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BRIEF REVIEW

SPHINGOSINE KINASE SIGNALLING IN IMMUNE CELLS

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SUMMARY

1. Sphingolipids are potent second messengers modulating biochemical intracellular events and acting as ligands to mediate extracellular systems. Sphingosine kinase (SPHK) is the enzyme that phosphorylates sphingosine into sphingosine-1-phosphate (S1P), a potent bioactive sphingolipid.

2. The fact that SPHK is highly conserved from protozoa to mammals and is ubiquitous in living tissues reveals important roles of the SPHK pathway for the maintenance of health maintenance. This is also supported by comprehensive reviews on features of its main product, S1P, as having intracellular as well as extracellular roles, inducing a wide range of physiological responses from triggering Ca^{2+} release from internal stores to promoting growth and cell motility.

3. Immune cell activities have been shown to be modulated by the dynamic balance between ceramide, sphingosine and S1P, conceptualized as a rheostat. Cell proliferation, differentiation, motility and survival have been attributed to the regulatory actions of S1P. The properties of SPHK activity in immune cells are linked to the functions of triggered growth and survival factors, phorbol esters, hormones, cytokines and chemokines, as well as antigen receptors, such as FcγRI and FcεRI.

4. Mechanisms of the SPHK signalling pathway are explored as new targets for drug development to suppress inflammation and other pathological conditions.

Key words: immune cells, inflammation, lymphocytes, mast cells, monocytes/ macrophages, neutrophils, signalling, sphingosine kinase, sphingosine-1-phosphate.

INTRODUCTION

The elucidation of phospholipid chemical structures in cell membranes[1] was the seminal work for the current understanding and research on lipid signalling transduction. Early work showed that bioactive lipid metabolites, located at the plasma membrane, could activate the internal machinery of the cells, resulting in the stimulation of physiologically relevant events. Since then, many studies have supported the role of several lipid-derived molecules as key players in various signal transduction pathways.[2]

Polar sphingolipids originate from sphingomyelin (SM), a membrane phospholipid. The genitive sphingo derives from the Greek word sphinx (*σφινξ*). Sphingosine was the name suggested by Thudichum for a new lipid that evokes the enigmatic sphinx owing to its chemical nature containing both amine and alcohol groups, but insoluble in water.[3] Sphingosine is the backbone of all sphingolipids, which were initially studied for their structural roles in cell membranes. Sphingolipids have now been identified as potent second messengers that modulate diverse physiological processes.[4]

Over the past few years, it has become clear that sphingolipids are sources of important signalling molecules. In particular, sphingolipid metabolites, such as ceramide and sphingosine-1-phosphate (S1P), have emerged as a new class of potent bioactive molecule. They have been implicated in a variety of cellular processes, including cell differentiation, apoptosis and proliferation.[4–7] Sphingomyelin, a major membrane sphingolipid, is the precursor of these bioactive molecules. Through the hydrolysis of SM by sphingomyelinases (SMase), ceramide is formed. Ceramide can be hydrolysed very rapidly by ceramidases to yield sphingosine and sphingosine, in turn, is phosphorylated by sphingosine kinase (SPHK) to yield S1P (Fig. 1). In immune cells, S1P has been shown to be associated with signals for immune cell activation and differentiation,[8] such as calcium mobilization, cytoskeletal reorganization and chemotaxis.[9,10]

Several biological effectors have been shown to promote the synthesis of S1P, including growth factors, cytokines, antigens and G-protein-coupled receptor agonists.[11–14] Recently, much interest has been focused on the two distinct cellular roles of S1P as an extracellular agonist and as an intracellular second messenger.[15] This phospholipid is capable of acting as an extracellular ligand to activate specific G-protein-coupled receptors and as an intracellular second messenger that can trigger the release of calcium from internal stores.[16] Many studies have enforced the notion of S1P as an important intracellular second messenger. First, the activation of various plasma membrane receptors, such as the platelet-derived growth factor (PDGF) receptor,[11,17,18] FcεRI and FcγRI antigen receptors,[19–21] as well as the *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) and C5a receptors,[22–24] was found to increase rapidly intracellular S1P production through the activation of SPHK. Second, inhibition of SPHK strongly reduced or even inhibited cellular events triggered by these receptors, such as receptor-stimulated DNA synthesis, Ca^{2+} mobilization and

