

## Review Article

# The Many Facets of Sphingolipids in the Specific Phases of Acute Inflammatory Response

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Received 1 October 2017; Accepted 20 December 2017; Published 6 February 2018

Academic Editor: Vinod K. Mishra

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This review provides an overview on components of the sphingolipid superfamily, on their localization and metabolism. Information about the sphingolipid biological activity in cell physiopathology is given. Recent studies highlight the role of sphingolipids in inflammatory process. We summarize the emerging data that support the different roles of the sphingolipid members in specific phases of inflammation: (1) migration of immune cells, (2) recognition of exogenous agents, and (3) activation/differentiation of immune cells.

## 1. Introduction

*1.1. What Are Sphingolipids, How They Are Metabolized, and Where They Are Located?* Sphingolipids are an important class of lipids that play fundamental roles in cell life. The main sphingolipids include sphingomyelin (SM), ceramide (Cer), ceramide-1-phosphate (C1P), sphingosine (Sph), sphingosine-1-phosphate (S1P), glucosylceramide (GluCer), lactosylceramide (LacCer), gangliosides, and galactocerebrosides. All sphingolipids are metabolically interconnected in the equilibrium within the cells; each of them is rapidly produced to be used as a structural molecule and/or as a lipid mediator in response to a stimulus based on cellular needs. The metabolic pathway of sphingolipids includes (1) the de novo sphingolipid biosynthesis pathway with all intermediate bioactive molecules and (2) the SM catabolic pathway with all intermediate and final bioactive molecules (Figure 1).

SM is one of the most abundant sphingolipids in mammalian cell membranes. De novo synthesis of sphingolipids starts by the action of serine palmitoyltransferase that transfers the palmitic fatty acid to serine to form ketosphinganine that by ketosphinganine reductase is transformed into dihydroSph (also known as sphinganine). The dihydroSph is N-acetylated by ceramide synthases (CerS) that exists in 6

isoforms (CerS1 to CerS6), which add fatty acyl chains of defined chain length to dihydroSph to generate dihydroCer. DihydroCer is converted to Cer by the action of dihydroCer desaturase. Cer can be converted by the glucosylceramide synthase (GluCer-synthase) to GluCer and further to LacCer by the action of lactosylceramide synthase (LacCer-synthase). The complex glycosylated ceramides are generated by different glycosyltransferases which are specific to sugar residues that they transfer to generate gangliosides. In addition, Cer can be converted by the Cer galactosyltransferase into galactocerebroside. Cer is also a precursor for the synthesis of SM by the SM-synthase that exists in 2 isoforms SM-synthase 1 and SM-synthase 2, by adding to Cer the phosphorylcholine (PPC) of phosphatidylcholine (PC) [1]. In the *salvage pathway*, SM is hydrolysed by the sphingomyelinase (SMase) to PPC and Cer [1]. SMases are distinguished on the basis of their optimal pH and Km values in neutral (n-SMase), acid (a-SMase), and alkaline (alk-SMase) sphingomyelinase [2]. n-SMase is responsible for the degradation of SM of cell membranes and cytosol, a-SMase in lysosomes, and alk-SMase at intranuclear level [2, 3]. SM can also be used to synthesize PC as the donor of PPC that is added to diacylglycerol by the reverse sphingomyelin synthase (RSM-synthase) [3]. Cer generated by the SMases can be

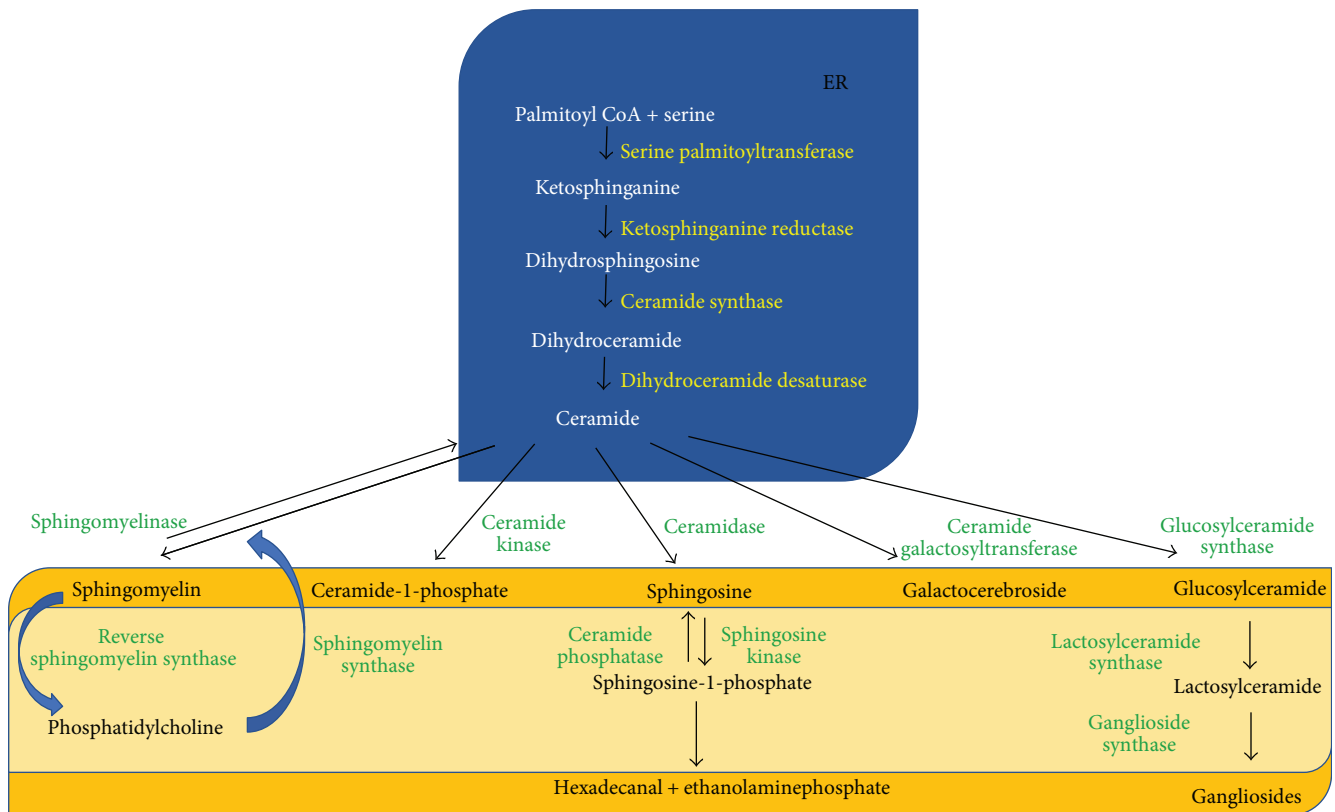


FIGURE 1: Sphingolipid pathways. The de novo synthesis occurs in endoplasmic reticulum (ER). Other biochemical pathways occur in the plasma, lysosome, and nucleus membranes.

either degraded to Sph and free fatty acids by the ceramidases [4] or directly converted to C1P by the Cer kinase (CerK) [5, 6]. C1P is generated at the inner plasma membrane of cells and transported to different intracellular compartments by the human lipid transfer protein CPTP (ceramide-1-phosphate transfer protein) [7]. In the plasma membranes, Sph is formed by neutral ceramidase in the presence of divalent cations at neutral pH [8]. Five human ceramidase genes have been identified, including *ASAH1*, *ASAH2*, *ACER1*, *ACER2*, and *ACER3*, and their protein products are classified as the acid (*ASAH1*), neutral (*ASAH2*), and alkaline ceramidase (*ACER1-3*) subtypes according to their pH optimum for their catalytic activity [9]. Ceramidases have diverse functions depending on their subcellular location and the local pH [10]. Acid ceramidase is responsible for the degradation of Cer within lysosomes [11]. Sph generated by the ceramidases can be phosphorylated to S1P by sphingosine kinase (SphK) [12]. There are 2 isoenzymes of the enzyme, SphK1 and SphK2. SphK1 is the major enzyme responsible for S1P formation [13, 14]. SphK1 is distributed in the cytosol, and SphK2 is localized in the nuclei [15]. The reaction is reversible thanks to SphPh. S1P can be irreversibly broken down by S1P lyase (S1PL) to ethanolamine phosphate and hexadecanal.

Sphingolipids and their metabolizing enzymes are expressed in almost all tissues of the mammalian organism and are distributed in different structures of the cells. CerK is particularly expressed in the brain, kidney, and liver, and

it is very low in the colon [16]. Free Sph is present in the liver [17], HL60 cells [18], neutrophils [19], membranes, and purified nuclei [20, 21]. S1P is expressed at a very low amount in fibroblasts [22].

**1.1.1. Sphingolipids in Lipid Rafts.** In cell membranes, sphingolipids are associated with sterols to form specialized plasma membrane microdomains called lipid rafts that facilitate ligand-receptor interaction, cellular signal transduction, and membrane protein trafficking [23, 24]. At the intranuclear level, the lipid microdomains are rich in SM and cholesterol and n-SMase is associated to the inner nuclear membrane of the liver [25] and embryonic hippocampal cells [26].

