimulators (84). It is resistant to pancreatic proteases and can be released from the mucosa by bile salts and by tryptic digestion of a C-terminal peptide, which anchors the enzyme to the brush border (85).

Intestinal N-CDase was purified from a bile salt eluate from rat intestine mucosa and from human ileostomy content (86, 87). The enzymes are identical to neutral ceramidases identified in the apical membrane of rat renal tubular cells (88), in the mouse small intestine (89), and in the human liver (90). Like alk-SMase the N-CDase has a longitudinal distribution that coincides with the main site of ceramide digestion (91). Although bile salt stimulated lipase (BSSL) present in human milk and in pancreatic juice was shown to hydrolyze ceramide (92), the digestion of ceramide in most parts of the small intestine is not decreased in BSSL (-/-) mice (93), whereas N-CDase knockout mice show a marked increase

of ceramide excretion in the feces (94). The conclusion is that N-CDase is more important for the digestion of ceramide than BSSL.

Although alk-SMase, lactase phlorizine hydrolase and N-CDase are important enzymes in sphingolipid digestion, they may also hydrolyze endogenous sphingolipid substrates in the apical membrane of epithelial cells, thereby generating messengers that influence mucosal functions. In support of this hypothesis, over expression of alk-SMase in Cos 7 cells caused a 30% reduction of SM in the cells (79). As in other tissues acid SMase, glucosyl- and galactosylceramidase, and acid ceramidase localized primarily to the lysosomes, are, however, also expressed, and it has not been excluded that these enzymes may play a role as well.

The course of SM digestion and thereby the exposure of distal small intestine and colon to SM and its metabolites can be influenced by the amount of SM given, the presence of bile salts and other lipids, and by the levels of the enzymes involved. Early studies indicated an extended course and a limited capacity of SM digestion. The recovery of SM ^{14}C stearic acid in chyle was incomplete and about 25% of the sphingosine appeared in feces, mainly as ceramide (64). Increasing the dose of SM increased the proportion and amount of undigested SM and ceramide in the lower half of the gut, and increased output of SM and ceramide in feces (95). In a human study, Hertervig found that feeding a dose of 250 mg milk SM in a standardized meal increased output of both ceramide and intact SM in ileostomy content in humans (96), indicating an incomplete digestion and absorption of SM. When rats were fed radio-labeled SM orally together with cholesterol or sitosterol the digestion of SM was delayed and colonic exposure increased (97). In vitro studies with purified alk-SMase showed that the presence of glycerolipids or sterols, but not of free fatty acids inhibited SM hydrolysis by alk-SMase (98). The higher alk-SMase in jejunum and ileum than in duodenum and the higher concentrations of other dietary lipids in the upper part of small intestine may thus explain why SM digestion primarily occurs in middle and lower small intestine.

Expression of alk-SMase and ceramidase can be affected by dietary factors and drugs. Alk-SMase was increased by psyllium and ursodeoxycholic acid (99-101) and decreased by a fat rich diet. The effects of ursodeoxycholic acid are stronger on polarized colonic cells than on monolayer cells (102). Psyllium, a water soluble dietary fiber, increases alk-SMase and decreases N-CDase activity, and may thus increase the ceramide levels in the gut. Variations in the key apoptotic enzyme caspase 3 correlates positively to alk-SMase but negatively to acid SMase (99). Several compounds with anticancer and anti-inflammatory properties such as 5-ASA, boswellic acid and ursolic acid increase alk-SMase activity (103) In view of the

strict bile salt dependence of alk-SMase, the question has been raised whether bile or bile acids may be important for the expression of the enzyme in the intestine. The alk-SMase activity in feces and gut lumen was significantly decreased by biliary diversion but not the mucosal activity (104). The overall conclusion is that the alk-SMase level can be significantly influenced by external factors.

In conclusion dietary SM is sequentially hydrolyzed by alk-SMase and N-CDase. The digestion is extended and occurs primarily in the middle and lower level of the small intestine. From a teleological point of view the protease resistance and the release of the enzymes by trypsin and bile salts makes possible an extended intraluminal digestion, with minimal risk of mucosal damage caused by unintentional hydrolysis of mucosal sphingolipids.

