

Review

Beyond a Structural Component: Sphingolipids in Immunology

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Abstract. Two major classes of lipids participating in signaling cascades in immune cells are known today. One comprises glycerol-based lipids with diacylglycerol as its most prominent member that mediates the activation of classical and novel protein kinase C molecules. The second group contains the sphingolipids, with the best-investigated representatives being sphingosine, sphingosine-1-phosphate, and ceramide. In the last years the latter two molecules have especially received considerable attention for their modulatory capacity in the course of an apoptotic response. Today it is clear that sphingolipids are ubiquitously distributed in all eukaryotic cells, especially in cellular membranes, where they were previously thought to fulfil an exclusively structural role. Recent findings, however, have demonstrated functions beyond this. Sphingolipid specific G-protein coupled receptors were identified and their role as intracellular second messengers has been further elucidated. In addition, glycosphingolipids, in particular, are enriched in certain membrane compartments, known as detergent resistant membranes. These serve as entry sites for several receptor-mediated signaling events by stabilizing receptor/kinase interactions, suggesting an involvement in the initiation of signaling cascades. Altogether, these findings have led to new insights into both the role of these lipids in signaling as well as the underlying pathology of several diseases with imbalances in the sphingolipid metabolism. The development of these disorders has mainly been attributed to the toxic potential of lysosphingolipids up to now. In addition, attempts have been made to develop compounds and drugs containing the sphingolipid backbone for influencing diseases associated with unwanted cell activation (e.g. cancer, inflammatory processes). These novel findings and developments are reviewed in the following.

Key words: cell signaling; detergent resistant membranes; intracellular second messengers; sphingolipids.

Introduction to Sphingolipids

In Greek mythology the sphinx is a monster that poses a riddle to all it encounters and kills those who do not answer the riddle. In analogy, THUDICHUM⁷³

named the backbone of sphingolipids, “sphingosine” (S), for all the enigmas this group of lipids presented to him in 1884. In the following years newly discovered sphingolipids were mainly named according to the tissue from which they were isolated (e.g.: sphingo-

Abbreviations used: AD – atopic dermatitis, DRMs – detergent resistant membranes, EDG – endothelial differentiation gene, GPCR – G-protein coupled receptor, IL – interleukin, IP-3 – inositol-tris-phosphate, MAPK – mitogen activated protein kinase, PLC – phospholipase, PKC – protein kinase C, RBL – rat basophilic leukemia, S – sphingosine, S1P – sphingosine-1-phosphate, SK – sphingosine kinase, SM – sphingomyelin, SMase – sphingomyelinase, TNF- α – tumor necrosis factor α .

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myelin (SM), cerebroside), giving the impression that they were primarily components of the central nervous system⁴⁴.

Nowadays it is proven that sphingolipids are expressed ubiquitously in all eukaryotic cells and that they are a major component of cellular membranes. In addition to this structural role, however, they exert a modulatory capacity on cells by acting at three different levels. First, they serve as extracellular ligands for G-protein coupled receptors (GPCRs), which are linked to the activation of phospholipase C (PLC), phospholipase A2 (PLA2), mitogen activated protein kinase (MAPK) pathways, and elevated Ca^{2+} levels in a wide variety of cell types^{34, 51}. Second, they act intracellularly as second messenger molecules and are, in this respect mainly implicated in regulating cell growth and apoptosis as well as being an alternative to inositol-trisphosphate (IP-3)^{9, 75}. Third, glycosylated derivatives are constituents of detergent resistant membranes (DRMs), which serve as entry level for many signaling cascades and have recently been recognized as being important to the activation of tyrosine kinases such as lyn and syk^{24, 66}.

Compared to glycerol-based lipids, the structure of sphingolipids is characterized by a long chain base (sphingoid) backbone. Commonly, 18–20 carbon atoms with two or three hydroxyl groups and a trans double bond in position 4 are found. The simplest types are lysosphingolipids, characterized by a free amino group such as S, dihydro-S or galactosyl-S. Acylation of the amine with a fatty acid or 2-hydroxy fatty acid results in compounds such as ceramide (N-palmitoyl-S; for structures see Fig. 1). More complex sphingolipids (cerebrosides or gangliosides) are formed if polar head groups are added at position 1 of ceramide. These derivatives together give rise to more than 60 different sphingols and over 300 either glycosylated or more complex forms of lipids (for review see⁴⁴).