**1.2. Phases of Acute Inflammation and Mediators.** Inflammation can arise as a response of the immune system to damage caused by foreign bodies and/or infectious, chemical, physical agents with the aim to protect the organism. The acute inflammatory response envisages a series of specific phases that requires the involvement of different cells and molecules [27]. It begins with transient and nonconstant vasoconstriction due to the release of catecholamines, serotonin, thromboxane A<sub>2</sub>, and prostacyclin by different cells followed by vasodilation due to the release of nitric oxide, bradykinin, histamine, and E and I series prostaglandins resulting in slow blood flow. An increase in vascular permeability allows granulocytes (neutrophils, eosinophils, and basophils) or mast

cells, in relation to the stimulus that induced the inflammation, to interact with endothelium. The following sequence of events involves margination, rolling, adhesion, and transmigration of the immune cells to the damaged tissue to exercise their defense role. Circulating monocytes from the blood migrate to the inflamed tissue and transform into macrophages. Each phase requires a set of specific bioactive molecules [27]. For the resolution of inflammation, the following fundamental stages occur: reepithelization, angiogenesis, granulation tissue formation, and collagen deposition. If inflammation does not resolve, B-lymphocytes are transformed into plasma cells that produce antibodies against specific antigens of the exogenous agent that has caused the damage. However, inflammation could also be directed against autoantigens leading to an autoimmune response. In addition to the release of antibodies by B-lymphocytes, a hallmark of inflammation is the release of cytokines and chemokines by different cell types. Of particular importance in several inflammatory and autoimmune diseases is the cytokine tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) produced by activated monocytes and macrophages [28, 29]. TNF- $\alpha$  has been characterized as a pleiotropic cytokine critical for cell trafficking and inflammation [30] and host defense against various pathogens [31–33]. It is associated with several autoimmune and inflammatory diseases, such as rheumatoid arthritis [34], septic shock [29], and inflammatory bowel diseases [35].

## 2. Sphingolipids in Cell Pathophysiology

Sphingolipids are fundamental molecules for cell life since they play both structural and functional roles either in cell membranes or in the nucleus. As actors in cell structure, sphingolipids influence the fluidity of the cell membrane [36], nuclear membrane [2], and nuclear matrix [37] and form lipid rafts, as reported above. Functionally, sphingolipids act as second messengers in various signaling pathways, for example, via the activation or inhibition of several kinases and phosphatases [38–46]. In particular, Sph is capable to induce GTP cyclohydrolase [47], to inhibit NADPH oxidase by preventing the translocation of 47-phox, a cytosolic component of the enzyme, to the membranes [48], to inhibit CTP:phosphocholine cytidyltransferase [49], and to activate phospholipase D [50]. Activation of various plasma membrane receptors, such as the PDGFR [22, 51], the Fc $\epsilon$ RI, and Fc $\gamma$ RI [52] as well as the C5aR [53], was found to rapidly increase intracellular S1P production through the stimulation of the SphK. Inhibition of SphK stimulation strongly reduced or even prevented cellular events such as receptor-stimulated DNA synthesis, Ca<sup>2+</sup> mobilization, and vesicular trafficking. Interest in S1P focused recently on two distinct cellular actions of this lipid, namely, its function as an extracellular ligand, activating specific G protein-coupled receptors, and its role as an intracellular second messenger [54]. S1P acts through five specific receptors (S1P1, S1P2, S1P3, S1P4, and S1P5) [55–57]. Moreover, numerous publications demonstrate the ability of Sph [58–66] and S1P [67] to induce mobilization of Ca<sup>2+</sup> from intracellular stores. Ca<sup>2+</sup> seems to be an important regulator of CerK activity most likely by the interaction with calmodulin (CaM); the binding

of CaM to CerK enhances CerK activity and the formation of C1P intracellular [65]. In this way, sphingolipids are now known to mediate cell proliferation [66, 68], differentiation [69], apoptosis [70, 71], stress response [72, 73], neuronal physiopathology [74], platelet aggregation [75], inhibition of blood coagulation [76], and cancer [77].

## 3. Roles of Sphingolipids in Specific Phases of Acute Inflammation

Sphingolipids have different roles in fundamental phases of the acute inflammatory response such as migration of immune cells, recognition of exogenous agents, and activation/differentiation of immune cells.

**3.1. Migration of Immune Cells.** The infiltration of immune cells into the sites of lesion and further their migration to proximate lymph nodes requires their exit from the blood stream and their migration across the basement membrane, a process that involves the interaction of selectins and subsequent integrins on immune cells with glycoprotein ligands on endothelial cells [78]. This process requires both sphingolipids as intermediates of the de novo sphingolipid biosynthesis pathway and sphingolipids as intermediates of the SM catabolic pathway (Figure 2).

**3.1.1. Sphingolipids as Intermediates of the De Novo Biosynthesis Pathway.** Inhibition of sphingolipid de novo synthesis in THP-1 monocytes reduces their migration toward MCP-1 (monocyte chemoattractant protein 1). This could be achieved by knockdown of either serine palmitoyl-transferase subunit 1 or partitioning defective protein 3 (Par3) in these cells [79]. In CerS2 knockout mice, migration of neutrophils is impaired that is possibly related to reduced production of very long chain glycosphingolipids and a reduced G-CSF expression as well as Lyn signaling in these mice [80]. Glycosphingolipids on human myeloid cells stabilize the binding of these cells to E-selectin [81, 82]. Downregulation of GluCer synthase (UGCG) in HL-60 cells reduced rolling of HL-60 on E-selectin but not on P-selectin bearing human umbilical vein endothelial cells (HUVEC). This leads to a reduced cell transmigration of UGCG-downregulated HL-60 cells across a HUVEC monolayer [83]. Also, Iwabuchi et al. have shown that migration of human neutrophils depends on LacCer at the plasma membrane [84]. Binding of a specific anti-Lac-Cer antibody (T5A7) to neutrophils induces migration. This is likely due to the activation of Src-family kinase Lyn and phosphoinositol 3 kinase (PI3K). But possibly also a G $\alpha$ - (i/o) coupled receptor is involved [84]. Especially in this work, it was demonstrated that there are distinct differences between human and mouse neutrophils. They detected a ~20-fold lower LacCer content in plasma membranes in mouse than in human neutrophils [84]. Furthermore, the immune system of mice differs fundamentally from humans. For instance, in humans, neutrophils constitute with approximately 50–70% of the major population of circulating leukocytes, whereas in mice, neutrophils represent only 5–10% of blood leukocytes [85]. Therefore, comparing data generated in mice to human pathological

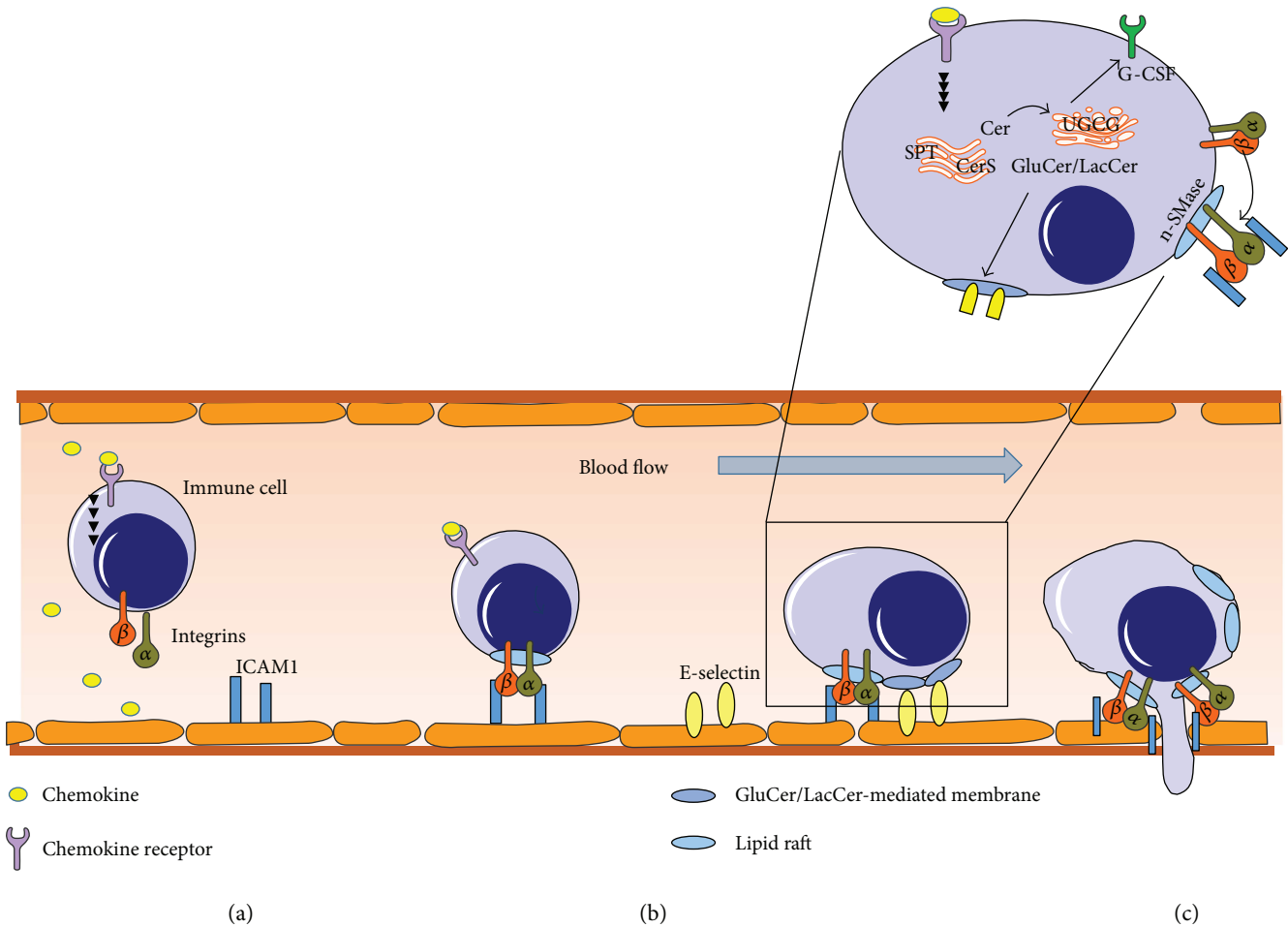


FIGURE 2: Activation, migration, and invasion of immune cells from the blood are influenced by several sphingolipids. (a) Initial adhesion step mediated by activation of immune cells by cytokines or chemokines and subsequent activation of integrins. (b) Activated integrins translocate into lipid rafts and bind to endothelial receptors like ICAM1. GluCer-enriched membranes are important for interaction with E-selectin. (c) Migration of immune cells is dependent on n-SMase and C1P.

conditions is critical, as the genetic or chemical-induced mouse disease models are only in part comparable to the situation in humans.