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vesicular trafficking.[17–24] Moreover, S1P is released by activated platelets during platelet activation[14] and can be detected in significant amounts in the serum.[25] Other studies have demonstrated a role for S1P in chemotaxis and in the prevention of apoptosis.[26,27]

As an extracellular mediator, several reports have shown that S1P binds a number of G-protein-coupled receptors encoded by endothelial differentiation genes (EDG), collectively known as the EDG receptors.[28–32] Binding of S1P to these receptors triggers a wide range of cellular responses, including proliferation, enhanced extracellular matrix assembly, stimulation of adherent junctions, the formation of actin stress fibres and the inhibition of apoptosis induced by either ceramide or growth factor withdrawal.[33–37]

Over the past few years, activation of SPHK and the consequent formation of S1P have been reported extensively for different cell types. In the present review, we will focus on the role of SPHK in mediating cellular responses in different immune cell types, as well as its potential role as a target for therapeutic intervention.

SPHINGOSINE KINASE

Sphingosine kinases are new members of the class of lipid kinases, including ceramide kinases, diacylglycerol kinases (DAGK) and phosphatidylinositol 3-kinases, which mediate cellular responses. Sphingosine kinases from seven different organisms have been cloned:[38] from mouse,[39,40] the yeast *Saccharomyces cerevisiae*,[41–43] the ciliated protozoan *Tetrahymena pyriformis*,[44] the plant *Arabidopsis thaliana*,[45,46] the worm *Caenorhabditis elegans,*[47] the fly *Drosophyla melanogaster*[48] and two human homologues.[40,49] Sphingosine kinase is evolutionarily well conserved and holds common regions in protozoans, yeasts, plants and mammals. There is a high degree of homology between the murine and human enzymes.[40,49] Sphingosine kinase is found to share similar a phosphate donor-binding site and phosphorylation mechanism with other kinases, such as the DAGK.[50]

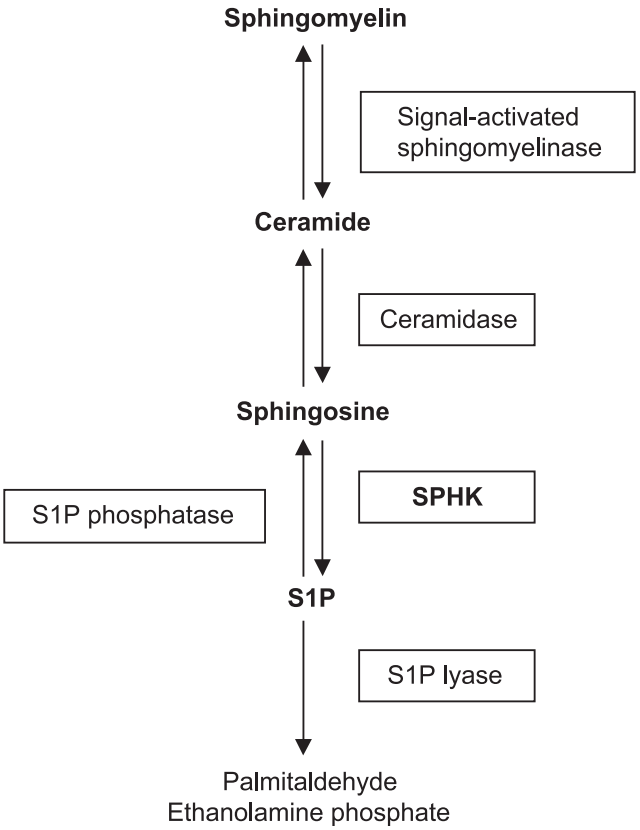

Fig. 1 The sphingolipid metabolic pathway. Sphingomyelyn is hydrolysed by sphingomyelinase to ceramide. Ceramide is metabolized by ceramidase to generate sphingosine. Sphingosine kinase (SPHK) phosphorylates sphingosine into sphingosine-1-phosphate (S1P), which is further cleaved by S1P lyase to a fatty aldehyde and ethanolamine phosphate.

