Sphingosine kinase and sphingosine 1-phosphate in asthma

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Synopsis
Sphingolipids are amphipathic molecules ubiquitously expressed in all eukaryotic cell membranes. Initially characterized as structural components of cell membranes, sphingolipids have emerged as sources of important signalling molecules over the past decade. Sphingolipid metabolites, such as ceramide and S1P (sphingosine 1-phosphate), have been demonstrated to have roles as potent bioactive messengers involved in cell differentiation, proliferation, apoptosis, migration and angiogenesis. The importance of SphK (sphingosine kinase) and S1P in inflammation has been demonstrated extensively. The prevalence of asthma is increasing in many developed nations. Consequently, there is an urgent need for the development of new agents for the treatment of asthma, especially for patients who respond poorly to conventional therapy. Recent studies have demonstrated the important role of SphK and S1P in the development of asthma by regulating pro-inflammatory responses. These novel pathways represent exciting potential therapeutic targets in the treatment of asthma and are described in the present review.

Key words: asthma, cytokine, inflammation, sphingolipid, sphingosine 1-phosphate (S1P), sphingosine kinase (SphK)

INTRODUCTION

SphK (sphingosine kinase) is a key enzyme in the sphingolipid metabolic pathway which phosphorylates sphingosine into S1P (sphingosine 1-phosphate), thus providing an essential checkpoint that regulates the relative levels of ceramide, sphingosine and S1P [1]. To date, two mammalian SphKs have been cloned, sequenced and characterized. These kinases are encoded by two genes SPHK1 [1–3], and SPHK2 [4]. Human SPHK1 localizes to chromosome 17 (17q25.2), whereas SPHK2 maps to chromosome 19 (19q13.2). Although the two mammalian isoforms possess five evolutionarily conserved domains found in all SphKs and are highly similar in amino acid sequence, they differ in kinetic properties and also in temporal and spatial distribution, implying that they may have distinct physiological functions.

Lysoospholipid S1P can mediate diverse biological processes, mainly due to its ability to act as an intracellular second messenger as well as extracellularly as a specific and high-affinity ligand for a family of GPCRs (G-protein-coupled receptors) known previously as the EDG (endothelial differentiation gene) family. To date, five S1P receptors have been identified, which include EDG-1, EDG-3, EDG-5, EDG-6 and EDG-8, and these receptors are now collectively known as S1P1–S1P5 [5]. Binding of S1P to these receptors triggers a wide range of cellular responses, including proliferation, enhanced extracellular matrix assembly, stimulation of adherent junctions, formation of actin stress fibres and inhibition of apoptosis [6,7]. These receptors mediate their diverse cellular functions through differential coupling to various heterotrimeric G-proteins and through heterogeneity in their expression patterns [5]. S1P has also been proposed to play an intracellular role as a second messenger after observations that stimulation of various plasma-membrane receptors, such as PDGFR (platelet-derived growth factor receptor), FcγRI (Fcγ receptor 1) and FcεRI (Fcε receptor 1) antigen receptors, the fMLP (N-formylmethionyl-leucyl-phenylalanine) receptor, the C5a receptor and TNFR [TNF (tumour necrosis factor)-α receptor], trigger the rapid production of S1P through SphK activation [6,7]. Moreover, inhibition of SphK strongly reduced cellular...
events triggered by these receptors, such as receptor-triggered DNA synthesis, Ca\(^{2+}\) mobilization and vesicular trafficking.

**ASTHMA**

Asthma is a chronic inflammatory disorder associated with AHR (airway hyper-responsiveness) that leads to recurrent episodes of wheezing, breathlessness and coughing. It represents a serious global health problem, with an estimated 300 million individuals affected. The most effective anti-asthmatic drugs currently available include inhaled \(\beta_2\)-receptor agonists and glucocorticoids, which control asthma in approx. 90–95% of patients [8]. Clinically, asthma may be divided into an allergic and non-allergic origin, distinguished by the presence or absence of IgE antibodies to common environmental allergens. However, in both forms of the disease, the airway is infiltrated by Th2 cells, which secrete cytokines such as IL (interleukin)-4, IL-5, and IL-13. Th2 cytokines orchestrate airway inflammation by further stimulating mast cells and macrophages, driving eosinophilia, promoting leukocytosis and enhancing B-cell IgE production [9]. These inflammatory cells, together with the resident airway cells, interact with one another to perpetuate airway inflammation, leading to AHR and remodelling [10].