4. Intestinal sphingolipids and inflammatory bowel diseases

Inflammatory bowel disease (IBD), primarily Crohn's disease and ulcerative colitis, are common diseases caused by an interaction between genetic predisposition and environmental factors. An excess of polymorphonuclear leukocytes, eosinophils, macrophages and different subtypes of B- and T-lymphocytes are present in varying proportions and with different tissue location depending on the type and stage of disease. At the site of inflammation, cytokines, eicosanoids and glycerolipid- and sphingolipid messengers are produced by the epithelium and by the immunocompetent cells. Current hypotheses for the pathogenesis of IBD emphasize the deregulation of the immune response to normal gut bacteria (22). Sphingolipid involvement in IBD can be related both to mucosal integrity, barrier and receptor functions and formation of sphingolipid messengers in epithelium and inflammatory cells. Bacterial and viral infections may trigger the onset or relapse of IBD. Interactions of bacteria and virus with sphingolipid receptors are therefore relevant both to the actions of intestinal pathogens and to IBD.

4-1. The receptor function and anti-infectious effects of sphingolipids.

Since targeting of sphingolipids to the apical membrane is an important feature of the barrier formation, any abnormality in this process may be of interest in relation to the pathogenesis of IBD. These aspects of IBD are poorly investigated.

One may, however, postulate that glucosylceramide and other neutral glycol-SLs have an important barrier role. It is therefore interesting to note, that multidrug resistance protein 1 (MDR1) participates both in transfer of glucosylceramide to the apical membrane of polarized cells (105) and in the translocation of glucosylceramide in the Golgi complex, making it available for the synthesis of other neutral glyco-SLs as lactosylceramide and globotriaosylceramide (106). The reason is that IBD has been linked to a downregulation and genetic polymorphism in IBD (107) and the MDR1a-/- mouse appears to be an appropriate model for spontaneous colitis (108). Further exploration of the complex connections between glucosylceramide synthesis and MDR1 expression (109) in relation to IBD is undoubtedly needed.

Some microorganisms and microbial toxins use glyco-SLs to attach to the host cell as a way of mediating pathogenic effects. Viruses can bind to host cells via glyco-SLs. Dietary sphingolipids may thus compete for the attachment sites and promote elimination of pathogenic organisms and toxins from the intestine and counteract bacterial translocation. Milk fat consumption was shown to be related to a reduced number of food-borne infections (110, 111). Since enteropathogenic bacteria and viruses, and possibly with normal commensal in a predisposed individual, may trigger onset or relapse of IBD, the interaction between these agents and mucosal sphingolipid receptors is briefly summarized in this context.

Numerous studies demonstrate important receptor functions of gangliosides for pathogenic bacteria, toxins and virus (112). The gangliosides provide a negative charge to the surface, serving as specific binding sites for cholera toxins, enteropathogenic *Escherichia coli* toxin, and rotavirus etc and to be involved in the immune response to oral vaccination with several antigens via receptor function at the M-cells of the Peyers patches. GM1 is the major ganglioside in the small intestine and was shown to be a receptor for cholera toxin, the binding being essential for triggering the diarrhea response. In case of enterohaemorrhagic *E coli* the toxin exerts a specific pathogenic mechanism. Shiga toxins are virulence factors produced by certain bacteria as E. coli O157:H7 (113), that cause hemorrhagic colitis (114).   The binding site for Shiga toxins is a Glyco-SLs (Gb3) (115), which is not present in normal colonic epithelial cells but highly expressed in metastatic colon cancer (116).

Exogenous gangliosides such as in milk may be potentially important during suckling, as they may protect breast fed infants from infections. It has been shown that milk gangliosides can inhibit enterotoxins, inhibit adhesion of *E coli* and suppress

the growth of *E coli* and potentially other pathogenic microorganisms in the intestine of premature babies, and stimulate growth of bifidobacteriae. Milk gangliosides also inhibit cholera toxin adherence to Caco2 cells (117). Enriching infant formula with ganglioside changed the microflora. *E coli* in feces of preterm infants decreased and bifidobacteriae increased, and the growth of bifidobacteriae is promoted by sialic acid (118). Gangliosides may be essential for neonatal growth of the nervous system (119) and may enhance uptake of lipids in the weanling rat (110). Interestingly addition of gangliosides to the diet reduced infection with *Giardia muris*, a close relative to the human intestinal pathogen *Giardia lamblia*, in mice (120).