Isoforms

The first members of the SPHK family were cloned, almost simultaneously, in budding yeast,[43] plant[44] and mouse.[39] Following the purification[51] and subsequent cloning of murine SPHK1,[39] the first human homologue was cloned.[49]

Being evolutionarily very much conserved, SPHK possesses a conserved kinase catalytic domain that contains an ATP-binding site, as well as five other conserved domains, namely C1 through to C5.[8,20] These domains may play a role in substrate recognition because they are different to domains found in other lipid kinases, such as ceramide kinases.[52]

To date, two mammalian SPHK have been cloned, sequenced and characterized. These kinases are encoded by two genes, *SPHK1*[39,49,53] and *SPHK2*.[40] At the protein level, SPHK1 is smaller than SPHK2. In terms of kinetics, SPHK1 exhibits higher enzymatic activity than SPHK2.[54] Other SPHK isoforms have been suggested to exist and to possess different properties and cellular distribution.[55]

Substrate specificity

Sphingosine kinases appear to be highly specific in their substrate preference. Both SPHK1 and SPHK2 are capable of phosphorylating *erythro*-sphingosine, dihydrosphingosine and phytosphingosine; however, no other phospholipids appear to be significantly phosphorylated by these enzymes.[39,49,53] Although the SPHK could be inhibited by a number of compounds, the best known inhibitors are analogues of sphingosine, such as DL-*threo*-dihydrosphingosine (DHS) and *N*,*N*-dimethylsphingosine (DMS).[56,57]

Tissue distribution

At the organ level, SPHK1 is found to be most abundant in lung, liver and spleen, whereas SPHK2 is expressed predominatly in the liver and heart.[39,49,53] Immunohistochemical analysis has shown cell type-specific localization of SPHK1 in the white matter of the cerebrum and cerebellum, the red nucleus and cerebral peduncle in the midbrain, the uriniferous tubules in the kidney, the endothelial cells in vessels of various organs and in megakaryocytes and platelets.[55,58–60] The tissue distribution of human SPHK mRNA by northern blots showed highest expression in adult lung and spleen, followed by peripheral blood leucocyte, thymus, kidney, brain and heart.[49]

Subcellular localization of SPHK

Sphingosine kinase was initially thought to be a cytosolic enzyme.[51] Studies later showed that subcellular distribution of

SPHK varies according to organs: it is cytosolic in the liver, blood and brain, but SPHK activity is associated with the insoluble fraction of lymph nodes and Peyer's patches.[51] Furthermore, it has been shown that SPHK can translocate from the cytosol to the plasma membrane.[20,23,24] A role for Ca^{2+}/calmodulin has been proposed for SPHK1 translocation, which is not necessary for activity.[61]

Sphingosine kinase 1 is generally localized to the cytoplasm and it translocates to the plasma membrane upon activation,[20,23,24] but no nuclear translocation has been shown for SPHK1. In contrast, at least one study has shown that SPHK2 can be localized to the nucleus[62] and SPHK activity has also been shown in nuclear extracts from cells stimulated with PDGF.[62] Another study showed the extracellular export of SPHK1.[63]