**3.1.2. Sphingolipids as Intermediates of the SM Catabolic Pathway.** Treatment of neutrophils with the chemotaxin, formylmethionylleucylphenylalanine, leads to a translocation of n-SMase to plasma membranes where it is involved in the spreading and the extension of pseudopods. In these cells, n-SMase seems to influence the distribution of Rac 1/2 and RhoA to the leading edge of migration as this polarized distribution is totally lost when n-SMase was inhibited [86]. In line with these findings, factors associated with n-SMase activity-deficient leukocytes show also a disrupted chemotactic response. They protrude pseudopodia in all directions instead of having one clear leading edge, indicating that these cells are impaired in their navigation capacity to chemokines [87]. a-SMase is involved in mast cell migration [88].

In CerK-deficient macrophages, the MCP-1/CCR2 signaling pathway is attenuated implicating that C1P plays a role in macrophage migration [89]. Incubation of macrophages

with C1P stimulates cell migration in a G(i) protein-dependent manner that causes phosphorylation of extracellularly regulated kinases (ERK) 1 and 2, protein kinase B, and activation of phospholipase C- $\beta$ 2 (PLC- $\beta$ 2) [90, 91]. Also, metalloproteinase- (MMP-) 2 and MMP-9 are upregulated in a PI3K and ERK 1/2- dependent manner after stimulation of macrophages with C1P [92]. Further studies show that C1P induces the release of macrophage chemoattractant protein-1 (MCP-1/CCL2 (CC-chemokine ligand 2)), which binds to the CCR2 or CCR4 receptor and influences thereby monocyte migration [93]. The role of S1P in immune cell activation and migration is already summarized in another review within this special issue, to which we want to refer here [94].

**3.2. Recognition of Exogenous Agents.** Toll-like receptors (TLRs) together with Nod-like receptors (NLRs) belong to a group of receptors (pattern recognition receptors (PRRs)) that are able to indicate the presence of several pathogen-associated molecular patterns (PAMPs) to immune cells which enable them to distinguish foreign



organisms such as viruses, bacteria, fungi, and parasites from host cells [95–97]. Many receptors, important for the immune response, are clustered in lipid rafts upon activation [98–100]. The regulation of T-cell receptor signaling occurs in lipid raft [101–103]. Nevertheless, complex sphingolipids can also act as direct recognition receptors for microorganisms. However, binding of pathogens to a single saccharide is only weak, but adherence to multiple saccharides, as they are observed in lipid rafts, is strong [104, 105]. Various TLRs exhibit a cholesterol or sphingolipid binding-like sequence in their transmembrane region, indicating that they directly interact with specific lipids associated with rafts in the membrane [106]. Increased virus uptake was related to an enhanced expression of CD150 in lipid rafts at the cell surface [107]. The binding of pathogens to its cellular recognition receptors involves both sphingolipids as intermediates of the de novo biosynthesis pathway and sphingolipids as intermediates of the SM catabolic pathway.

**3.2.1. Sphingolipids as Intermediates of the De Novo Biosynthesis Pathway.** Knockout animals of the subunit 2 of the serine palmitoyltransferase and of SM-synthase 1 or SM-synthase 2 in macrophages influence TLR signaling by preventing its proper translocation to the plasma membrane [108–110]. In CerS2 knockout mice, we could demonstrate that these mice develop more severe colitis after dextran sodium salt (DSS) treatment than CerS2 WT mice. CerS2-ko mice show significant changes in several sphingolipids like a drop in very long-chain CerS/(dh)-CerS and an increase in long-chain CerS/(dh)-CerS. These changes are associated with a loss of the tight junction protein ZO-1 in colon epithelial cells leading to weakened endogenous defense against the microbiome and an increase in several immune cells in the colon [111]. Blocking of the dihydroCer desaturase, leading to the accumulation of dihydroCer in cultured cells, inhibits the infection of cells with HIV-1 [112]. GluCer or LacCer form membrane microdomains for the recognition and phagocytosis of microorganism. Microorganisms bind to PRRs at dendritic cells which undergo a conformational change, resulting in the translocation of the receptors into LacCer-enriched platforms [113]. Additionally, it has been shown that LacCer and complex glycosphingolipids of cellular membranes such as Gb3 and GM1 are direct binding structures for bacteria and viruses (like *Haemophilus influenzae*, *Neisseria meningitidis*, and *Polyomavirus*) [114, 115]. Berenson et al. could show that binding of *E. coli* enterotoxin LT-IIc to glycosphingolipids requires the whole glycosphingolipid and that neither the oligosaccharide nor the Cer alone is sufficient for binding. Furthermore, they demonstrated that also the chain length of the glycosphingolipid is important for the binding of LT-IIc [116].

**3.2.2. Sphingolipids as Intermediates of the SM Catabolic Pathway.** The activation of the TLR4 by *Helicobacter pylori* or lipopolysaccharide (LPS) is dependent on the activation of a-SMase and Cer formation [117]. CerS are necessary and sufficient to mediate TLR4 translocation to the plasma membrane in a Src-dependent manner [117]. Avota and

coworkers demonstrated that binding of measles virus to pattern recognition receptor on DCs leads to an activation of a-SMase and enhanced virus uptake into DCs [107]. In fact, it induces SMase activity that subsequently increases Cer-rich membrane platforms and initiated intracellular signaling processes by clustering different receptors into these platforms. Also, infection of human epithelial cells by rhinoviruses is dependent on a-SMase activity, as pharmacological inhibition or genetic deficiency of a-SMase prevents this infection [99]. This group showed further that activation of a-SMase comprises its translocation from intracellular compartments onto the cell surface that takes place by a microtubule- and microfilament-dependent transport mechanism. Also, infection with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Neisseria gonorrhoeae* requires the activation of a-SMase and subsequently the formation of Cer-enriched membrane platforms [114, 118, 119]. Treatment of mice with the a-SMase inhibitor amitriptyline and antibiotics prevents lethal *Staphylococcus aureus*-induced sepsis and death [120]. This observation leads to a phase II randomised, double-blind, placebo-controlled trial investigating the a-SMase inhibitor amitriptyline in patients with cystic fibrosis. The amitriptyline-treated CF patients showed a significant increase in lung function and weight after treatment for 1–3 years in comparison to placebo-treated patients [120]. These data indicate that inhibition of a-SMase might be a new therapy option for patients with cystic fibrosis, who suffer from perpetual infections.

**3.3. Activation/Differentiation of Immune Cells.** After binding of microorganism, their toxins, or cytokines to extracellular receptors, the immune cells get activated and reprogrammed to distinct subtypes. This reprogramming is a cell type-specific process and includes metabolic changes, DNA rearrangements, and differentiation. Furthermore, it leads to the production and release of cytokines and chemokines by these cells. The inflammasomes are multimeric protein complexes in macrophages and neutrophils that are involved in the production of the proinflammatory cytokine IL-1 $\beta$  and activated after the binding of microbes to these cells [117]. Also, activation and differentiation of immune cells involve both sphingolipids of the de novo biosynthesis pathway and the sphingolipid metabolic pathway from SM catabolism.

**3.3.1. Sphingolipids as Intermediates of the De Novo Biosynthesis Pathway.** In hepatocytes, overexpression of CerS6, which is responsible for the production of C16-Cer, leads to an elevated TNF- $\alpha$  secretion via the activation of the p38 mitogen-activated protein kinase (MAPK) [121]. In line with these data, Ali et al. observed an enhanced activity of the TNF- $\alpha$ -converting enzyme (TACE) after the treatment of CerS2 knockout mice with LPS [122]. This results in elevated TNF- $\alpha$  level and worsens the outcome of LPS-induced septic shock in CerS2-ko mice. CerS2 knockout mice show also an upregulation of C16-Cer as a compensation mechanism to the loss of C24:0- and C24:1-Cer [123]. The activation of inflammasomes resulting in the release of IL-1 $\beta$  in macrophages seems to be independent from the sphingolipid de novo synthesis [124].