The effects of sphingosine and ceramide on the pathogenic bacterial strains *E coli* O157:H7, *Salmonella enteritidis*, *Campylobacter jejuni* and *Listeria monocytogenes* was investigated *in vitro*. It was found that sphingosine but not ceramide has strong antibacterial effects on all strains (111). Thus in the small intestine where digestion of sphingolipids is fastest, the continuous generation of sphingosine may be important to control bacterial growth.

In conclusion, these antibacterial and antiviral mechanisms of dietary sphingolipids may be important but the in vivo relevance is often hard to evaluate.

4-2. Sphingolipid signaling in intestinal inflammation.

Although the roles of ceramide, sphingosine, S1P and C1P as lipid messengers in tumor and inflammation in general have been the subject of many previous investigations (13), current information on the specific involvement of sphingolipids in intestinal inflammation is sparse. Current views emphasize that sphingolipid metabolites may contribute to both the proinflammatory and antiinflammatory response, and that S1P formation and action may be targets for IBD therapy.

Ceramide, although it functions as a proapoptotic molecule in many cell types, may also induce inflammatory response. Previous studies have shown that the proinflammatory response to TNFα and IL-1 involves activation of neutral SMase, leading to the formation of ceramide (121). NF-κB is a key molecule with proinflammatory properties and its activity was increased by ceramide in the small intestinal epithelial cells, due to reduction of IκB-a and IκB-b (122). Similar activation of NF-κB was also identified in HT29 colon cells after SMase treatment

and the cells appear more sensitive to acid SMase than N-SMase (123). In addition, the formation of ceramide may be involved in the assembly of Toll-like receptor (TLR) in response to bacterial toxin such as lipopolysaccharide (LPS) (124). Several microbial ligands such as LPS, p-fimbriae and the B-subunit of Shiga toxin were shown to increase the levels of ceramide and trigger a TLR4 dependent response in either leukaemia cells or epithelial cells (125, 126)

However, as has been reviewed previously (127), the role of ceramide often varies with the site and mechanism of formation and with cell type. In aortic endothelial cells, ceramide formed by neutral SMase in response to an oxidized phosphatidylcholine was reported to inhibit the LPS-induced IL-8 response (128). Of particular interest is a recent study showing that hydrolysis of SM at the apical membrane of intestinal Caco-2 cells by SMase attenuates the intoxication of the host cells by cholera toxin (129). The study indicates a protective effect of intestinal SMase, particularly alk-SMase, as it is an ecto-enzyme anchoring on the surface of microvilli membrane and also present in the intestinal content, with good access to SM at the apical membrane of mucosal cells. A reduction of alk-SMase in human longstanding ulcerative colitis was previously reported (130).

S1P has primarily proinflammatory properties and acts as a chemo attractant for neutrophils and macrophages. It has antiapoptotic effects on macrophages, induces mast cell degranulation and regulates lymphocyte functions and traffic. S1P also induces COX 2 expression, thus influencing production of eicosanoid inflammatory mediators. Formation of PGE2, a major prostaglandin in the gastrointestinal tract, may thereby be increased. How this relates to the gastrointestinal tract is presently unknown. One may speculate about both harmful and beneficial implications, since COX inhibition induces mucosal ulcerations and makes colitis worse (28), but does also counteract colon cancer development. S1P may thus be good during the progress of the inflammation and not during the resolution. Interestingly Sph K1 knockout mice exhibited normal inflammatory response in acute peritonitis and chronic collagen induced arthritis models (131). Tissue levels of S1P were not increased in inflammation.

Emphasizing the role of S1P in gut inflammation, orally administered sphingosine kinase inhibitors were recently shown to suppress colitis induced by dextran sulphate (132). Exploiting current knowledge about the key role of S1P in

lymphocyte traffic, the S1P receptor modulator FTY720 was shown to alleviate colitis in a number of experimental colitis models (133-135)

Sphingosine on the other hand may counteract S1P induced priming of neutrophils and induce apoptosis in leukocytes and macrophages, and was also shown to stimulate PGE2 production in fibroblasts and to enhance TNFα induced PGE2 production (136). There are thus several mechanisms by which sphingolipid feeding might favour anti-inflammatory as well as proinflammatory mechanisms in the gut. Regarding intestine much information is, however, lacking today. For instance the expression of Sph K1 and 2, S1P-lyase and S1P-phosphatases and the regulation and distribution of these enzymes along the gastrointestinal tract need to be studied in much more detail. Studies on the regulation of intracellular and external levels of sphingolipid metabolites in normal and inflamed tissue are needed.