**SphK SIGNALLING IN MAST CELLS**

Mast cells are effector cells that play a central role in allergic diseases. In an acute allergic attack, interaction of allergens with IgE antibodies bound to the high-affinity IgE-receptor FceRI on mast cells leads to the activation of various biochemical pathways, resulting in mast cell degranulation and the release of a wide range of bioactive mediators and cytokines [11]. Mast cells are also known to play a role in chronic allergic responses such as asthma. In addition to mediator release, mast cells are also being recognized for their ability, by direct cell-to-cell contact, to alter ASM (airway smooth muscle) cells, mucus production and T-cell activation, leading to AHR and eventually eliciting an inflammatory infiltrate [12]. Mast-cell-derived mediators such as histamine, LTD\(_4\) (leukotriene D\(_4\)), PGD\(_2\) (prostaglandin D\(_2\)) and tryptase have been found in BAL (bronchoalveolar lavage) from patients with allergic asthma [13], supporting the role of these cells in the early allergic reaction in asthma.

One of the biochemical pathways triggered by FceRI activation is the SphK signalling pathway. In 1996, Choi et al. [14] first discovered that FceRI stimulation induces SphK activity, leading to the increase in SIP levels in the rat mast cell line RBL. Furthermore, FceRI-triggered Ca\(^{2+}\) mobilization was inhibited in the presence of the SphK inhibitor DHS (D,L-threo-dihydrosphingosine). The FceRI/SphK signalling pathway is clathrin-dependent and involves signalling through PI3K (phosphoinositide 3-kinase) [15]. It has also been proposed that the differential ratio of sphingosine to SIP, in the cytosol, regulates the activity of mast cells [16]. In mouse mast cells, these two lipids have been demonstrated to have opposing effects, with sphingosine being inhibitory and SIP activating the MAPK (mitogen-activated protein kinase) pathway, resulting in degranulation and leukotriene release triggered by FceRI activation [16]. FceRI stimulation leads to SphK1-dependent Ca\(^{2+}\) mobilization in human mast cells, as well as SphK1-dependent mast cell degranulation [17]. Using specific siRNA (small interfering RNA) knockdown of the isoforms, Spiegel et al. [18] also found that SphK1 plays a critical role in IgE/antigen-induced degranulation, migration towards an antigen and CCL2 (CC chemokine ligand 2) secretion from human mast cells [18]. Furthermore, studies using a chemical antagonist of SIP\(_2\) or antisense knockdown of either SIP\(_1\) or SIP\(_2\) have defined specific and non-redundant roles for each of the receptors in mast cells [19,20]. In mouse bone-marrow-derived mast cells, activation of SIP\(_1\) is important for cytoskeletal rearrangements and migration of mast cells towards an antigen, but they are dispensable for FceRI-triggered degranulation. However, SIP\(_2\), whose expression is up-regulated by FceRI cross-linking, was required for degranulation and inhibited migration towards an antigen [19]. In rat basophilic leukaemia cell line RBL-2H3, the SIP\(_2\) antagonist ITE-013 blocked SIP-induced mast cell migration [20]. Controversy surrounding the role of SphK1 and SphK2 in mast cells has also been documented. Olivera et al. [21] reported that SphK2 regulates mast cell activation, whereas SphK1 enhances susceptibility to antigen challenge *in vivo* in knockout mouse models. Taken together, these data suggest a key role for SphK and/or its product SIP in the responses triggered by activated mast cells.

**SphK/SIP AND ASM**

ASM cells function as the primary effector cells that modulate bronchomotor tone. However, beyond its contractile functions, ASM also contributes to the pathogenesis of asthma in various ways. Airway instillation of bacterial LPS (lipopolysaccharide) into rat lungs enhanced ICAM-1 (intercellular adhesion molecule-1) expression in ASM [22]. Lazaar et al. [23] demonstrated the constitutive expression of CD44 on human ASM in culture as well as in human bronchial tissue transplanted into SCID (severe combined immunodeficiency) mice. Levels of ICAM-1 and VCAM-1 (vascular cell adhesion molecule-1) expression are also induced on ASM by inflammatory mediators such as TNF-\(\alpha\) and may play a role in cell–cell interactions with other inflammatory cells, such as T-cells [23], eosinophils [24] and mast cells [25]. Under the influence of inflammatory cytokines such as IL-6, ASM may be induced to undergo hyperplasia and hypertrophy and contribute ultimately to airway obstruction [26]. ASM can also secrete various inflammatory mediators such as GM-CSF (granulocyte/macrophage colony-stimulating factor) and IL-5, which contribute to the
proliferation and survival of eosinophils [27,28]. ASM also plays prominent role in the recruitment of inflammatory cells, as it has also been demonstrated to be a source of various chemokines such as eotaxin [29], MCP-1 (monocyte chemoattractant protein-1) [30] and RANTES (regulated upon activation, normal T-cell expressed and secreted) [31]. SphK1 is mainly localized to the smooth muscles of the peripheral airways in the mouse. Furthermore, muscarine-induced peripheral airway constriction was reduced by inhibition of SphK using DHS and DMS (N,N-dimethylphosphosine), suggesting that the SphK/S1P signalling pathway contributes to cholinergic constriction of murine peripheral airways [32].