MECHANISMS OF SPHK ACTIVATION IN MAMMALIAN CELLS

Many studies have shown that external stimuli activate SPHK, resulting in an increase in S1P levels, in a number of cell types. Growth factors, such as epidermal growth factor (EGF),[12] PDGF,[11] nerve growth factor (NGF)[64] and vascular endothelial growth factor (VEGF),[65] have been shown to increase SPHK activity. Vitamin D_3 and serum are also activators of SPHK.[66,67] Furthermore, cytokines, chemotactic peptides and Fc receptors have been reported to activate SPHK activity in different inmmune–effector cells.[13,14,19,20,23,24,68,69]

Regulation of SPHK1 appears to occur at both the transcriptional and post-transcriptional level.[70,71] At the post-transcriptional level, SPHK1 has been shown to be regulated by phosphorylation and translocation,[23,24,72,73] whereas the regulation of SPHK2 activity remains unclear. It has been suggested that the molecular mechanism of SPHK1 activation correlated with its phosphorylation at Ser225, which is also necessary for translocation of the enzyme from the cytosol to the plasma membrane.[73]

ROLES OF SPHK AND S1P IN DIFFERENT CELLULAR PROCESSES

Importance of SPHK in calcium signalling and mobilization

Perhaps the best studied intracellular function involving SPHK activity in immune cell activation is its potential role in the regulation of intracellular calcium signals.[14,19–24,74,75]

Cytosolic Ca^{2+} is a ubiquitous intracellular messenger, pivotal in many signal transduction pathways. It is used to control a variety of cellular activities, ranging from proliferation and differentiation to cell death.[76] Resting cells have a Ca^{2+} concentration of approximately 100 nmol/L; this amount of intracellular Ca^{2+} is insufficient to trigger substantial cellular activities. Upon stimulation, the level of intracellular Ca^{2+} can rise very quickly, reaching up to 1 μmol/L, and Ca^{2+} triggered cellular activities occur.[76] It is well established that the increase in cytosolic Ca^{2+} can be temporally and spatially complex. This is because of the fact that different cells respond differently to a particular stimulus, whereas each stimulus triggers a particular cell type differently. Hence, intracellular Ca^{2+} signals could be of a single burst, short-lived, long-lasting or oscillatory. All these may occur in a localized microenvironment and be triggered as a widespread event.[77]

Cells generate their Ca^{2+} signals by using both internal and external sources of Ca^{2+}. Internally, Ca^{2+} is stored in specialized compartments, such as the endoplasmic reticulum (ER), sarcoplasmic reticulum (SR), mitochondria or in small compartments known as calciosomes.[78] It is also known that endosomes and phagosomes can store calcium.[79] The Ca^{2+} signals are controlled by the generation of intracellular second messengers, which bind to specific receptors/channels. There are several intracellular second messengers known to increase cytosolic levels of Ca^{2+}, including inositol 1,4,5-trisphosphate (IP_3), cyclic adenosine 5′-diphosphoribose (cADPR), nitric oxide, hydrogen peroxide, superoxide, nicotinic acid adenine dinucleotide phosphate (NAADP), diacylglycerol, arachidonic acid, phosphatidic acid, sphingosine, S1P and Ca^{2+} itself.[77] Of these intracellular second messengers, some act on intracellular Ca^{2+} channels found on internal compartments for Ca^{2+} release, some act on Ca^{2+}-entry channels found on the plasma membrane, whereas others may act on both the release and entry of Ca^{2+}.[76,77] Owing to the specificity of these second messengers, it is likely that there are several different types of intracellular Ca^{2+} release channels. However, only IP_3 receptors and ryanodine receptors (RYR) have been well characterized to date. Upon stimulation of cell surface receptors, the second messengers generated will determine which Ca^{2+} channels are activated. For instance, IP_3 generated binds to IP_3 receptors on the ER, resulting in the release of Ca^{2+} from the ER.[76] The activity of the RYR is modulated by the generation of cADPR;[78] NAADP acts on a separate, uncharacterized channel.[79] Exactly how S1P causes Ca^{2+} release from intracellular stores remains unclear.