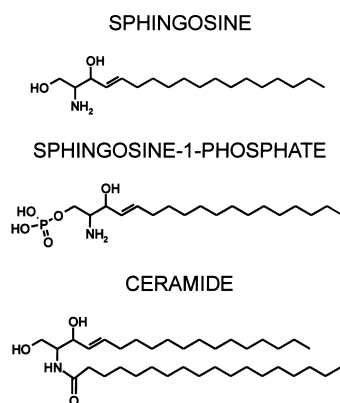


Fig. 1. Chemical structure of sphingosine, sphingosine-1-phosphate, and ceramide

Due to the anabolism and catabolism of sphingolipids, which favor rather complex structures such as SM and ganglioside GM3, the concentration of free sphingoid bases (e.g.: S, sphinganine) is usually low inside of cells⁴⁴. They mostly arise as a result of the turnover of more complex structures or from dietary sources such as milk, butter, cheese and meat, where their concentration ranges from 0.3 $\mu\text{mol/g}$ to 1 $\mu\text{mol/g}$ ⁴⁴.

A Short History of Sphingolipids in Cell Activation/Inhibition

Interest in sphingolipids was revived by the discovery of their role as cellular messengers in the mid 80's with the finding that D-erythro-S potently inhibits protein kinase C (PKC) by competitively inhibiting diglycerides, phorbol dibutyrate and Ca^{2+} activation^{19, 20}. Multiple enzymes such as Na/K ATPase⁵⁰, calcium/calmodulin-dependent protein kinase³⁰, insulin receptor kinase¹ and many more were described in the following years as being targets either of stimulatory or inhibitory effects. All these studies used exogenously applied S and, therefore, dealt with this compound class as a kind of "drug" applied from the outside. In 1988, phorbol ester (PMA) activation of neutrophils was shown to lead to the elevation of free S intracellularly, producing the first example of an agonist-stimulated change reported for this lipid⁷⁷. This initiated a series of investigations which shifted the focus to a biologically existing intracellular second messenger function, instead of an exogenously applied compound (see later).

S was originally described as a negative growth regulator. At concentrations above 15 μM it is toxic, most likely due to its detergent properties and its potent inhibition of PKCs. At concentrations between 1–10 μM , however, it stimulates DNA synthesis and increases the number of Swiss fibroblasts a fact that is attributed to the conversion of S to sphingosine-1-phosphate (S1P) inside cells⁶⁹. This hypothesis is supported by initial studies using S1P, which was found to be more mitogenic than S alone and acts synergistically with other growth factors in promoting proliferation⁴⁴. Since then, effects of both lipids have been investigated in many cell types measuring a variety of parameters; however, a fully coherent picture has so far not been achieved. There is seemingly conflicting data with respect to activation and inhibition of different cell types and targets by one particular lipid. A preliminary explanation could be the concept of counter-regulatory lipids, as recently shown for T cells (apoptosis)¹⁰ and mast cells (allergic triggering)⁵⁹.