**3.3.2. Roles of Sphingolipids as Intermediates of the SM Catabolic Pathway.** Both in the Cfr-deficient mice (mouse model for cystic fibrosis) and in the high-fat diet- (HFD-) induced glomerular injury mouse model, the activation of aSMase is associated with enhanced activity of inflammasomes. Knockout of aSMase or caspase 1 inhibition protected Cfr-deficient mice from lung inflammation and kidney from HFD-induced injury [123]. Furthermore, knockdown of aSMase in both mouse models prevents the production and release of IL-1. These data indicate that the activation of the aSMase is an essential event in the activation of inflammasomes and subsequent production of proinflammatory cytokines. To which extent the generation of CerS by the aSMase itself is important for the formation of the inflammasome is not known as very recently it has been shown that activation of the S1PR1 contributes to the expression of NLRP3 inflammasome. As mentioned above, all sphingolipids are metabolically interconnected; therefore, CerS generated by the aSMase are subsequently degraded by the ceramidase to sphingosine which can be phosphorylated to S1P that subsequently can activate different receptors. Weichand et al. have shown that the knockdown of the S1PR1 in tumor-associated macrophages leads to a reduced NLRP3 expression and reduced IL-1 $\beta$  levels [125], indicating that S1P might be the important player in the activation of the inflammasome. However, Wang et al. demonstrated that loss of acid ceramidase 3 (Acer3), leading to an elevation in C18:1-Cer in blood mononuclear cells (BMCs), aggravates DSS-induced colitis, which is related to the hyperactivation of the innate immune system [126]. *In vitro*, Wang et al. could demonstrate that Acer3 deficiency enhanced and prolonged LPS-induced increases in the mRNA levels of IL-1 $\beta$ , IL-6, IL-23a, and TNF- $\alpha$  [126]. Activation of bone marrow-derived mast cells (BMMCs) by antigen/IgE leads to a 2.5-fold increase in aSMase activity, an increase in [Ca<sup>2+</sup>]<sub>i</sub>, and the release of  $\beta$ -hexosaminidase. All these were impaired in antigen/IgE-stimulated aSMase (-/-) BMMCs or by cotreatment with the aSMase inhibitor, amitriptyline [88]. These data indicated that aSMase-generated CerS are important for the activation of immune cells and the production of proinflammatory cytokines. CerK is also expressed in peripheral blood leukocytes. Here, it plays a role in phagocytosis and promotes phagolysosomal formation and fusion in polymorphonuclear leukocytes in a Ca<sup>2+</sup>-dependent manner [127, 128]. The degranulation of mast cells after binding to IgE is not only associated to an activation of aSMase but also positively influenced by the Ca<sup>2+</sup>-dependent CerK activation and consequent C1P production [16, 65]. C1P is also involved in the release of various proinflammatory prostanoids like PGE<sub>2</sub> (prostaglandin E<sub>2</sub>) as the endogenous generation of C1P binds to the C2 domain of the cytosolic phospholipase A2 $\alpha$  (cPLA2 $\alpha$ ) promoting thereby cPLA2 $\alpha$  translocation to cellular membranes [129–131]. Interestingly, PGE<sub>2</sub> can either promote or inhibit mast cell degranulation, dependent on the EP2/EP3 (E-prostanoid) receptor status of the cells [132]. This means that under some circumstances C1P might also inhibit mast cell degranulation. Unfortunately, all these mechanisms seem only slightly to be influenced in CerK<sup>-/-</sup> mice [133], which calls

the importance of C1P and CerK for mast cell function and eicosanoid synthesis into question. However, a detailed lipid analysis in these mice demonstrated that C1P levels are unchanged in the plasma of CerK<sup>-/-</sup> mice [134], indicating that an adaptation mechanism takes place in these mice that compensates for the loss of CerK. However, Wijesinghe et al. already assumed that C1P subspecies especially not only d(18:1/18:0) but also C16:0 and C24:0 or C24:1 C1P are generated by alternative pathways besides CerK [135], but until now, it is not known how. Additionally, to intracellularly generated C1P, also, extracellular C1P influences immune cell activation. So the addition of C1P to LPS-activated neutrophils inhibits LPS-induced IL-8 production and NF $\kappa$ B activation [136]. Also *in vivo*, in the LPS-induced acute lung injury mouse model, C1P attenuates the LPS-induced inflammation [136]. These data indicate that intracellular- and extracellular-generated C1P influences immune cells thereby rather leading to contrary effects. Binding of invaders to glycosphingolipids is important for entry into host cells by phagosomes and seems to prevent their fusion with lysosomes [137]. Sph is capable to inhibit phosphatidic acid phosphohydrolase in neutrophils [138] and the release of Ca<sup>2+</sup> from endothelial cells [139]. Among SphKs, SphK1 is the isoform activated by proinflammatory cytokines [48] and plays an essential role in the TNF- $\alpha$ -triggered intracellular Ca<sup>2+</sup> signal, degranulation, cytokine production, and activation of NF $\kappa$ B, thus suggesting a pivotal role for SphK1 on the proinflammatory responses triggered by TNF- $\alpha$  [140, 141]. It is known that some of the effects of TNF- $\alpha$  are orchestrated by sphingolipid metabolites [142]. TNF- $\alpha$  stimulates the elevation of Cer and Sph, which has been shown to play a role in apoptosis in various cell types [143]. We found that Sph accumulates in the liver of mice treated with recombinant TNF- $\alpha$  [144]. The observed relationship between the toxicity of TNF- $\alpha$  mutants, the toxicity of Sph, and the extent of its accumulation in a murine liver provides evidence suggesting that Sph may be a mediator of TNF- $\alpha$ -induced cell damage and death [145]. TNF- $\alpha$  activates SM cycle during the induction of apoptosis [145]. Stimulation of HL60 cells with TPA (12-O-tetradecanoylphorbol-13-acetate) and simultaneous treatment with radioactive labelled serine leads to an increase in radiolabelled GluCer, LacCer, and GM3 in these cells after 48 h [146]. Receptors in LacCer-enriched platforms interact with the Src family kinase Lyn initiating the phagocytosis of the microorganism. The interaction between LynK and G protein can be influenced by the LacCer chain length thereby impacting the activation of neutrophils [113, 147].

#### 4. Conclusions

In conclusion, when we investigate the role of a specific sphingolipid in physiological or pathophysiological processes, we have to keep in mind that sphingolipids are in a distinct equilibrium in the cell. Using mice, which bear a knockout for a specific gene of the sphingolipid pathway, the concentration of the sphingolipids depends on both the specific enzyme which is downregulated and various other sphingolipids which are a precursor or derivative of this

sphingolipid. Several compensation mechanisms are induced by the accumulation of one specific sphingolipid, due to the knockdown of an enzyme using it as a substrate. This leads to an increase in sideways which also metabolizes this substrate leading to an upregulation of other sphingolipids. Therefore, we have to keep in mind that the observed effects in specific knockout mice might be related to the deregulation of various sphingolipids and/or the disturbance of an equilibrium and it is very likely that various sphingolipids together influence inflammatory processes.

## Abbreviations

a-SMase:	Acid sphingomyelinase
alk-SMase:	Alkaline sphingomyelinase
C1P:	Ceramide 1-phosphate
Cer:	Ceramide
CerK:	Ceramide kinase
CerS:	Ceramide synthases
C1TP:	Ceramide-1-phosphate transfer protein
DAG:	Diacylglycerol
GluCer-synthase:	Glucosylceramide synthase
GluCer:	Glucosylceramide
LacCer-synthase:	Lactosylceramide synthase
LacCer:	Lactosylceramide
LynK:	Lyn kinase
n-SMase:	Neutral sphingomyelinase
PAMPs:	Pathogen-associated molecular patterns
PC:	Phosphatidylcholine
PPC:	Phosphorylcholine
PRRs:	Pattern recognition receptors
RSM-synthase:	Reverse sphingomyelin synthase
S1P:	Sphingosine-1-phosphate
S1PL:	S1P lyase
SM:	Sphingomyelin
SM:	Synthase
SMase:	Sphingomyelinase
Sph:	Sphingosine
SphK:	Sphingosine kinase
SphPh:	Sphingosine phosphatase.

## Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Authors' Contributions

Sabine Grösch and Alice V. Alessenko contributed equally to this work.

## Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) [SFB 1039/1, B05] and DFG Project [GR2011/3-2].

## References

- [1] E. Albi, C. A. M. La Porta, S. Cataldi, and M. V. Magni, "Nuclear sphingomyelin-synthase and protein kinase C  $\delta$  in melanoma cells," *Archives of Biochemistry and Biophysics*, vol. 438, no. 2, pp. 156–161, 2005.
- [2] E. Albi and M. P. Viola Magni, "The role of intranuclear lipids," *Biology of the Cell*, vol. 96, no. 8, pp. 657–667, 2004.
- [3] E. Albi, R. Lazzarini, and M. V. Magni, "Reverse sphingomyelin-synthase in rat liver chromatin," *FEBS Letters*, vol. 549, no. 1-3, pp. 152–156, 2003.
- [4] N. Coant, W. Sakamoto, C. Mao, and Y. A. Hannun, "Ceramidases, roles in sphingolipid metabolism and in health and disease," *Advances in Biological Regulation*, vol. 63, pp. 122–131, 2017.
- [5] R. N. Kolesnick and M. R. Hemer, "Characterization of a ceramide kinase activity from human leukemia (HL-60) cells. Separation from diacylglycerol kinase activity," *The Journal of Biological Chemistry*, vol. 265, no. 31, pp. 18803–18808, 1990.
- [6] Y. A. Hannun and L. M. Obeid, "The ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind," *The Journal of Biological Chemistry*, vol. 277, no. 29, pp. 25847–25850, 2002.
- [7] D. K. Simanshu, R. K. Kamlekar, D. S. Wijesinghe et al., "Non-vesicular trafficking by a ceramide-1-phosphate transfer protein regulates eicosanoids," *Nature*, vol. 500, no. 7463, pp. 463–467, 2013.
- [8] C. W. Slife, E. Wang, R. Hunter et al., "Free sphingosine formation from endogenous substrates by a liver plasma membrane system with a divalent cation dependence and a neutral pH optimum," *The Journal of Biological Chemistry*, vol. 264, no. 18, pp. 10371–10377, 1989.
- [9] C. Mao and L. M. Obeid, "Ceramidases: regulators of cellular responses mediated by ceramide, sphingosine, and sphingosine-1-phosphate," *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, vol. 1781, no. 9, pp. 424–434, 2008.
- [10] N. Okino, X. X. He, S. Gatt, K. Sandhoff, M. Ito, and E. H. Schuchman, "The reverse activity of human acid ceramidase," *The Journal of Biological Chemistry*, vol. 278, no. 32, pp. 29948–29953, 2003.
- [11] K. P. Bhabak, B. Kleuser, A. Huwiler, and C. Arenz, "Effective inhibition of acid and neutral ceramidases by novel B-13 and LCL-464 analogues," *Bioorganic & Medicinal Chemistry*, vol. 21, no. 4, pp. 874–882, 2013.
- [12] P. P. Van Veldhoven and G. P. Mannaerts, "Subcellular localization and membrane topology of sphingosine-1-phosphate lyase in rat liver," *The Journal of Biological Chemistry*, vol. 266, no. 19, pp. 12502–12507, 1991.
- [13] T. Kohama, A. Olivera, L. Edsall, M. M. Nagiec, R. Dickson, and S. Spiegel, "Molecular cloning and functional characterization of murine sphingosine kinase," *The Journal of Biological Chemistry*, vol. 273, no. 37, pp. 23722–23728, 1998.
- [14] C. A. Oskeritzian, S. E. Alvarez, N. C. Hait, M. M. Price, S. Milstien, and S. Spiegel, "Distinct roles of sphingosine kinases 1 and 2 in human mast-cell functions," *Blood*, vol. 111, no. 8, pp. 4193–4200, 2008.
- [15] N. Igarashi, T. Okada, S. Hayashi, T. Fujita, S. Jahangeer, and S. C. Nakamura, "Sphingosine kinase 2 is a nuclear protein and inhibits DNA synthesis," *The Journal of Biological Chemistry*, vol. 278, no. 47, pp. 46832–46839, 2003.