Immune receptor activation triggers an increase in intracellular Ca^{2+}: A role for SPHK

Calcium is a key second messenger in leucocyte activation. It mediates activation, at least in part, of the respiratory burst and secretion of microbicidal granule constituents.[80,81] In immune cells, the resting cytosolic free Ca^{2+} concentration (intracellular Ca^{2+}) hovers in the 100 nmol/L range and is acutely elevated upon the engagement of immune receptors.[82,83] Release of Ca^{2+} stored in the ER and opening of store-operated channels are largely responsible for this elevation. However, Ca^{2+} is also now thought to be released by early and late endosomes, lysosomes and the yeast vacuole.[79,84] Along the same lines, it is entirely conceivable that Ca^{2+} trapped in the lumen of forming phagosomes, or accumulated afterwards by plasmalemmal Ca^{2+} pumps, may be released at critical stages of immune cell activation. Indeed, preliminary evidence to this effect has been presented.[85] For example, in phagocytic cells, a localized periphagosomal increase in intracellular Ca^{2+} has been recorded,[82] although this was attributed to the preferential distribution of ER in the immediate vicinity of phagosomes.

The release of Ca^{2+} from internal stores following receptor engagement in immune cells, is triggered by phospholipid-derived second messengers. Inositol 1,4,5-trisphosphate is the best-characterized second messenger responsible for triggering Ca^{2+} release from internal stores.[86] In some studies, receptor-triggered Ca^{2+} release from internal stores in neutrophils, mast cells and monocytes/macrophages has also been shown to be IP_3 independent.[19–24,74,87,88] There is indirect evidence suggesting that

L-plasmin, an actin-binding protein, is phosphorylated in response to phagocytosis and may participate in the IP_3-independent Ca^{2+} increase mediated by FcγRIIa in neutrophils.[89]

Recently, it has been shown that S1P is an important trigger responsible for the release of Ca^{2+} from internal stores stimulated by FcγRI aggregation in monocytes or FcεRI aggregation in mast cells and chemotactic peptides in neutrophils and macrophages.[14,19–21,23,24,74,88] In contrast, FcγRI triggers phospholipase C γ activation and Ca^{2+} signals that are IP_3 dependent in another macrophage model.[74] Of interest, we have recently reported that, in human mast cells, FcεRI triggers a dual calcium response.[20] In that study, mast cells were shown to concurrently use different messengers, both IP_3 and S1P, to generate the Ca^{2+} signals that led to the synthesis and release of inflammatory mediators.[20] Essentially, the FcεRI antigen receptor on these cells could trigger multiple signalling pathways. Other than the hydrolysis of phosphoinositide, which leads to IP_3 production, phospholipase D is activated to hydrolyse phosphatidylcholine into phosphatidic acid and choline. It has been suggested that phosphatidic acid could activate SPHK, resulting in phosphorylation of sphingosine to S1P.[90] In this dichotomy of Ca^{2+} signalling, SPHK mediates a fast and transient peak, whereas IP_3 mediates the sustained plateau.[91]