Sphingolipids and Diseases Including Toxins

Lipid storage diseases (sphingolipidoses) arise due to inborn genetic defects in enzymes involved in the sphingolipid metabolism. The onset of these diseases is usually in the neonatal period of childhood, leading to organomegaly or progressive mental and neurological disorders, based on neurological degeneration, brain atrophy, gliosis and demyelination. They are characterized by the accumulation of lipid molecules proximal to the genetic defect, at substantial concentrations in tissues and primarily in lipid storing cells as found in spleen, liver, lung, lymph nodes and bone marrow. Accumulated lysosphingolipids, which lack the amide linked fatty acid in the 2-amino position of the sphingoid base, are proposed as the functional link to the pathogenesis of disorders such as Krabbe, Gaucher, Niemann-Pick, Tay-Sach's and others (see Table 1). The potent inhibition of PKCs at lower concentrations ($< 15 \mu\text{M}$) and the toxicity at higher concentrations ($> 15 \mu\text{M}$), are speculated to be the prime cause for the overall symptoms and the progressive and sometimes fatal neurological appearance¹⁹. Therefore, it is very likely that the "Psychosine Hypothesis" by MIYATAKE and SUZUKI for Krabbe disease can be extended to other lysosphingolipids and their corresponding disorders. Recent findings, however, indicate an additional immunological component in at least some of these diseases, such as Krabbe and Gaucher. In an authentic animal model for Krabbe disease, the so-called Twitcher (Twi/Twi) mice, a kind of "allergic neuritis" with eosinophils, polymorphonuclear cells and degranulating mast cells infiltrating into nerve fibers was described^{36, 37, 57}. In *in vitro* studies, the corresponding lysosphingolipid galactosyl-S (psychosine) primes and partially activates mouse mast cells. This occurs via an IgE-independent relocation of the two tyrosine kinases lyn and syk into DRMs, leading to their corresponding activation (see later)⁵⁸. In Gaucher patients, where glucosyl-S is accumulating, not only lipid laden macrophages comprise the lipid storing cells but, in addition, elevated levels of pro-inflammatory cytokines such as tumor necrosis factor α (TNF- α , interleukin 8 (IL-8), IL-1 β and IL-6 are found in the serum. Their levels have been linked to the severity and progression of the disease^{2, 23, 47}.

Besides lipid storage diseases where specific sphingolipids accumulate, other disorders are also accompanied by changes in the (sphingo) lipid balance. For years it has been known that atopic dermatitis (AD), an inflammatory skin disorder beginning with a predominant Th2-type T cell involvement, is accompanied by

Table 1. Sphingolipidoses and associated lysosphingolipids

Disease	Lysosphingolipid
Krabbe's disease	Galactosylsphingosine
Gaucher's disease	Glucosylsphingosine
Lactosylceramidosis	Lactosylsphingosine
Niemann-Pick's disease	Sphingosylphosphorylcholine
Metachromic leukodystrophy	Sulfogalactosylsphingosine Sulfolactosylsphingosine
Fabry's disease	Globotriosylsphingosine Galabiosylsphingosine
GM ₁ gangliosidosis	LysoGM ₁
Tay-Sach's disease	LysoGM ₂
Sandhoff's disease	LysoGA ₂
GM ₃ gangliosidosis	LysoGM ₃ LysoGD _{1α}
Other gangliosidosis	Lysotriosialoganglioside Lysogloboside sphingosyltrihexoside

changes in the lipid composition in the horny layer of the epidermis²⁶. While cholesterol based lipids are found in enhanced concentrations, sphingolipids, especially ceramide, are reduced in the lesions as well as in non-lesional skin of AD patients¹². This may be based on the observed abnormal expression of a SM deacylase, which converts SM into sphingosylphosphorylcholine, thereby bypassing the natural degradation to ceramide by sphingomyelinase (SMase)⁴⁹. As an alternative, one may also attribute the loss of ceramide to the bacterial skin flora in AD patients (e.g., *Pseudomonas aeruginosa* (AN17)), which contributes to enhanced ceramidase levels⁵². In the past these changes were thought to be the basis for trans-epidermal water loss (TEWL), with the clinical appearance of dry skin in affected individuals. However, a number of newer indirect findings suggest that the observed imbalance is not an epiphenomena accompanying the disorder, but contributes to the progression and severity of this disease, which is primarily observed in atopic children. Interestingly, the generated sphingosylphosphorylcholine stimulates the expression of ICAM-1 and TNF- α in keratinocytes via an activation of the MAPK pathway, a hallmark of an ongoing inflammation of the skin²⁷. This is in agreement with older findings, where the reduced skin barrier function due to the loss of ceramide, was shown to be accompanied by increased DNA synthesis and epidermal hyperplasia⁶⁰.