- [16] M. Sugiura, K. Kono, H. Liu et al., "Ceramide kinase, a novel lipid kinase. Molecular cloning and functional characterization," *The Journal of Biological Chemistry*, vol. 277, no. 26, pp. 23294–23300, 2002.
- [17] S. A. Rusakov, G. N. Filippova, M. I. Pushkareva, V. G. Korobko, and A. V. Alesenko, "The effect of tumor necrosis factor on the free sphingosine level and sphingomyelinase in murine liver cells and nuclei," *Biokhimiia*, vol. 58, pp. 724–732, 1991.
- [18] A. H. Merrill Jr., A. M. Sereni, V. L. Stevens, Y. A. Hannun, R. M. Bell, and J. M. Kinkade Jr., "Inhibition of phorbol ester-dependent differentiation of human promyelocytic leukemia (HL-60) cells by sphinganine and other long-chain bases," *The Journal of Biological Chemistry*, vol. 261, no. 27, pp. 12610–12615, 1986.
- [19] E. Wilson, E. Wang, R. E. Mullins et al., "Modulation of the free sphingosine levels in human neutrophils by phorbol esters and other factors," *The Journal of Biological Chemistry*, vol. 263, no. 19, pp. 9304–9309, 1988.
- [20] A. V. Alesenko, E. A. Pantaz, M. I. Pushkareva, S. A. Rusakov, and G. N. Filippova, "Change in the level of endogenous sphingosine in cell nuclei from regenerating rat liver," *Biokhimiia*, vol. 58, no. 3, pp. 461–470, 1993.
- [21] A. V. Alesenko, P. I. Boikov, L. B. Drobot, S. A. Rusakov, and G. N. Filippova, "Change in the level of sphingosine in rat liver nuclei and cells during oncogene superexpression induced by cycloheximide," *Biokhimiia*, vol. 59, no. 7, pp. 1076–1087, 1994.
- [22] A. Olivera and S. Spiegel, "Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens," *Nature*, vol. 365, no. 6446, pp. 557–560, 1993.
- [23] T. Kobayashi, M. Takahashi, Y. Nagatsuka, and Y. Hirabayashi, "Lipid rafts: new tools and a new component," *Biological and Pharmaceutical Bulletin*, vol. 29, no. 8, pp. 1526–1531, 2006.
- [24] K. Simons and E. Ikonen, "Functional rafts in cell membranes," *Nature*, vol. 387, no. 6633, pp. 569–572, 1997.
- [25] G. Cascianelli, M. Villani, M. Tosti et al., "Lipid microdomains in cell nucleus," *Molecular Biology of the Cell*, vol. 19, no. 12, pp. 5289–5295, 2008.
- [26] E. Bartoccini, F. Marini, E. Damaskopoulou et al., "Nuclear lipid microdomains regulate nuclear vitamin D<sub>3</sub> uptake and influence embryonic hippocampal cell differentiation," *Molecular Biology of the Cell*, vol. 22, no. 17, pp. 3022–3031, 2011.
- [27] M. B. Serra, W. A. Barroso, N. N. da Silva et al., "From inflammation to current and alternative therapies involved in wound healing," *International Journal of Inflammation*, vol. 2017, Article ID 3406215, 17 pages, 2017.
- [28] M. El Alwani, B. X. Wu, L. M. Obeid, and Y. A. Hannun, "Bioactive sphingolipids in the modulation of the inflammatory response," *Pharmacology & Therapeutics*, vol. 112, no. 1, pp. 171–183, 2006.
- [29] B. Beutler and A. Cerami, "The biology of cachectin/TNF—a primary mediator of the host response," *Annual Review of Immunology*, vol. 7, no. 1, pp. 625–655, 1989.
- [30] G. Kassiotis and G. Kollias, "Uncoupling the proinflammatory from the immunosuppressive properties of tumor necrosis factor (TNF) at the p55 TNF receptor level: implications for pathogenesis and therapy of autoimmune demyelination," *Journal of Experimental Medicine*, vol. 193, no. 4, pp. 427–434, 2001.
- [31] K. Pfeffer, T. Matsuyama, T. M. Kundig et al., "Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *L. monocytogenes* infection," *Cell*, vol. 73, no. 3, pp. 457–467, 1993.
- [32] J. Rothe, W. Lesslauer, H. Lotscher et al., "Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by *Listeria monocytogenes*," *Nature*, vol. 364, no. 6440, pp. 798–802, 1993.
- [33] J. L. Flynn, M. M. Goldstein, J. Chan et al., "Tumor necrosis factor- $\alpha$  is required in the protective immune response against mycobacterium tuberculosis in mice," *Immunity*, vol. 2, no. 6, pp. 561–572, 1995.
- [34] M. Feldmann, "Development of anti-TNF therapy for rheumatoid arthritis," *Nature Reviews Immunology*, vol. 2, no. 5, pp. 364–371, 2002.
- [35] L. B. Dudnik, A. N. Tsiupko, M. A. Shupik et al., "Relationship of tumor necrosis factor  $\alpha$  expression with activation of sphingomyelinase and lipid peroxidation after removal of cholesterol factor," *Biology Bulletin*, vol. 34, no. 1, pp. 5–13, 2007.
- [36] F. C. Santos, A. S. Fernandes, C. A. C. Antunes et al., "Reorganization of plasma membrane lipid domains during conidial germination," *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, vol. 1862, no. 2, pp. 156–166, 2017.
- [37] E. Albi, S. Cataldi, G. Rossi, and M. V. Magni, "A possible role of cholesterol-sphingomyelin/phosphatidylcholine in nuclear matrix during rat liver regeneration," *Journal of Hepatology*, vol. 38, no. 5, pp. 623–628, 2003.
- [38] A. Conway, N. J. Pyne, and S. Pyne, "Ceramide-dependent regulation of p42/p44 mitogen-activated protein kinase and c-Jun N-terminal-directed protein kinase in cultured airway smooth muscle cells," *Cell Signal*, vol. 12, no. 11–12, pp. 737–743, 2000.
- [39] T. E. Fox, K. L. Houck, S. M. O'Neill et al., "Ceramide recruits and activates protein kinase C  $\zeta$  (PKC $\zeta$ ) within structured membrane microdomains," *The Journal of Biological Chemistry*, vol. 282, no. 17, pp. 12450–12457, 2007.
- [40] I. Galve-Roperh, A. Haro, and I. Diaz-Laviada, "Ceramide-induced translocation of protein kinase C  $\zeta$  in primary cultures of astrocytes," *FEBS Letters*, vol. 415, no. 3, pp. 271–274, 1997.
- [41] F. Jiang, K. Jin, S. Huang et al., "Liposomal C6 ceramide activates protein phosphatase 1 to inhibit melanoma cells," *PLoS One*, vol. 11, no. 9, article e0159849, 2016.
- [42] R. T. Dobrowsky, C. Kamibayashi, M. C. Mumby, and Y. A. Hannun, "Ceramide activates heterotrimeric protein phosphatase 2A," *The Journal of Biological Chemistry*, vol. 268, no. 21, pp. 15523–15530, 1993.
- [43] M. Verheij, R. Bose, X. H. Lin et al., "Requirement for ceramide-initiated SAPK/JNK signalling in stress-induced apoptosis," *Nature*, vol. 380, pp. 75–79, 1996.
- [44] B. Yao, Y. Zhang, S. Delikat, S. Mathias, S. Basu, and R. Kolesnick, "Phosphorylation of Raf by ceramide-activated protein kinase," *Nature*, vol. 378, no. 6554, pp. 307–310, 1995.
- [45] G. C. Globe, S. Stribling, L. M. Obeid, and Y. A. Hannun, "Protein kinase C isoenzymes: regulation and function," *Cancer Survivor*, vol. 27, pp. 213–248, 1996.
- [46] Y. A. Hannun and R. M. Bell, "Regulation of protein kinase C by sphingosine and lysosphingolipids," *Clinica Chimica Acta*, vol. 185, no. 3, pp. 333–345, 1989.