Role of SPHK in angiogenesis and control of cell adhesion molecule expression

Angiogenesis or neovascularization refers to the formation of new blood vessels. Cells that are activated during tissue injury, such as platelets, have been shown to release S1P into the serum, where it can act as a mitogen promoting cell growth and potential neovascularization. For instance, it has been shown that, in human platelets, basal SPHK activity is high and that S1P is stored in high concentrations inside human platelets; following stimulation, S1P is released from platelets in the micromolar range.[92] The release of S1P may serve as an autocrine and/or paracrine factor with potential roles in endothelial cell injury, inflammation and angiogenesis. In addition, S1P is a high-affinity agonist for EDG-1, a gene induced during angiogenesis.[93] Sphingosine-1-phosphate exerts profound endothelial cell responses associated with angiogenesis, including the liberation of endothelial cells from established monolayers, chemotactic migration, proliferation, adherens junction assembly and morphogenesis into capillary like structures.[63,94,95] Furthermore, VEGF stimulates SPHK activity, which has been proposed to be critical for the processes of angiogenesis.[65] In another study, tumour necrosis factor (TNF)-α stimulation of endothelial cells was shown to rapidly stimulate SPHK activity and S1P generation,[13] leading to the expression of the cell adhesion molecules vascular cell adhesion molecule-1 and E-selectin. The mechanism by which TNF-α couples the activation of SPHK to cell adhesion molecule expression was demonstrated through the use of an analogue of sphinosine, namely DMS. This inhibitor strongly inhibited the TNF-α-induced activation of extracellular signal-regulated kinase and nuclear factor-κB, as well as expression of cell adhesion molecules.[13]

Anti-apoptotic effects of SPHK

Apoptosis refers to programmed cell death and leads to the resolution of inflammation without disgourgement of cytotoxic cellular contents. This is an evolutionary conserved cell suicide process, required in tissue homeostasis to prevent uncontrolled cell proliferation.[96] Ceramide and its catabolite sphingosine (Fig. 1) prevent cell proliferation and promote apoptosis, whereas opposing effects are mediated by S1P. Sphingosine-1-phosphate promotes cell survival in response to apoptotic stimuli, such as TNF-α, Fas ligand, serum deprivation, ionizing radiation or anticancer drugs.[97,98] In SPHK-overexpressing cells, in which high levels of basal S1P are found, the sphingosine/S1P balance is shifted towards an anti-apoptotic setting.[99] Interestingly, it has also been shown that neuronal cell apoptosis induced by the withdrawal of tropic factor could be suppressed in cells that overexpress SPHK1.[100]

Chemotaxis

Chemotaxis is the directed migration of cells towards the gradient of a chemoattractant. In purified human peripheral blood neutrophils, monocytes and macrophages, it has been shown that SPHK plays a central role in leucocyte chemotaxis.[14,23,24] Furthermore, the formation of S1P may integrate the activation of downstream signals via the paracrine/autocrine activation of EDG-1, regulating directional movement triggered by non-G-protein-coupled receptor chemoattractants, such as PDGF or phorbol esters.[101,102] Moreover, S1P has also been shown to enhance glioblastoma cell adhesion; it is possible that autocrine or paracrine signalling by S1P, through its receptors, enhances both glioma cell proliferation and invasiveness.[103]

SPHINGOSINE KINASE ACTIVITY IN DIFFERENT IMMUNE CELLS

Role of SPHK in neutrophils

Neutrophils are terminally differentiated immune cells which are essential for defence against microbes. Conversely, they are also involved in tissue damage resulting from inflammation. Activated neutrophils generate a broad and vigorous set of alterations in gene expression, shaping the inflammatory response.[104] Sphingosine kinase regulates neutrophil priming to provide an essential defence against infections.[105] In the early acute neutrophil-mediated inflammatory response, neutrophils may cause tissue damage owing to their overactivation or prevention from apoptosis control. Next, recruitment of circulating neutrophils to damaged tissue plays a critical role in a number of physiological and pathophysiological events, such as protection from invading pathogens and the development of the inflammatory process. During an inflammatory response, circulating neutrophils attach to the activated endothelial cells in blood vessels through various adhesion molecules. They would then migrate through the endothelial cell layer into the injured sites, where they release toxic substances, such as active oxygen radicals and active enzymes that destroy infected, as well as surrounding normal, tissue. Studies have shown that SPHK plays key roles in neutrophil activation, including neutrophil chemotaxis.[14,91] Moreover, it has also been reported that TNF-α and fMLP stimulated superoxide production via SPHK activity, because the SPHK inhibitor DHS inhibited TNF-α induced superoxide production.[106] We have recently reported that C5a-triggered responses, such as chemotaxis, degranulation and superoxide

generation, in primary human neutrophils are inhibited by the more specific SPHK inhibitor DMS.[24] These reports demonstrate a key role for SPHK in regulating physiologically relevant events of human neutrophils.