Elevated levels of S1P in BAL fluid were recovered from allergic asthma patients after ragweed antigen challenge and may play a role in both acute bronchoconstriction and airway remodelling through its direct action on ASM cells [33]. On the basis of a model of human ASM cells embedded in collagen matrices, Rosenfeldt et al. [34] demonstrated that S1P induced the formation of stress fibres, contraction of individual ASM cells and stimulated myosin light chain phosphorylation in a Rho-kinase-dependent manner [34]. In another study [35] using guinea-pig tracheal smooth muscle strips, pretreatment with S1P markedly enhanced methacholine-induced contraction. This effect was inhibited in the presence of a Rho-kinase inhibitor. Exogenous addition of S1P to human bronchial smooth muscle cells increased the level of active RhoA (GTP-RhoA) and phosphorylation of MYPT1 (myosin phosphatase target subunit 1). These findings demonstrate that exposure of ASM to S1P results in airway hyper-reactivity mediated via RhoA and inactivation of myosin phosphatase [35].

In addition, Roviezzo et al. [36] evaluated the effects of exogenous S1P administration on isolated bronchi and whole lungs harvested from Balb/c mice sensitized to OVA (ovalbumin) by measuring bronchial reactivity and lung resistance. They found that, in OVA-sensitized mice, S1P causes a dose-dependent contraction of isolated bronchi and increased airway resistance in the whole lung system. This was accompanied with an enhanced expression of SphK1, SphK2, S1P2 and S1P3 [36].

**SphK/S1P and Lung Epithelial Cells**

A key component of asthma is the structural change that involves all of the elements of the airway wall. There has been increasing evidence that the airway epithelium plays a crucial and fundamental role in asthma development [37,38]. Activation of cPLA2α (cytosolic phospholipase A2α) is a crucial step in the production of AA (arachidonic acid) and subsequent eicosanoid formation. It has been demonstrated in the human lung epithelial cell line A549 that AA release can be induced via S1P/S1P3. The S1P-induced Ca2+ flux and AA release was associated with SphK1 expression and activity. Furthermore, Rho-associated kinase, downstream of S1P1, was crucial for S1P-induced cPLA2α activation [39]. In a recent study by Kono et al. [40], normal human bronchial epithelial cells were cultured in an air/liquid interface and treated with IL-13 to induce their differentiation into goblet cells. IL-13 treatment increased the expression of SphK1 and MUC5AC (mucin 5AC) at both the protein and mRNA levels, whereas SphK2 expression was not modified. Furthermore, treatment with DMS decreased MUC5AC expression up-regulated by IL-13 treatment. IL-13-induced ERK1/2 (extracellular-signal-regulated kinase 1/2) phosphorylation was also inhibited by DMS, whereas neither p38 MAPK nor STAT6 (signal transducer and activator of transcription 6) phosphorylation was affected. These results suggest that SphK1 is involved in MUC5AC production induced by IL-13 upstream of ERK1/2 phosphorylation and is independent of STAT6 phosphorylation [40].