A further link between an imbalance in the sphingolipid metabolism and disease comes from the observation of mycotoxins, termed fumonisins, isolated from *Fusarium moniliforme* and related fungi. They are prevalent on corn, sorghum, millet and other agricultural products worldwide. Today, 5 different toxins, FB1,

B2, B3, B4 and C1, fall into this class and are related to a second group, the AAL-toxins, which have a free amino group at the C-2 position. The notable similarity of their structure to sphinganine may be the basis for their potent inhibition of ceramide synthase, an enzyme involved in the *de novo* synthesis of more complex sphingolipids (for review see⁴⁴). As a result, an accumulation of free sphingoid bases and a block in the formation of more complex sphingolipid structures is observed. Farm and laboratory animals exposed to these fumonisins develop disorders such as porcine pulmonary edema and equine leukoencephalomalacia (farm animals), hepatotoxicity and liver tumor formation (laboratory animals, rats). For humans, a correlation between esophageal cancer and toxin-contaminated corn in regions of China and Southern Africa has been described (for review see⁴⁰). In this context it is interesting to note that fumonisins were shown to attenuate apoptosis induced by a variety of agents and stimuli, such as daunorubicin, camptothecin, fatty acids, TNF- α and phorbol esters⁵⁶. As a mechanism one could envision either the inhibition of ceramide synthesis (a second messenger in apoptosis, see later) or alternatively the accumulation of free sphingoid base, primarily S1P, which is mitogenic. The recently isolated fungal metabolite myriocin, which potently inhibits the sphingolipid synthesis pathway at the earliest step of condensation of serine with palmitoyl CoA (serine palmitoyltransferase), should provide further clarification⁵⁶. The second group of toxins, AAL, although similar in structure and with the same inhibition profile, have, surprisingly, been found to be extremely phytotoxic without causing any harm to animals.

Drugs (in Development) Based on the Sphingolipid Backbone for Immunologically Driven Diseases

Originally, most compounds and derivatives of this class were claimed to have anti-proliferative and apoptotic properties and, therefore, were planned as therapeutics in cancer and diseases characterized by elevated cell proliferation (e.g., Sphinx profiled L-threodihydroS (Sanfingol) to support doxorubicin in cancer^{65, 67}; Beiersdorf profiled N-octanoyl-S for keratosis^{3, 25}). Newer findings show a wide variety for potential applications of these lipids in hematopoietic and lymphoid disorders which are currently being explored. Three selected examples to illustrate this fact: John P. Roberts Research Institute claims a pharmaceutically active ceramide that increases hematopoiesis. After 8 days of

treatment with the specific compound N-butyrylsphinganine, mice exhibited a 20% increase in platelet number compared to control animals. Hematocrit samples at days 8 and 15 showed a 30–50% increase in the buffy coat. Bristol-Myers Squibb claims a series of malonic acid derivatives that are o-dicarboxylated α - and β -glycolipid compounds⁵. They act as inhibitors of cell adhesion with an IC₅₀ of 8.2 μ M against P-selectin binding. *In vivo*, 41% inhibition of vascular permeability and 96% inhibition of neutrophil release at 3 mg/rat intravenous application are observed. The related structure BMS-190394 was found to be a potent inhibitor of the dermal immune complex induced reverse Arthus reaction in rats if given intravenously or intraperitoneally. The effective dose (ED₅₀) here compares favorably to that of dexamethasone⁷⁴. Yoshitomi claims a compound, which is structurally related to sphingofungin B, as an immunosuppressant, isolated from the fungus *Isalia sinclairi*, which is highly active in a mouse mixed lymphocyte reaction (MLR)^{15, 82}. Improved derivatives of this structure (FTY720) display remarkable immunosuppressive activity in the mouse allogeneic mixed lymphocyte reaction in the range of IC₅₀ = 6.1 nM⁸³. FTY720 prolonged, dose-dependently, skin allograft survival in rats after intraperitoneal application (0.3–3 mg/kg), intravenous application (0.1–10 mg/kg) and if given orally (0.1–30 mg/kg) with no apparent toxicity. Newer mechanistic studies *in vivo* suggest that this compound might influence the migration of lymphocytes, leading to leukocytopenia in the blood and an accumulation in lymph nodes and Payer's patches^{8, 78}.

Effects of Sphingolipids on Signal Transduction Events in Immune Cells

Today a picture emerges of sphingolipids participating in 3 ways in signal transduction events. Firstly, as ligands of corresponding GPCRs (see later), secondly as alternative intracellular messenger molecules to IP-3 and components of intracellular signaling pathways⁹, and thirdly (for glycosphingolipids) as constituents of the DRMs (see later).