- [47] P. Z. Anastasiadis, D. M. Kuhn, J. Blitz, B. A. Imerman, M. C. Louie, and R. A. Levine, "Regulation of tyrosine hydroxylase and tetrahydrobiopterin biosynthetic enzymes in PC12 cells by NGF, EGF and IFN- $\gamma$ ," *Brain Research*, vol. 713, no. 1-2, pp. 125-133, 1996.
- [48] J. I. Sasaki, M. Yamaguchi, S. Saeki, H. Yamane, N. Okamura, and S. Ishibashi, "Sphingosine inhibition of NADPH oxidase activation in a cell-free system," *The Journal of Biochemistry*, vol. 120, no. 4, pp. 705-709, 1996.
- [49] Y. Lavie, O. Piterman, and M. Liscovitch, "Inhibition of phosphatidic acid phosphohydrolase activity by sphingosine. Dual action of sphingosine in diacylglycerol signal termination," *FEBS Letters*, vol. 277, no. 1-2, pp. 7-10, 1990.
- [50] Y. Lavie and M. Liscovitch, "Activation of phospholipase D by sphingoid bases in NG108-15 neural-derived cells," *The Journal of Biological Chemistry*, vol. 265, no. 7, pp. 3868-3872, 1990.
- [51] K. E. Bornfeldt, L. M. Graves, E. W. Raines et al., "Sphingosine-1-phosphate inhibits PDGF-induced chemotaxis of human arterial smooth muscle cells: spatial and temporal modulation of PDGF chemotactic signal transduction," *The Journal of Cell Biology*, vol. 130, no. 1, pp. 193-206, 1995.
- [52] R. Alemany, D. M. Zu Henringdorf, C. J. van Koppen, and K. H. Jacobs, "Formyl peptide receptor signaling in HL-60 cells through sphingosine kinase," *The Journal of Biological Chemistry*, vol. 274, no. 7, pp. 3994-3999, 1999.
- [53] F. B. M. Ibrahim, S. J. Pang, and A. J. Melendez, "Anaphylatoxin signaling in human neutrophils: a key role for sphingosine kinase," *The Journal of Biological Chemistry*, vol. 279, no. 43, pp. 44802-44811, 2004.
- [54] D. Meyer zu Heringdorf, C. J. van Koppen, and K. H. Jacobs, "Molecular diversity of sphingolipid signalling," *FEBS Letters*, vol. 410, no. 1, pp. 34-38, 1997.
- [55] C. Donati and P. Bruni, "Sphingosine 1-phosphate regulates cytoskeleton dynamics: implications in its biological response," *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 1758, no. 12, pp. 2037-2048, 2006.
- [56] A. Matecki and T. Pawelczyk, "Regulation of phospholipase C  $\delta 1$  by sphingosine," *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 1325, no. 2, pp. 287-296, 1997.
- [57] K. Wong and L. Kwan-Yeung, "Sphingosine mobilizes intracellular calcium in human neutrophils," *Cell Calcium*, vol. 14, no. 6, pp. 493-505, 1993.
- [58] A. Olivera, H. Zhang, R. O. Carlson, M. E. Mattie, R. R. Schmidt, and S. Spiegel, "Stereospecificity of sphingosine-induced intracellular calcium mobilization and cellular proliferation," *The Journal of Biological Chemistry*, vol. 269, no. 27, pp. 17924-17930, 1994.
- [59] S. Sakano, H. Takemura, K. Yamada, K. Imoto, M. Kaneko, and H. Ohshika, "Ca<sup>2+</sup> mobilizing action of sphingosine in Jurkat human leukemia T cells. Evidence that sphingosine releases Ca<sup>2+</sup> from inositol trisphosphate- and phosphatidic acid-sensitive intracellular stores through a mechanism independent of inositol trisphosphate," *The Journal of Biological Chemistry*, vol. 271, no. 19, pp. 11148-11155, 1996.
- [60] D. H. Needleman, B. Aghdasi, A. B. Seryshev, G. J. Schroepfer Jr., and S. L. Hamilton, "Modulation of skeletal muscle Ca<sub>2</sub>(+)-release channel activity by sphingosine," *American Journal of Physiology-Cell Physiology*, vol. 272, no. 5, pp. C1465-C1474, 1997.
- [61] W. C. Huang and S. H. Chueh, "Calcium mobilization from the intracellular mitochondrial and nonmitochondrial stores of the rat cerebellum," *Brain Research*, vol. 718, no. 1-2, pp. 151-158, 1996.
- [62] S. J. Pandol, M. S. Schoeffer-Payne, A. S. Gukovskaya, and R. E. Rutherford, "Sphingosine regulates Ca<sup>2+</sup>-ATPase and reloading of intracellular Ca<sup>2+</sup> stores in the pancreatic acinar cell," *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 1195, no. 1, pp. 45-50, 1994.
- [63] M. Mattie, G. Brooker, and S. Spiegel, "Sphingosine-1-phosphate, a putative second messenger, mobilizes calcium from internal stores via an inositol trisphosphate-independent pathway," *The Journal of Biological Chemistry*, vol. 269, no. 5, pp. 3181-3188, 1994.
- [64] T. K. Ghosh, J. Bian, and D. L. Gill, "Sphingosine 1-phosphate generated in the endoplasmic reticulum membrane activates release of stored calcium," *The Journal of Biological Chemistry*, vol. 269, no. 36, pp. 22628-22635, 1994.
- [65] S. Mitsutake and Y. Igarashi, "Calmodulin is involved in the Ca<sup>2+</sup>-dependent activation of ceramide kinase as a calcium sensor," *The Journal of Biological Chemistry*, vol. 280, no. 49, pp. 40436-40441, 2005.
- [66] A. Alessenko and S. Chatterjee, "Neutral sphingomyelinase: localization in rat liver nuclei and involvement in regeneration/proliferation," *Molecular and Cellular Biochemistry*, vol. 143, no. 2, pp. 169-174, 1995.
- [67] G. Wang, S. D. Spassieva, and E. Bieberich, "Ceramide and S1P signaling in embryonic stem cell differentiation," *Methods in Molecular Biology*, vol. 1697, pp. 153-171, 2018.
- [68] S. Cataldi, M. Codini, G. Cascianelli et al., "Nuclear lipid microdomain as resting place of dexamethasone to impair cell proliferation," *International Journal of Molecular Science*, vol. 15, no. 11, pp. 19832-19846, 2014.
- [69] M. Garcia-Gil, A. Lazzarini, R. Lazzarini et al., "Serum deprivation alters lipid profile in HN9.10e embryonic hippocampal cells," *Neuroscience Letters*, vol. 589, pp. 83-87, 2015.
- [70] E. Albi, S. Cataldi, G. Rossi et al., "The nuclear ceramide/diacylglycerol balance depends on the physiological state of thyroid cells and changes during UV-C radiation-induced apoptosis," *Archives of Biochemistry and Biophysics*, vol. 478, no. 1, pp. 52-58, 2008.
- [71] A. V. Alessenko, P. Ya Boikov, G. N. Filippova, A. V. Khrenov, A. S. Loginov, and E. D. Makarieva, "Mechanisms of cycloheximide-induced apoptosis in liver cells," *FEBS Letters*, vol. 416, no. 1, pp. 113-116, 1997.
- [72] M. Cui, Y. Wang, J. Cavaleri, T. Kelson, Y. Teng, and M. Han, "Starvation-induced stress response is critically impacted by ceramide levels in *Caenorhabditis elegans*," *Genetics*, vol. 205, no. 2, pp. 775-785, 2017.
- [73] W. D. Jarvis, F. A. Fornari Jr., K. L. Auer et al., "Coordinate regulation of stress- and mitogen-activated protein kinases in the apoptotic actions of ceramide and sphingosine," *Molecular Pharmacology*, vol. 52, no. 6, pp. 935-947, 1997.
- [74] M. Garcia-Gil and E. Albi, "Nuclear lipids in the nervous system: what they do in health and disease," *Neurochemical Research*, vol. 42, no. 2, pp. 321-336, 2017.
- [75] Y. A. Hannun, C. R. Loomis, A. H. Merrill Jr., and R. M. Bell, "Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding in vitro and in human platelets,"