While ceramide, sphingosine and S1P have divergent effects on intestinal inflammation, ganglioside, particularly galactosylceramide (GalCer) was reported to inhibit inflammation. Alpha-GalCer was found in vivo to inhibit *Toxoplasma gondii*-induced ileitis by overexpression of IFN-γ via a specific interaction with NKT cells, which regulate the immune response (137). Similar inhibition by alpha-GalCer on allergic airway inflammation was also reported (138). The inhibitory effects of GalCer may be of species specificity, as one analogue of GalCer, CCL-34 but not GalCer, was found to stimulate NF-κB in a TLR4 dependent manner in Raw 264.7 cells (139).

4-3. Interaction of sphingolipid signaling with eicosanoid and glycolipid signalings

The roles of eicosanoids in inflammation in general and in IBD have been extensively investigated. An important progress in the recent years was the identification of the cross-communication between sphingolipid and eicosanoid signaling (Fig. 3). In eicosanoid metabolism, the key enzymes are PLA2 and COX2. PLA2 triggers the hydrolysis of phosphatidylcholine (PC) leading to increased formation of both arachidonic acids and lysoPC. Arachidonic acid is the precursor of PGE2 and leukotrienes, and the lysoPC will further be converted to lysophosphatidic acid (LPA). PGE2, leukotriene, and LPA are potent factors promoting inflammatory responses. As shown in Fig 2, SM was reported previously to inhibit the PLA2

activity, indicating a protective effect of the lipids on pathogenesis of inflammation (140). More importantly, C1P and S1P, the phosphorylated form of ceramide and sphingosine, were recently reported to activate PLA2 and induce expression of COX2, respectively (19). C1P and S1P seem to act concertedly to stimulate the formation of PGE2 and promote the inflammation. Thus the kinases catalyzing the phosphorylation of ceramide and sphingosine may be novel targets for the development of antiinflammatory drugs.

PLA2 is also a key enzyme responsible for production of platelet activating factor (PAF), as it cleaves alkyl-acyl-glycerophosphocholines to form lyso-PAF, a precursor of PAF. In addition to the crosstalk between sphingolipid messengers and eicosanoids there may also be an interaction between sphingolipid- and glycerolipid messengers in the gut. PAF can be synthesized and released from the inflammatory mucosa and is implicated in IBD such as Cohn's disease, ulcerative colitis, and necrotizing enteritis in the newborn, which are associated with high extracellular levels of PAF. It is therefore of great interest that alk-SMase was shown to hydrolyze and inactivate PAF (141). PAF is considered to be hydrolyzed primarily by PAF-deacetylase. The effects of alk-SMase on PAF represents an additional pathway which is well suited to act in the gut because of its location at the mucosal surface and the resistance of the enzyme to proteases. In addition, LPA derived from lyso-PC under the actions of lysophospholipase D is another lipid messenger that stimulates cell migration and inflammation. Alk-SMase can also hydrolyze lyso-PC with a phospholipase C activity and thus may compete with lysophospholipase D and reduce the formation of LPA (79).

5. Intestinal sphingolipids and colonic carcinogenesis

5-1. Link of sphingolipid metabolism with colonic tumorigenesis.

Since ceramide and sphingosine are regulators of cell growth, differentiation and apoptosis, the questions have been asked whether the metabolites formed from dietary or membrane SM may influence the cell cycle of the gut epithelium under normal and tumorigenic conditions, and whether sphingolipid metabolites regulate normal proliferation and differentiation in crypt cell progenitor compartment and cell fate along the crypt villous axis. Analytical studies more than two decades ago

identified differences in sphingolipid composition between tumor tissue and normal tissues of the gastrointestinal tract. When animals were injected with dimethylhydrazine, a colonic chemical carcinogen, SM was found to be increased in the colonic tissues before the appearance of adenomas, accompanied by a reduction of SMase, indicating a decreased SM hydrolysis prior to malignant transformation (142). Furthermore this colon carcinogen was found to also decrease glyco-SLs and to induce changes of the glyco-SL pattern in the intestine (143). However, in human colon cancer tissues, the levels of SM are reduced as measured by 31P magnetic resonance spectroscopy (144), together with reduced ceramide levels by about 50% (145). Similar changes were also found in many other cancer tissues such as ovarian tumors, lung cancers and head and neck cancers (12). Multiple factors may be responsible for the changes of SM and ceramide in colonic tissues, such as de novo biosynthesis of sphingolipids, the hydrolysis of SM by different SMases, and the glycosylation of ceramide.