**MURINE ASTHMA MODELS**

Murine allergic asthma represents an ideal model with which to explore the diverse inflammatory effects of SphK blockade. In our study using OVA-induced allergic asthma mouse model [41], we observed that DMS, a sphingosine kinase mouse model, has inhibitory actions on inflammatory cell infiltration into the lungs, as revealed by histological examination, and a significant fall in total cells and eosinophil counts in BAL fluids. This suppressive action of DMS on leukocyte migration is consistent with the role of S1P in the chemotaxis of various immune cells [6], and could also be partially due to a direct effect on cellular migratory machinery, such as Ca2+ mobilization and expression of adhesion molecules including VCAM-1 and E-selectin [42]. In addition, an indirect effect mediated through the suppression of chemokine production is also possible, as reduced eotaxin BAL levels were observed in mice receiving DMS. Data from our murine studies have also shown that the anti-inflammatory effect of SphK1 blockade is, at least in part, mediated through a suppressive action on T-lymphocytes, as OVA-specific cell proliferation, and IL-4 and IL-5 production was reduced in thoracic lymph node cultures from DMS-treated mice [41]. A reduction in BAL fluid IL-4, IL-5 and eotaxin levels was also observed in these mice. The anti-inflammatory effects of SphK blockade may extend to resident ASM cells, as DMS administration significantly suppressed OVA-induced AHR to methacholine in a dose-dependent manner. The suppression of AHR by DMS could be due in part to the direct inhibition of ASM contraction, and also be associated with the reduced tissue eosinophilia and Th2 cytokine levels via SphK pathway inhibition [41].

To establish that the inhibitory effects of DMS were indeed mediated by direct SphK inhibition rather than non-specific off-target side effects [43] and to determine the efficacy of SphK1 blockade in murine asthma, we extended our study using an siRNA knockdown approach. Intranasal administration of SphK1 siRNA substantially reduced eosinophilic infiltration into...
**Table 1 Potential pathogenic role of S1P/SphK in asthma**

CCL2, CC chemokine ligand 2; RANTES, regulated upon activation, normal T-cell expressed and secreted.

<table>
<thead>
<tr>
<th>Cell type/system</th>
<th>Effect</th>
<th>Enzyme</th>
<th>Receptor</th>
<th>Reference(s) (species)</th>
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<tbody>
<tr>
<td>Mast cell</td>
<td>Degranulation</td>
<td>SphK1</td>
<td>S1P₂</td>
<td>[17,18] (human) and [19] (mouse)</td>
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<td></td>
<td>Chemotaxis</td>
<td>SphK1</td>
<td>S1P₁</td>
<td>[18] (human) and [19] (mouse)</td>
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<td></td>
<td>CCL2 production</td>
<td>SphK1</td>
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<td>[18] (human)</td>
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<td>TNF-α production</td>
<td>SphK1 and SphK2</td>
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<td>[18] (human)</td>
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<td>Airway epithelial cell</td>
<td>AA release</td>
<td>SphK1</td>
<td>S1P₃</td>
<td>[39] (human)</td>
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<td></td>
<td>Mucin production</td>
<td>SphK1</td>
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<td>[40] (human)</td>
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<tr>
<td>ASM cell</td>
<td>Contraction</td>
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<td>[34] (human)</td>
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<td></td>
<td>Proliferation</td>
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<td>[33] (human)</td>
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<td></td>
<td>RANTES and IL-6 production</td>
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<td>[33] (human)</td>
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<tr>
<td>Respiratory airway</td>
<td>Airway resistance and compliance</td>
<td>SphK1 and SphK2</td>
<td>S1P₂ and S1P₃</td>
<td>[36,41] (mouse)</td>
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<td>In vivo asthma mouse model</td>
<td>Leucocyte infiltration into lungs</td>
<td>SphK1</td>
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<td></td>
<td>IL-4, IL-5, IL-13 and eotaxin production</td>
<td>SphK1</td>
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<td>[41,45,46]</td>
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<td></td>
<td>Serum IgE</td>
<td>SphK1</td>
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<td>[41]</td>
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<td></td>
<td>Airway hyper-reactivity</td>
<td>SphK1</td>
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<td>[41,45,46]</td>
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<td></td>
<td>Eosinophilia</td>
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<td>Eosinophil peroxidase activity</td>
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<td>Goblet cell hyperplasia</td>
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corticosteroids. Elevated levels of S1P in BAL fluid were recovered from allergic asthma patients after ragweed antigen challenge. The S1P/SphK signalling pathway has been shown to play critical roles in immune cells that drive asthma pathogenesis as well as resident airway cells (summarized in Table 1). Furthermore, on the basis of recent data from in vivo murine asthma models, blockade of SphK activity can effectively suppress airway inflammation, Th2 cytokine and chemokine secretion, and markedly attenuate AHR in sensitized animals. These data indicate that SphK pathways may find therapeutic utility in allergic asthma.

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