- The Journal of Biological Chemistry*, vol. 261, no. 27, pp. 12604–12609, 1986.
- [76] P. R. Conkling, K. L. Patton, Y. A. Hannun, C. S. Greenberg, and J. B. Weinberg, “Sphingosine inhibits monocyte tissue factor-initiated coagulation by altering factor VII binding,” *The Journal of Biological Chemistry*, vol. 264, no. 31, pp. 18440–18444, 1989.
- [77] A. Lazzarini, A. Macchiarulo, A. Floridi et al., “Very-long-chain fatty acid sphingomyelin in nuclear lipid microdomains of hepatocytes and hepatoma cells: can the exchange from C24:0 to C16:0 affect signal proteins and vitamin D receptor?,” *Molecular Biology of the Cell*, vol. 26, no. 13, pp. 2418–2425, 2015.
- [78] B. A. Imhof and M. Aurrand-Lions, “Adhesion mechanisms regulating the migration of monocytes,” *Nature Reviews Immunology*, vol. 4, no. 6, pp. 432–444, 2004.
- [79] N. Z. Tamehiro, Z. Mujawar, S. Zhou et al., “Cell polarity factor Par3 binds SPTLC1 and modulates monocyte serine palmitoyltransferase activity and chemotaxis,” *The Journal of Biological Chemistry*, vol. 284, no. 37, pp. 24881–24890, 2009.
- [80] J. Barthelmes, A. M. de Bazo, Y. Pewzner-Jung et al., “Lack of ceramide synthase 2 suppresses the development of experimental autoimmune encephalomyelitis by impairing the migratory capacity of neutrophils,” *Brain, Behavior, and Immunity*, vol. 46, pp. 280–292, 2015.
- [81] N. Mondal, G. Stolfa, A. Antonopoulos et al., “Glycosphingolipids on human myeloid cells stabilize E-selectin-dependent rolling in the multistep leukocyte adhesion cascade,” *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 36, no. 4, pp. 718–727, 2016.
- [82] M. P. Espallat, R. R. Kew, and L. M. Obeid, “Sphingolipids in neutrophil function and inflammatory responses: mechanisms and implications for intestinal immunity and inflammation in ulcerative colitis,” *Advances in Biological Regulation*, vol. 63, pp. 140–155, 2017.
- [83] M. R. Stroud, K. Handa, K. Ito et al., “Myeloglycan, a series of E-selectin-binding poly lactosaminolipids found in normal human leukocytes and myelocytic leukemia HL60 cells,” *Biochemical and Biophysical Research Communication*, vol. 209, no. 3, pp. 777–787, 1995.
- [84] K. Iwabuchi, H. Masuda, N. Kaga et al., “Properties and functions of lactosylceramide from mouse neutrophils,” *Glycobiology*, vol. 25, no. 6, pp. 655–668, 2015.
- [85] A. Viola, S. Schroeder, Y. Sakakibara, and A. Lanzavecchia, “T lymphocyte costimulation mediated by reorganization of membrane microdomains,” *Science*, vol. 283, no. 5402, pp. 680–682, 1999.
- [86] R. G. Sitrin, T. M. Sassanella, and H. R. Petty, “An obligate role for membrane-associated neutral sphingomyelinase activity in orienting chemotactic migration of human neutrophils,” *American Journal of Respiratory Cell and Molecular Biology*, vol. 44, no. 2, pp. 205–212, 2011.
- [87] A. Boecke, D. Sieger, C. D. Neacsu, H. Kashkar, and M. Kronke, “Factor associated with neutral sphingomyelinase activity mediates navigational capacity of leukocytes responding to wounds and infection: live imaging studies in zebrafish larvae,” *The Journal of Immunology*, vol. 189, no. 4, pp. 1559–1566, 2012.
- [88] W. Yang, E. Schmid, M. K. Nurbaeva et al., “Role of acid sphingomyelinase in the regulation of mast cell function,” *Clinical & Experimental Allergy*, vol. 44, no. 1, pp. 79–90, 2014.
- [89] S. Mitsutake, T. Date, H. Yokota, M. Sugiura, T. Kohama, and Y. Igarashi, “Ceramide kinase deficiency improves diet-induced obesity and insulin resistance,” *FEBS Letters*, vol. 586, no. 9, pp. 1300–1305, 2012.
- [90] M. H. Granado, P. Gangoiiti, A. Ouro et al., “Ceramide 1-phosphate (C1P) promotes cell migration involvement of a specific C1P receptor,” *Cell Signalling*, vol. 21, no. 3, pp. 405–412, 2009.
- [91] M. Adamiak, M. Suszynska, A. Abdel-Latif, A. Abdelbaset-Ismail, J. Ratajczak, and M. Z. Ratajczak, “The involvement of hematopoietic-specific PLC- $\beta$ 2 in homing and engraftment of hematopoietic stem/progenitor cells,” *Stem Cell Reviews*, vol. 12, no. 6, pp. 613–620, 2016.
- [92] M. Ordonez, I. G. Rivera, N. Presa, and A. Gomez-Munoz, “Implication of matrix metalloproteinases 2 and 9 in ceramide 1-phosphate-stimulated macrophage migration,” *Cell Signalling*, vol. 28, no. 8, pp. 1066–1074, 2016.
- [93] L. Arana, M. Ordonez, A. Ouro et al., “Ceramide 1-phosphate induces macrophage chemoattractant protein-1 release: involvement in ceramide 1-phosphate-stimulated cell migration,” *The American Journal of Physiology-Endocrinology and Metabolism*, vol. 304, no. 11, pp. E1213–E1226, 2013.
- [94] E. Olesch, C. Ringel, B. Brüne, and A. Weigert, “Beyond immune cell migration: the emerging role of the sphingosine-1-phosphate receptor S1PR4 as a modulator of innate immune cell activation,” *Mediators of Inflammation*, vol. 2017, Article ID 6059203, 12 pages, 2017.
- [95] E. Meylan, J. Tschopp, and M. Karin, “Intracellular pattern recognition receptors in the host response,” *Nature*, vol. 442, no. 7098, pp. 39–44, 2006.
- [96] D. Y. Lu, H. C. Chen, M. S. Yang et al., “Ceramide and toll-like receptor 4 are mobilized into membrane rafts in response to *Helicobacter pylori* infection in gastric epithelial cells,” *Infection and Immunity*, vol. 80, no. 5, pp. 1823–1833, 2012.
- [97] P. S. Tawadros, K. A. Powers, M. Ailenberg et al., “Oxidative stress increases surface toll-like receptor 4 expression in murine macrophages via ceramide generation,” *Shock*, vol. 44, no. 2, pp. 157–165, 2015.
- [98] H. Grassme, A. Riehle, B. Wilker, and E. Gulbins, “Rhinoviruses infect human epithelial cells via ceramide-enriched membrane platforms,” *The Journal of Biological Chemistry*, vol. 280, no. 28, pp. 26256–26262, 2005.
- [99] S. Dreschers, P. Franz, C. Dumitru, B. Wilker, K. Jahnke, and E. Gulbins, “Infections with human rhinovirus induce the formation of distinct functional membrane domains,” *Cellular Physiology and Biochemistry*, vol. 20, no. 1–4, pp. 241–254, 2007.
- [100] M. B. Fessler and J. S. Parks, “Intracellular lipid flux and membrane microdomains as organizing principles in inflammatory cell signaling,” *The Journal of Immunology*, vol. 187, no. 4, pp. 1529–1535, 2011.
- [101] W. Wu, X. Shi, and C. Xu, “Regulation of T cell signalling by membrane lipids,” *Nature Reviews Immunology*, vol. 16, no. 11, pp. 690–701, 2016.
- [102] P. Varshney, V. Yadav, and N. Saini, “Lipid rafts in immune signalling: current progress and future perspective,” *Immunology*, vol. 149, no. 1, pp. 13–24, 2016.
- [103] T. M. Razzaq, P. Ozegbe, E. C. Jury, P. Sembi, N. M. Blackwell, and P. S. Kabouridis, “Regulation of T-cell receptor signalling by membrane microdomains,” *Immunology*, vol. 113, no. 4, pp. 413–426, 2004.