The distinction between lipid receptor mediated effects and intracellular mechanisms became evident when endothelial differentiation gene 1 (EDG-1; the first receptor) was cloned. The name of these orphan receptor results from the initial cloning of EDG-1 from phorbol-ester-induced differentiated endothelial cells (endothelial differentiation gene)²². Binding of S1P to EDG-1 allowed only a subset of responses (inhibition

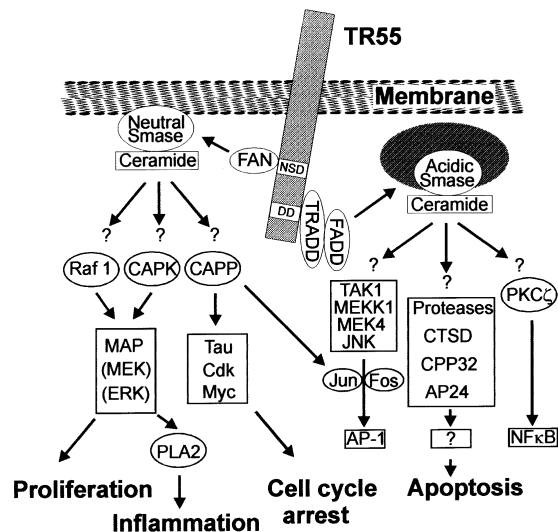


Fig 2. Function of ceramide in the TNF receptor (TR55) signaling pathways. Abbreviations: AP-1 – activator protein 1; AP24 – 24 kDa apoptotic protease; DD – death domain; CAPK – ceramide-activated protein kinase; CAPP – ceramide-activated protein phosphatase 2A; CPP32 – caspase 3; CTSD – cathepsin D; ERK – extracellular signal-regulated protein kinase; FAN – factor associated with neutral sphingomyelinase; JNK – c-jun-NH2 terminal kinase; MAP – mitogen-activated protein; MEK – MAPK kinase/ERK kinase; MEKK1 – mitogen-activated protein kinase kinase-1; NSD – neutral sphingomyelinase activation domain; PKC – protein kinase C; PLA2 – phospholipase A2; SMase – sphingomyelinase; TAK1 – transforming growth factor β -activating kinase

of forskolin-stimulated cAMP accumulation) and not all of the effects of S1P (mitogenesis, prevention of apoptosis⁷⁵). The aspect of sphingolipids as second messenger molecules is best illustrated by the recent description of S1P as an alternative to IP-3 with respect to intracellular Ca^{2+} mobilization in rat basophilic leukemia (RBL) cells⁹. In this cell-type, S1P is generated in the course of an Fc ϵ RI stimulation by S kinase (SK) directly from intracellular S⁹. Further studies showed that this conversion not only creates a second messenger molecule but serves as a kind of „permissive switch” converting an inhibitory principle (S) into an activating one (S1P)⁵⁹. Similar findings of two opposing, counter-regulatory sphingolipids, fine tuning a particular response, were also made in T cells¹¹. In this case, a rise in the concentration of ceramide resulted in the induction of apoptosis, a process that can be reversed by the addition of S1P¹⁰. This has led to a concept whereby one particular sphingolipid does not seem to have an assigned function *per se*, but that the context and, in particular, the balance between two (or more) sphingolipids in a cell determines the outcome of a signaling event.

Another example of sphingolipids as second messengers in signaling cascades comes from the SM path-