Role of SPHK in monocytes and macrophages

Monocytes and macrophages are key players of innate immunity in the host defence against invading pathogens. These cells contribute to a number of important immune processes, such as antigen presentation and the phagocytosis of infected cells, foreign particles and pathogens. Macrophages have been implicated in several pathologies, such as atherosclerosis,[107] rheumatoid arthritis, chronic inflammation and cancer.[108] Sphingosine kinase activity has been shown to be stimulated in macrophages by a wide range of stimuli. Its product, S1P, has been reported to advance the wound-healing processes *in vitro and in vivo*.[31] Other studies have demonstrated that SPHK activity modulates the expression of vascular cell adhesion molecules on endothelial cells,[13,36] This is essential for the recruitment of leucocytes during inflammation and progression of the inflammatory response. We have shown that activation of monocytes via the high-affinity IgG receptor (FcγRI) triggers SPHK activity that is essential for the release of calcium from internal stores and regulates monocyte NADPH oxidase activity;[21,74] because calcium is a key event in immune receptor signalling, the implications are that SPHK may regulate a variety of responses triggered by these receptors on monocytes and macrophages. More recently, we have shown that the anaphylatoxin uses SPHK in order to trigger several physiologically relevant events in human macrophages, such as calcium release from internal stores, chemotaxis, degranulation and cytokine production.[23] Furthermore, it has been shown that inhibition of SPHK by intracellular pathogens, on macrophages, may contribute to the survival of the pathogen. *Mycobacterium tuberculosis* is a highly successful pathogen that parasitizes the macrophages of its host. Following its ingestion by macrophages, *M. tuberculosis* inhibits phagosome maturation; this prevents the progression of the phagosome into an acidic, bactericidal lysosomic compartment.[109] It is well established that phagosome maturation is regulated by Ca^{2+} signals; it has recently been reported that *M. tuberculosis* blocks Ca^{2+} signalling and phagosome maturation in human macrophages via specific inhibition of SPHK,[75] suggesting a role for SPHK in phagosome maturation and, thus, on the proper disposal of intracellular pathogens by macrophages.

Role of SPHK in mast cells

Mast cells are effector cells that, upon activation, release and produce a wide range of bioactive mediators and cytokines. In particular, these contribute to the inflammatory responses triggered during allergic reactions. When allergens bind to sensitized mast cells, they trigger release of histamine-containing granules, as well as the release of other pro-inflammatory mediators.[110,111] Other than playing an important role in allergic responses, mast cells have been suggested to also play a significant role in both innate and acquired immunity against bacterial and parasitic infections.[112]

It has been proposed that the differential ratio of sphingosine to S1P in mast cells regulates the activity of mast cells. Activation of mast cells via the high-affinity IgE-receptor (FcεRI) leads to activation of SPHK, which results in increased formation of S1P,[19,20,113] Whereas sphingosine inhibits activation of the mitogen-activated protein kinase family, SAP-kinase and activator protein-1, S1P activates these molecules. Hence, activation of FcεRI stimulates mast cells, which, through the activation of SPHK, triggers the expression of the pro-inflammatory cytokines TNF-α and interleukin (IL)-5.[113] Moreover, we and others have shown that FcεRI activates SPHK-dependent Ca^{2+} mobilization in mast cells,[19,20] as well as SPH-dependent mast cell degranulation.[20] Put together, these data suggest a key role for SPHK and/or its product S1P on the responses triggered by activated mast cells.