- [104] H. Nakayama, H. Ogawa, K. Takamori, and K. Iwabuchi, "GSL-enriched membrane microdomains in innate immune responses," *Archivum Immunologiae Et Therapiae Experimentalis*, vol. 61, no. 3, pp. 217–228, 2013.
- [105] C. L. Schengrund, "Multivalent" saccharides: development of new approaches for inhibiting the effects of glycosphingolipid-binding pathogens," *Biochemical Pharmacology*, vol. 65, no. 5, pp. 699–707, 2003.
- [106] J. M. Ruysschaert and C. Loney, "Role of lipid microdomains in TLR-mediated signalling," *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 1848, no. 9, pp. 1860–1867, 2015.
- [107] E. Avota, E. Gulbins, and S. Schneider-Schaulies, "DC-SIGN mediated sphingomyelinase-activation and ceramide generation is essential for enhancement of viral uptake in dendritic cells," *PLoS Pathogens*, vol. 7, no. 2, article e1001290, 2011.
- [108] T. K. Hailemariam, C. Huan, J. Liu et al., "Sphingomyelin synthase 2 deficiency attenuates NFκB activation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 8, pp. 1519–1526, 2008.
- [109] Z. Li, T. K. Hailemariam, H. Zhou et al., "Inhibition of sphingomyelin synthase (SMS) affects intracellular sphingomyelin accumulation and plasma membrane lipid organization," *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, vol. 1771, no. 9, pp. 1186–1194, 2007.
- [110] Z. Li, Y. Fan, J. Liu et al., "Impact of sphingomyelin synthase 1 deficiency on sphingolipid metabolism and atherosclerosis in mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 32, no. 7, pp. 1577–1584, 2012.
- [111] S. Oertel, K. Scholich, A. Weigert et al., "Ceramide synthase 2 deficiency aggravates AOM-DSS-induced colitis in mice: role of colon barrier integrity," *Cellular and Molecular Life Sciences*, vol. 74, no. 16, pp. 3039–3055, 2017.
- [112] C. R. Vieira, J. M. Munoz-Olaya, J. Sot et al., "Dihydro-sphingomyelin impairs HIV-1 infection by rigidifying liquid-ordered membrane domains," *Chemistry & Biology*, vol. 17, no. 7, pp. 766–775, 2010.
- [113] K. Iwabuchi, H. Nakayama, C. Iwahara, and K. Takamori, "Significance of glycosphingolipid fatty acid chain length on membrane microdomain-mediated signal transduction," *FEBS Letters*, vol. 584, no. 9, pp. 1642–1652, 2010.
- [114] S. Hugosson, J. Angstrom, B. M. Olsson et al., "Glycosphingolipid binding specificities of *Neisseria meningitidis* and *Haemophilus influenzae*: detection, isolation, and characterization of a binding-active glycosphingolipid from human oropharyngeal epithelium," *The Journal of Biochemistry*, vol. 124, no. 6, pp. 1138–1152, 1998.
- [115] B. Tsai, J. M. Gilbert, T. Stehle, W. Lencer, T. L. Benjamin, and T. A. Rapoport, "Gangliosides are receptors for murine polyoma virus and SV40," *The EMBO Journal*, vol. 22, no. 17, pp. 4346–4355, 2003.
- [116] C. S. Berenson, H. F. Nawar, R. L. Kruzel, L. M. Mandell, and T. D. Connell, "Ganglioside-binding specificities of *E. coli* enterotoxin LT-IIc: importance of long-chain fatty acyl ceramide," *Glycobiology*, vol. 23, no. 1, pp. 23–31, 2013.
- [117] S. Kesavardhana and T. D. Kanneganti, "Mechanisms governing inflammasome activation, assembly and pyroptosis induction," *International Immunology*, vol. 29, no. 5, pp. 201–210, 2017.
- [118] H. Grassme, V. Jendrosseck, A. Riehle et al., "Host defense against *Pseudomonas aeruginosa* requires ceramide-rich membrane rafts," *Nature Medicine*, vol. 9, no. 3, pp. 322–330, 2003.
- [119] H. Peng, C. Li, S. Kadow et al., "Acid sphingomyelinase inhibition protects mice from lung edema and lethal *Staphylococcus aureus* sepsis," *Journal of Molecular Medicine*, vol. 93, no. 6, pp. 675–689, 2015.
- [120] C. Adams, V. Icheva, C. Deppisch et al., "Long-term pulmonary therapy of cystic fibrosis-patients with amitriptyline," *Cellular Physiology and Biochemistry*, vol. 39, no. 2, pp. 565–572, 2016.
- [121] C. D. Camell, K. Y. Nguyen, M. J. Jurczak et al., "Macrophage-specific *de Novo* synthesis of ceramide is dispensable for inflammasome-driven inflammation and insulin resistance in obesity," *The Journal of Biological Chemistry*, vol. 290, no. 49, pp. 29402–29413, 2015.
- [122] M. Ali, A. Saroha, Y. Pewzner-Jung, and A. H. Futerman, "LPS-mediated septic shock is augmented in ceramide synthase 2 null mice due to elevated activity of TNFα-converting enzyme," *FEBS Letters*, vol. 589, no. 17, pp. 2213–2217, 2015.
- [123] K. M. Boini, M. Xia, S. Koka, T. W. Gehr, and P. L. Li, "Instigation of NLRP3 inflammasome activation and glomerular injury in mice on the high fat diet: role of acid sphingomyelinase gene," *Oncotarget*, vol. 7, no. 14, pp. 19031–19044, 2016.
- [124] H. Grassme, A. Carpinteiro, M. J. Edwards, E. Gulbins, and K. A. Becker, "Regulation of the inflammasome by ceramide in cystic fibrosis lungs," *Cellular Physiology and Biochemistry*, vol. 34, no. 1, pp. 45–55, 2014.
- [125] B. Weichand, R. Popp, S. Dziumbala et al., "S1PR1 on tumor-associated macrophages promotes lymphangiogenesis and metastasis via NLRP3/IL-1β," *Journal of Experimental Medicine*, vol. 214, no. 9, pp. 2695–2713, 2017.
- [126] K. Wang, R. Xu, A. J. Snider et al., "Alkaline ceramidase 3 deficiency aggravates colitis and colitis-associated tumorigenesis in mice by hyperactivating the innate immune system," *Cell Death and Disease*, vol. 7, no. 3, article e2124, 2016.
- [127] V. Hinkovska-Galcheva and J. A. Shayman, "Ceramide-1-phosphate in phagocytosis and calcium homeostasis," *Advances in Experimental Medicine and Biology*, vol. 688, pp. 131–140, 2010.
- [128] V. Hinkovska-Galcheva, A. Clark, S. VanWay et al., "Ceramide kinase promotes Ca<sup>2+</sup> signaling near IgG-opsonized targets and enhances phagolysosomal fusion in COS-1 cells," *Journal of Lipid Research*, vol. 49, no. 3, pp. 531–542, 2008.
- [129] B. J. Pettus, A. Bielawska, P. Subramanian et al., "Ceramide 1-phosphate is a direct activator of cytosolic phospholipase A<sub>2</sub>," *The Journal of Biological Chemistry*, vol. 279, no. 12, pp. 11320–11326, 2004.
- [130] N. F. Lamour, P. Subramanian, D. S. Wijesinghe, R. V. Stahelin, J. V. Bonventre, and C. E. Chalfant, "Ceramide 1-phosphate is required for the translocation of group IVA cytosolic phospholipase A<sub>2</sub> and prostaglandin synthesis," *The Journal of Biological Chemistry*, vol. 284, no. 39, pp. 26897–26907, 2009.
- [131] R. V. Stahelin, P. Subramanian, M. Vora, W. Cho, and C. E. Chalfant, "Ceramide-1-phosphate binds group IVA cytosolic phospholipase a<sub>2</sub> via a novel site in the C2 domain," *The Journal of Biological Chemistry*, vol. 282, no. 28, pp. 20467–20474, 2007.
- [132] M. Serra-Pages, A. Olivera, R. Torres, C. Picado, F. de Mora, and J. Rivera, "E-prostanoid 2 receptors dampen mast cell degranulation via cAMP/PKA-mediated suppression of IgE-



- dependent signaling,” *Journal of Leukocyte Biology*, vol. 92, no. 6, pp. 1155–1165, 2012.
- [133] S. Mitsutake, H. Kumada, M. Soga et al., “Ceramide kinase is not essential but might act as a  $\text{Ca}^{2+}$ -sensor for mast cell activation,” *Prostaglandins & Other Lipid Mediators*, vol. 93, no. 3-4, pp. 109–112, 2010.
- [134] J. A. Mietla, D. S. Wijesinghe, L. A. Hoeflerlin et al., “Characterization of eicosanoid synthesis in a genetic ablation model of ceramide kinase,” *Journal of Lipid Research*, vol. 54, no. 7, pp. 1834–1847, 2013.
- [135] D. S. Wijesinghe, J. C. Allegood, L. B. Gentile, T. E. Fox, M. Kester, and C. E. Chalfant, “Use of high performance liquid chromatography-electrospray ionization-tandem mass spectrometry for the analysis of ceramide-1-phosphate levels,” *Journal of Lipid Research*, vol. 51, no. 3, pp. 641–651, 2010.
- [136] K. Baudiss, R. de Paula Vieira, S. Cicko et al., “C1P attenuates lipopolysaccharide-induced acute lung injury by preventing NF- $\kappa$ B activation in neutrophils,” *The Journal of Immunology*, vol. 196, no. 5, pp. 2319–2326, 2016.
- [137] A. Naroeni and F. Porte, “Role of cholesterol and the ganglioside  $\text{GM}_1$  in entry and short-term survival of *Brucella suis* in murine macrophages,” *Infection and Immunity*, vol. 70, pp. 1640–1644, 2002.
- [138] T. J. Mullmann, M. I. Siegel, R. W. Egan, and M. M. Billah, “Sphingosine inhibits phosphatidate phosphohydrolase in human neutrophils by a protein kinase C-independent mechanism,” *The Journal of Biological Chemistry*, vol. 266, no. 4, pp. 2013–2016, 1991.
- [139] S. Kim, V. Lakhani, D. J. Costa et al., “Sphingolipid-gated  $\text{Ca}^{2+}$  release from intracellular stores of endothelial cells is mediated by a novel  $\text{Ca}^{2+}$ -permeable channel,” *The Journal of Biological Chemistry*, vol. 270, no. 10, pp. 5266–5269, 1995.
- [140] D. A. Baker, J. Eudaly, C. D. Smith, L. M. Obeid, and G. S. Gilkeson, “Impact of sphingosine kinase 2 deficiency on the development of TNF- $\alpha$ -induced inflammatory arthritis,” *Rheumatology International*, vol. 33, no. 10, pp. 2677–2681, 2013.
- [141] A. Weigert, A. M. Johann, A. von Knethen, H. Schmidt, G. Geisslinger, and B. Brune, “Apoptotic cells promote macrophage survival by releasing the antiapoptotic mediator sphingosine-1-phosphate,” *Blood*, vol. 108, no. 5, pp. 1635–1642, 2006.
- [142] R. Kolesnick and D. W. Golde, “The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling,” *Cell*, vol. 77, no. 3, pp. 325–328, 1994.
- [143] H. Ohta, Y. Yatomi, E. A. Sweeney, S. Hakomori, and Y. Igarashi, “A possible role of sphingosine in induction of apoptosis by tumor necrosis factor- $\alpha$  in human neutrophils,” *FEBS Letters*, vol. 355, no. 3, pp. 267–270, 1994.
- [144] A. S. Soloviev, V. G. Korobko, L. N. Shingarova, S. V. Vasiliyeva, E. V. Makhova, and A. V. Alessenko, “Cytotoxic activity of tumor necrosis factor- $\alpha$  mutants: correlation of free sphingosine level in murine liver,” *Biokhimiia*, vol. 60, pp. 1283–1291, 1995.
- [145] A. V. Alessenko, E. I. Galperin, L. B. Dudnik et al., “Role of tumor necrosis factor alpha and sphingomyelin cycle activation in the induction of apoptosis by ischemia/reperfusion of the liver,” *Biochemistry*, vol. 67, no. 12, pp. 1347–1355, 2002.
- [146] K. Yokoyama, H. Nojiri, M. Suzuki, M. Setaka, A. Suzuki, and S. Nojima, “Clear differences in ceramide metabolism between glycosphingolipids and sphingomyelin in a human promyelocytic leukemia cell line HL-60 stimulated by a differentiation inducer,” *FEBS Letters*, vol. 368, no. 3, pp. 477–480, 1995.
- [147] K. Iwabuchi, H. Nakayama, A. Oizumi, Y. Suga, H. Ogawa, and K. Takamori, “Role of ceramide from glycosphingolipids and its metabolites in immunological and inflammatory responses in humans,” *Mediators of Inflammation*, vol. 2015, Article ID 120748, 10 pages, 2015.





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