way, which is used by a variety of distinct receptors such as the IL-1 β -R, the progesterone-R, the interferon γ receptor (IFN γ -R), and the TNF α -R as a downstream effector system (for review see^{18, 28, 68}). Pleiotropic effects including proliferation (fibroblasts), differentiation (promyelocytes) and modulation of effector functions (respiratory burst inhibition in neutrophils) are all regulated through this signaling cascade. A well-studied example of these multiple actions of the SM pathway is the TNF- α signaling (see Fig. 2). Proinflammatory events are mediated via neutral SMase, whereas apoptotic events are mediated via acid SMase⁷⁶. While ceramide in both pathways comprises the mediator, the location of the enzyme (neutral SMase plasma membrane bound; acidic SMase endolysosomal) and the time-point of induction are different^{64, 76}. Fas/CD95, γ -irradiation and lipopolysaccharide (LPS) also activate the apoptotic branch of the SM-pathway^{17, 21, 63}. Ceramide, which plays the key role in this signaling cascade, interacts with proteins such as ceramide-activated protein kinase (CAPK) and ceramide-activated protein phosphatase (CAPP), which comprise direct targets for this intracellular messenger^{13, 38, 41}. The membrane bound and proline directed CAPK was recently shown to mediate at least parts of the TNF- α triggering via phosphorylation of raf-1 and is postulated to be most likely identical to the kinase suppressor of ras (KSR)^{79, 84}.

Detergent Resistant Membranes (DRMs)

DRMs (also termed glycosphingolipid-cholesterol rafts or glycosphingolipid-enriched membrane domains) can be isolated by density gradient ultracentrifugation. While the majority of transmembrane proteins, as well as cytosolic proteins, are present in the higher density fractions, molecules implicated in initiating signaling cascades in leukocytes are found in the low-density proportion of such a gradient. These comprise the T cell receptor (TCR), the Fc ϵ RI, co-stimulatory molecules (CD4, CD8, and CD28), protein tyrosine kinases (PTKs, e.g., lyn), GPCRs, and some others (LAT, CD44, CD26, CD36). While some proteins, such as CD4 or CD8, are constitutively present in this compartment, others (e.g., Fc ϵ RI γ -chain, lyn) translocate into the DRMs in the process of cell activation. Especially for the kinases it has been shown that this translocation is a pre-requisite for their activity. The specific architecture of DRMs, which contain glycosphingolipids at the outer- and cholesterol at the inner leaflet, is thought to favor the interaction between immuno-receptor-based tyrosine activation motif (ITAM) bearing receptors and

corresponding kinases, by a protein : lipid or lipid : lipid interaction. In this complementary hypothesis to the former thinking of purely protein : protein based interactions, signaling starts at small semi-liquid "signaling" islands floating in the more liquid phospholipid-rich "ocean" of the leukocyte membranes (elegantly reviewed for the Fc ϵ RI recently by METZGER⁴⁵).

The assumption that DRMs are essentially involved in signal initiation has been further supported by the finding that certain antibodies directed against glycosphingolipids (such as the AA4 mAb against the α galactosyl derivative of GD1b) directly provoke a tyrosine phosphorylation of proteins such as p72 syk and PLC- γ in RBL cells⁷². The basis for these events might be the chemical structure of glycosphingolipids, which allows them to interact with the aliphatic proportion of cytoplasmic signaling molecules (p72 syk or PLC- γ). This interaction occurs via their predominantly saturated fatty acid residue(s) that penetrate(s) into the opposing cytoplasmic leaflet of the membrane. As a result, these molecules co-redistribute, leading to the transmission of the signal. A similar hypothesis exists for the initiation of signaling events at glycosylphosphatidylinositol (GPI) -anchored proteins such as CD87 (uPA-R), CD16b, CD14, and Thy-1 explaining how receptors and tyrosine kinases residing in opposite leaflets of the cell membrane, without direct contact, become co-translocated when externally triggered by antibodies or ligands, respectively²⁴. In RBL cells, crosslinking of Thy-1 by antibodies led to such a co-distribution with p53/56 lyn and further proteins forming 10 MDa large complexes in the DRMs¹⁴.

Proteins in the Sphingolipid Signal Transduction

A number of proteins interacting with sphingolipids have been described and the corresponding cDNAs have been cloned. Those include enzymes of the sphingolipid metabolism, the sphingolipid activator proteins (SAPs), the ryanodine binding protein from rabbit skeletal muscle and, important in the context of this review, sphingolipid-binding GPCRs as well as the SKs. From the sphingolipid-binding GPCRs known so far, (EDG-1 to EDG-5), EDG-1, EDG-3 and EDG-5 (H218, AGR-16) are S1P binders, while EDG-2 (VZG-1) and EDG-4 bind lysophosphatidic acid (LPA). The ligand for another family member, EDG-6, which is primarily expressed on hematopoietic cells, is not known up to now¹⁶. The three S1P receptors exhibit an overlapping as well as distinct pattern of expression in tissues with EDG-1 and EDG-5 being widely distributed. They are mostly either linked to Gi or Gq type proteins

for signal transduction, even though in HEK-293 cells increased cadherin expression via EDG-1 is reported to go via the low molecular mass G-protein Rho³⁵.