Role of SPHK in lymphocytes

It has been observed that enhanced SPHK activity promotes cell survival in Jurkat T cells in response to ceramide- or Fas-induced apoptosis.[99] In addition, individual immunological responses of T helper cells are modulated by sphingosine and the balance between counter-regulatory lipids in the serum.[8] One study has shown that stimulation of S1P receptors leads to sequestration of lymphocytes in secondary lymphatic tissues and this prevents their access to inflammatory lesions and graft sites.[101] Furthermore, it has been suggested that SPHK2 associates with the cytoplasmic region of the IL-12 receptor β-chain and it is likely to positively modulate the effect of IL-12 on the T cell response, which leads to the generation of interferon-γ.[114] Thus, although not very well characterized, the role of SPHK on lymphocytes appears to be significant.

FUTURE PERSPECTIVES AND THERAPEUTIC IMPLICATIONS

Understanding of sphingolipid signal transduction pathways has helped to provide potential new targets for the treatment of inflammatory conditions.[110] Pharmaceutical and therapeutic applications based on research of SPHK activation are envisaged to control the contribution of S1P to pathological states. Sphingosine kinase activity, through the generation of S1P, is likely to enhance inflammation by stimulating several immune effector cells, as well as to mediate the mitotic and chemotactic responses of immune cells and the activation of endothelial cells.[115] In addition, S1P was observed to be increased in the airways of asthmatics and SPHK activity has been shown to mediate degranulation of human mast cells.[20] Moreover, treatment with SPHK inhibitors has been proposed to reduce IgE/antigen-stimulated histamine release.[116]

Most studies on the role of SPHK in the inflammatory response have been centred on its role on cell adhesion molecule expression and its effect on neutrophil, mast cell and monocyte/macrophage functions, with very little data reported on the potential roles of SPHK in cell-mediated immune reactions. However, the available data show a role for SPHK in promoting proliferation and blocking apoptosis; this leads us to speculate that SPHK may enhance effects that depend on cell proliferation, such B and T cell clonal activation, but counter those that depend on apoptosis, such as activation-induced apoptosis or tolerance.

A further point of interest is that platelets have been shown to contain high levels of S1P and that following activation platelets release large amounts of S1P into the immediate environment. This gradient of S1P may act as a chemoattractant for monocytes, thus

initiating the inflammatory process important in promoting atherosclerosis and thrombosis.

Another interesting point is that several reports suggest that SPHK1 and S1P play a role not only in inflammation, but also in the interface with tumour immunology. For instance, TNF-α has been shown to use SPHK1 to induce cell adhesion molecule expression on endothelial cells, as well as to induce cyclooxygenase (COX)-2 activity and prostaglandin E_2 synthesis. All these mediators are involved in the inflammatory process and TNF-α is known to play a major role in inflammatory diseases, such as rheumatoid arthritis.[117] Moreover, it has been shown that COX-2 inhibitors can reduce tumour size and metastasis[118] and, thus, inhibition of SPHK1 could potentially be used as a novel therapeutic treatment not only in the case of inflammatory disorders, but also for cancer.

Finally, there is a potential role of SPHK1 in autoimmune diseases. An approximate 50 cM region on 17 q25, which harbours *stSG2854* (an expressed sequence tag found in the SPHK1-coding region), has been implicated in several autoimmune and inflammatory diseases, such as multiple sclerosis,[119] psoriasis and epidermodysplasia verruciformis.[120,121] Because the *SPHK1* gene has been mapped to this chromosomal region, SPHK1 is a possible candidate for disease susceptibility.[49]

The data discussed in the present review have summarized the considerable progress achieved in a few years of research on the physiological roles of SPHK, which, put together, reflect the importance of sphingolipid pathways in immune responses. With the aid of novel techniques, such as RNA interference (RNAi), antisense oligonucleotides and gene knock out, it is easier to consider that further explorations on the functions and applications of SPHK and its product S1P will help to elucidate the potential roles played by these bioactive molecules in health and disease. The data reviewed here strongly suggest a key role for SPHK in immune–inflammatory processes and indicate the SPHK as novel 'druggable targets' for the development of new therapeutics for inflammatory and other diseases.

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