The interest in the relation between sphingolipids and colorectal cancer was triggered in 1994 by a study, which showed that administration of SM inhibited the formation of aberrant crypt foci and reduced the malignant transformation in mice (146). The finding was thereafter confirmed and extended by other studies, which showed similar effects of natural and synthetic SM, as well as monoglucosyl ceramide, lactosyl ceramide, and ceramide analogues (147). SM was also found to enhance chemotherapeutic effects of anticancer drugs both in vivo and in vitro (148) and is both chemopreventive and chemotherapeutic (149). Hydrolysis of the sphingolipid to generate ceramide seems a key procedure for such anticancer effects. Particular attention has therefore been given to alk-SMase (NPP7), the key enzyme in the intestine responsible for hydrolysis of SM and generation of ceramide. Progressive reduction of alk-SMase activity with malignant transformation has been reported in human colonic inflammation and carcinogenesis (130, 150, 151). Recently loss of function mutation of alk-SMase has been found in colon cancer HT29 cells (152) and in liver cancer HepG2 cells (153). The mutations occur at the alternative splicing level, involving the deletion of exon 4, which encodes a critical His that participates in the formation of binding site for SM (154). However, how often the mutation occurs in association with tumorigenesis and what factors that affect or even correct the altered splicing are still unknown and should be the targets for further investigation.

5-2. Diverse effects of sphingolipid metabolites on colonic tumorigenesis.

While SM, ceramide and sphingosine have antiproliferative effects in colon cancer cells, S1P has emerged as a potent molecule that stimulates cell proliferation, inhibits apoptosis and promotes inflammation, cell migration, and angiogenesis (154). High levels of S1P correlate to a poor survival rate (155). In colon cancer cells, S1P has been shown to induce COX 2 expression (156, 157), and activate p38 MAPK and Erk (158). S1P can also increase the production of PDGF and VEGF and activate growth factor receptor. Visentin et al. recently showed that neutralizing S1P with specific antibody significantly inhibited the cancer progression through inhibiting the cell proliferation and blocking neovascularization in several cell lines including colon cancer HT29 cells (159). Sph K, the enzyme responsible for the formation of S1P is upregulated in many cancer tissues and also in AOM induced colonic adenocarcinoma (160). Down regulation of Sph K by siRNA inhibits COX2 expression and PGE2 production (160). A balance between ceramide/sphingosine and S1P has been considered as a rheostat mechanism that determines the cell survival and cell death. In addition, the balance is also determined by the enzymes that catabolize S1P, such as S1P lyase and S1P phosphatase. The normal intestinal epithelial cells have high levels of S1P lyase and S1P phosphatase. However, in colon cancer cells both S1P lyase and S1P phosphatase are down regulated and thus the catabolism of S1P is inhibited (161). Over expression of S1P lyase potentiates apoptosis via p53- and p38 dependent pathways. Comparing with S1P phosphatase, the levels of S1P lyase may be more important in regulating the balance between ceramide and S1P, as the removal of S1P by S1P lyase is irreversible. Interestingly, in the familial adenomatous polyposis Min mouse model, further knocking out of Sph K1 significantly decreases the adenoma size and cell proliferation(162). These inhibitory effects are not affected by knocking down of S1P receptors, indicating that the intracellular S1P plays an important role in tumorigenesis in these animals.

Although ceramide is in general antiproliferative, recent studies indicate that its effects vary with location and pathway for ceramide formation. For example ceramide formed by acid SMase is restricted to the lysosomes and cannot exert signaling effects at its site of formation (163). Too high acid SMase activity may in fact facilitate the cell growth as cancer cell growth requires not only vasculature to provide oxygen, but also an effective autophagy, i.e. a process by which cells

selectively digest potions of their interior, in order to grow. Autophagy, which is conducted by lysosomal enzymes, has been shown to be an important process to stimulate cell survival (164, 165). The acid SMase activity is high in rapidly dividing Caco-2 cells. Several anticancer compounds such as boswellic acid, psyllium, and curcumin inhibit acid SMase activity (99, 103, 166, 167). As mentioned in the previous section, C1P has also been reported as another proliferative and proinflammatory factor derived from sphingolipid metabolism (168, 169), by its ability to activate PLA2 and increase the production of arachidonic acid (170). Considering the stimulatory effects of S1P on COX2, C1P and S1P seem to act concertedly to stimulate the formation of PGE2 and promote the inflammation and carcinogenesis (16).