A further group of S-interacting proteins are the SKs, which mediate the conversion of S to S1P. Their first description appeared already in 1973 (blood platelets) and 1974 (*Tetrahymena pyriformis*)^{70, 71}, although, it took another 25 years until the corresponding mammalian cDNAs were cloned³². In the meantime, many reports about partial purification and characterization of the enzyme(s) from various cell types were published and SK was implicated in contributing and influencing multiple signaling cascades. The ubiquitous expression of an SK-like activity (e.g., platelets, 3T3 fibroblasts, monocytes, mast cells, HL-60 cells, PC12 cells, human erythroleukemia cells, RBL cells, rat periosteal cells) and the conservation through a variety of species (besides mammals it is also described in yeast) suggest that this enzyme class is of central importance in multiple processes^{6, 7, 9, 31, 33, 39, 42, 43, 53, 59, 61}. This is supported by its implication in regulating apoptosis^{10, 11, 31, 39}, proliferation and survival^{55, 75}, as well as mediating Ca²⁺ mobilization after receptor ligation^{9, 43, 46}. Activation of the enzyme itself is regulated by cyclic AMP levels (addition of forskolin or dibutyryl cyclic AMP as demonstrated in a rat periosteal cell line and in PC12 cells^{39, 61}), by a PKC-dependent mechanism (activation with phorbol ester and inhibition with staurosporin and calphostin as shown in human erythroleukemia cells^{6, 42}), or by acidic phospholipids (phosphatidylserine, phosphatidylinositol, phosphatidic acid, phosphatidylinositol bisphosphate and cardiolipin⁵⁴). Receptors that are linked to SK are either G-protein-coupled receptors (e.g., m2 and m3 muscarinic acetylcholine receptors), growth factor receptors (NGF, bFGF)⁶¹ or members of the FcR family (Fc γ RI, Fc ϵ RI)^{9, 43}. Increased levels of S1P were subsequently implicated in mediating the release of Ca²⁺ from internal stores and to counter-regulate sphingolipids such as ceramide or S, thereby preventing either apoptosis (as described in T cells)¹⁰ or allowing Fc ϵ RI initiated signaling (as described in mast cells)⁵⁹, respectively. SK therefore can be regarded as a kind of "permissive switch" initiated at several receptors (shown for the Fc ϵ RI) to generate an intracellular lipid milieu allowing protein based signaling cascades, such as MAPKs and PKCs to activate cells⁵⁹.

Sphingolipids as Released Mediators

All of the above described mechanisms of signaling events by sphingolipids, especially those attributed to

S1P, are strongly supported by the finding that the latter indeed comprises a naturally occurring and extracellularly secreted mediator of some cell types after activation. In platelets, triggering by prothrombotic stimuli was shown to result in the formation and secretion of S1P. The mechanism of export is so far not elucidated⁸¹. Similar findings were recently made for FcεRI stimulated mast cells, which after triggering release considerable amounts of S1P, but not S, into the medium⁵⁹. In agreement with these findings, concentrations of S1P in the serum amount up to 0.5 nM⁸⁰. It is important to note, in this respect, that no corresponding sphingolipidosis has been characterized so far for S1P, supporting the notion that it is a naturally used activating principle. This naturally occurring extracellular function of S1P is also suggested by one of the two enzymes degrading this molecule. Besides sphingosine-1-phosphatase (an integral membrane protein with seven predicted transmembrane domains), type 2 phosphatidic acid phosphatase (PAP1 or LLP1, an integral membrane protein with 6 transmembrane domains) isoform 2a has the catalytic site extracellularly located. This suggests that ectoenzymes exist which are supposed to keep the extracellular concentration of secreted sphingolipids in a kind of balance^{4, 62}.

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