Ceramide is the precursor of glyco-SLs. Important roles of glyco-SLs in tumorigenesis are emerging. Exogenously administered glyco-SLs such as glucosylceramide, lactosylceramide, and GD3 have been shown to inhibit the formation of aberrant crypt foci caused by chemical carcinogens (171). GM3 in cell culture was found to stimulate cell differentiation and inhibit cell proliferation through the activation of PTEN (172). Similar effects were also found in drug-induced synthesis of GM3. However, there is evidence showing that conversion of ceramide to glyco-SLs in colon cancer is related to cancer invasiveness, metastasis and most importantly, drug resistance (173, 174). The synthesis of UDP-Gal transporter is increased in human colon cancer and is correlated with Dukes-stage (175). In metastatic colon cancer, Gb3 levels were reported to be increased in correlation with invasiveness, which may relate to the function of Gb3 as receptor for Shiga toxin 1 (116). Overexpression of glucosylceramide synthase was identified in colon cancer cells resistant to drug treatment, associated with an increase of multidrug resistant phenotype and expression of P-glycoproteins (116, 176). Based on this information, the breakdown of ceramide by ceramidase may under some circumstances be critical to avoid the accumulation of glycoceramide and also the formation of C1P. Although the N-CDase has been cloned and the enzyme deficient mice have been generated (94), the dynamic changes of intestinal ceramidase in association with colon cancer development are still largely unknown.

In conclusion, colon cancer is associated with multiple changes of the enzymes responsible for sphingolipid metabolism, including the reductions of serine palmitoyl-CoA transferase, alk-SMase, and sphingosine lyase, and increases of

glucoceramide synthase, and Sph K, resulting in decrease in ceramide levels and increase in S1P levels (Fig 4). The changes of N-CDase and ceramide kinase in colon cancer have not been established. While inhibiting glucosylceramide synthase results in enhanced apoptosis (177, 178), knocking out N-CDase does not cause severe pathology, indicating that the conversion of ceramide to glucoceramide is a fundamental pathway for ceramide metabolism. Whether ceramide kinase is important for ceramide level in the intestine is not clear. The expression of the enzyme is reported to be high in human liver, but not intestine (179).

Future perspectives

Sphingolipids are both cellular constituents and dietary components. The intestinal tract is an organ that is rich in sphingolipids. Today important features have emerged from studies of the sphingolipid rich interface and of the metabolism and anticancer effects of dietary sphingolipids. New information has been gained which make S1P a potential target in the treatment of colorectal cancer and IBD. An immense complexity remains, however, to be structured. Sphingolipid digestion is an extended process with limited capacity and the exposure to colon of sphingolipids and their metabolites can be changed by dietary means, but the physiological effects on normal mucosal functions, inflammatory processes and tumorigenesis that this may have need further study. A question that should be asked is whether the high expression of alk-SMase and N-CDase in the middle and lower small intestine contributes to the low tumor incidence in this organ. The anti-inflammatory effects of alk-SMase need further investigation in view of its ability to hydrolyze and inactivate PAF even in the protease rich intestinal environment. The information linking C1P formation and eicosanoid signaling further emphasizes the importance of identifying of key points in the complex crosstalk between different lipid and peptide signals. Considering the dual role of sphingolipids as barrier and receptor components as well as precursors of important messengers the longitudinal distribution and the regulation of virtually every enzyme that participates in sphingolipid metabolism in the gastrointestinal tract needs further study. Focus should be not only on those participating in formation and partitioning between of sphingoid bases and their phosphorylated forms between the different pathways, but also on the enzymes that form and degrade sphingolipids during the differentiation of the apical membrane of the epithelial cells. Finally, although new information has emerged on the effects and the metabolism of milk sphingolipids in the suckling neonate, the knowledge on

this subject is still too fragmentary. A key issue in both the adult and the neonate is whether sphingolipids and the metabolites they generate can influence the function not only of the epithelial cells but also the immunological function of the gut.

Acknowledgement

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Table 1. Sphingolipid metabolic enzymes in the intestinal tract

Enzyme	Note	Ref
Serine palmitoyl-CoA transferase	Found in all tissue including intestine	[48]
Ceramide synthase	CerS 1, 2, 4, 5, 6 are found in intestine	[49]
Sphingomyelin synthase	SMS1 in Golgi and SMS2 in membrane.	[54]
Sphingomyelinase:		
Alkaline SMase	Major enzyme for SM digestion	[78,95]
Acid SMase	High in proliferative crypt cells	[102]
Neutral SMase	Low in the intestine	[104]
Ceramidase		
Neutral ceramidase	Major enzyme for ceramide degradation	[78]
Bile salt stimulated lipase	From pancreas and milk	[92]
Ceramide kinase	Low in intestine, high in liver	[179]
Sphingosine kinase	Two types. Type 1 accounts for 40-70%	[70,71]
Sphingosine-1-P lyase	High in physiological conditions	[72]
Sphingosine-1-P phosphatase	Present in the intestine (authors' data)	
Glucosylceramide synthase	High in stomach and intestine.	[55,56]
Glucosyltransferase	High in villous	[55,56]

Figure legends

Fig. 1. Signaling effects of SM metabolites. Metabolism of SM generates signaling molecules including ceramide, sphingosine, ceramide-1-phosphate (C1P), and sphingosine-1-phosphate (S1P). Ceramide and sphingosine are major lipid messengers that inhibit cell growth and inflammation, whereas C1P and S1P are major molecules with proliferative and inflammatory properties, through various signal transduction pathways. The open arrow indicates the chemical pathways and the solid line indicates the biological effects. The lines with arrows indicate stimulation and those with a blunt line indicate inhibition.

Fig. 2. Hydrolysis of SM in the intestinal lumen and mucosal cells. SM is hydrolyzed by alk-SMase and N-CDase to sphingosine, which is absorbed and converted to fatty acid and ceramide. The fatty acid and part of both endogenous and exogenous ceramide/SM will be incorporated into chylomicrons. The solid line with arrow indicates chemical reaction and the dashed line with arrow indicates translocation.

Fig. 3. Interaction of sphingolipid signaling with eicosanoid signaling in inflammation. Under the actions of PLA2 and Cox2, phosphatidylcholine (PC) is converted to PEG2 which stimulates inflammation and cell proliferation. This pathway is enhanced in one hand by S1P which stimulates the expression of Cox2 and , in the other hand, by C1P which enhances PLA2 transport and activity by binding to the enzyme.

Fig. 4. Changes of enzymes responsible for sphingolipids metabolism in colon cancer. SPT: serine palmitoyl transferase, Alk-SMase: alkaline SMase, SMS: SM synthase, GCS: glycoceramide synthase, CDase: ceramidase, SPK: sphingosine kinase, SPL: S1P lyase. ⊕ : increase, ⊖: decrease.
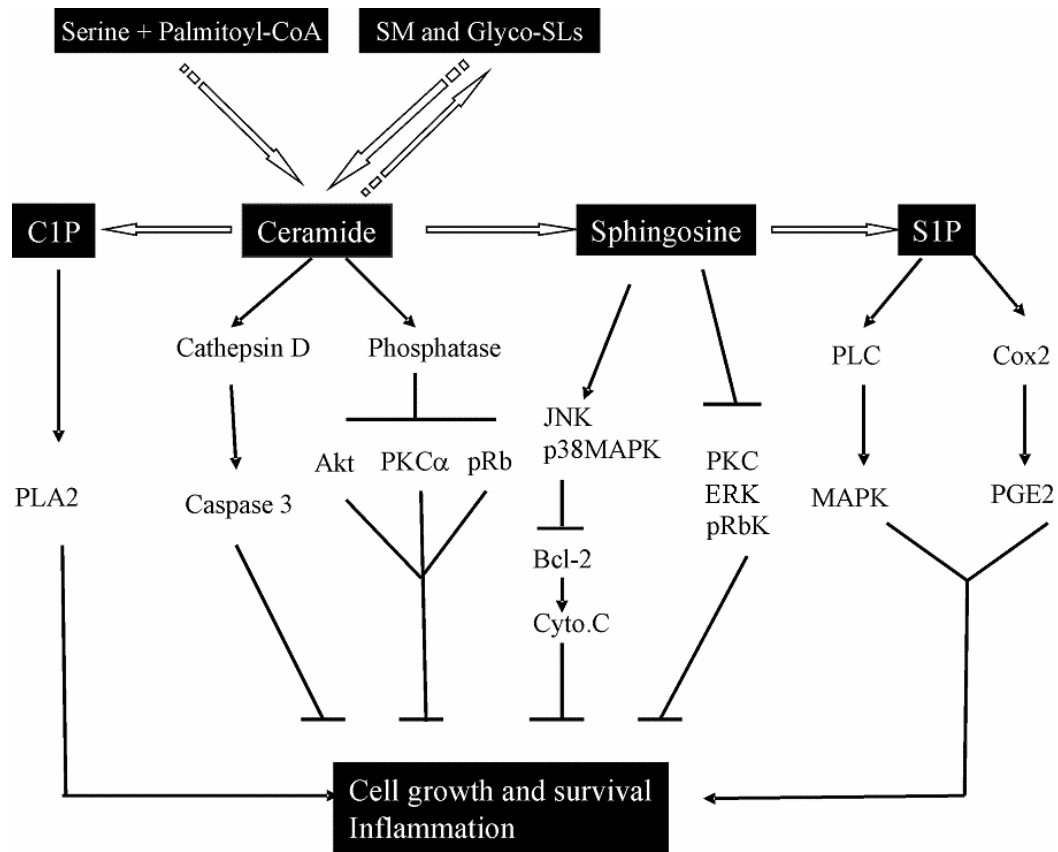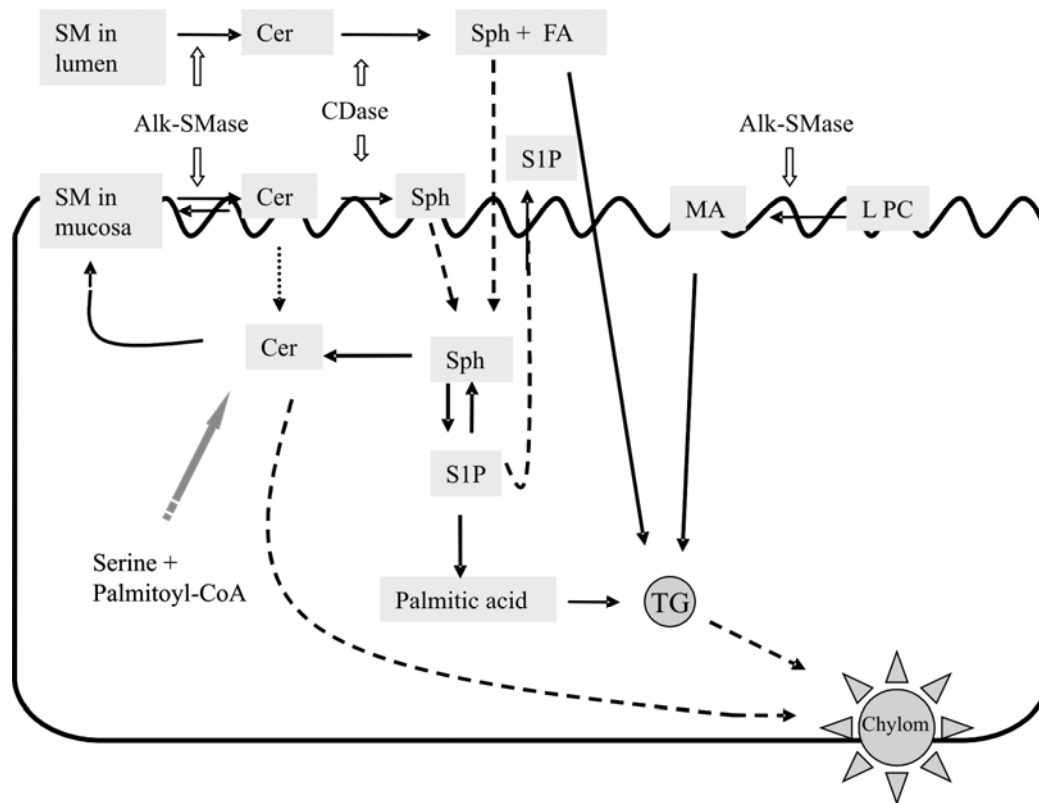
Fig. 1

Fig. 2

Fig. 3.

Fig. 4