

Allergy therapy: The therapeutic potential of targeting sphingosine kinase signalling in mast cells

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Mast cell activation is a central event in allergic diseases, and investigating the signalling pathways triggered during mast cell activation may lead to the discovery of novel therapeutic targets. Mast cells can be activated by a multitude of stimuli including antibodies/antigen, cytokines/chemokines and neuropeptides, resulting in a variety of responses including the immediate release of potent inflammatory mediators. Moreover, recent data suggest that mast cell-mediated responses are also influenced by the differential sphingolipids/sphingosine to sphingosine-1-phosphate ratio. The importance of sphingolipids as potent biological mediators of both intracellular and extracellular responses is being increasingly recognized and accepted; it is now appreciated that activation of mast cells, via the high-affinity IgE-receptor (FcεRI) leads to the activation of sphingosine kinases (SphK), resulting in increased formation of sphingosine-1-phosphate. Furthermore, FcεRI activates SphK-dependent calcium mobilization in mast cells, leading to degranulation, cytokine, and eicosanoid production, and chemotaxis. In the past two years a critical role for SphK in allergic responses *in vivo* has emerged. In this review, I focus on the current understanding of the role of sphingosine kinases during mast cell signalling *in vitro* and their role during hypersensitivity responses *in vivo*, and discuss the potential of these enzymes as novel therapeutic targets to treat allergic diseases.

Key words: Allergies · Mast cells · Sphingolipids · Sphingosine kinases · Therapeutic target

Introduction

The triggering event in the initiation of an acute allergic attack is the interaction of normally innocuous substances, *i.e.* allergens, with IgE antibodies bound to the surface of mast cells, via the high-affinity IgE-receptor, FcεRI. This initiates a number of biochemical pathways that ultimately result in mast cell degranulation, and the release of a variety of both pre-formed, and newly synthesized, inflammatory mediators [1]; however, mast cells are also known to play a role in delayed and/or chronic allergic responses such as asthma [1]. Figure 1 shows the principal pathways leading to acute and chronic allergic

reactions. Mast cell activation triggered by FcεRI leads to the activation of sphingosine kinase (SphK), which in turn changes the balance between sphingosine and sphingosine-1-phosphate (S1P), in favour of S1P [2–5]. These two lipids have opposite effects on immune cells [6]. In immune cells, sphingolipid metabolism is tightly related to stages of immune cell development, differentiation, activation, and proliferation – and manifested in the form of physiological responses such as survival, proliferation, calcium mobilization, cytoskeletal reorganization, chemotaxis, and eicosanoid and cytokine production [6–9].

Sphingosine-1-phosphate

Sphingolipids have emerged as a source of important signalling molecules potentially involved in pathophysiological

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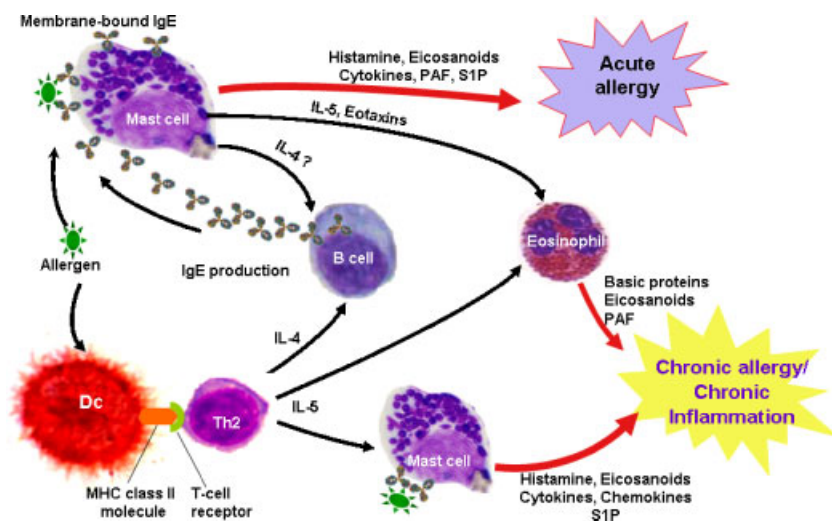


Figure 1. Pathways leading to acute and chronic allergic reactions. Mast cells play roles in both acute and chronic allergic reactions by secreting various proinflammatory mediators, including histamine, cytokines, chemokines and lipid products.

processes [6]. S1P belongs to a new class of potent bioactive molecules, which are involved in a variety of cellular processes, including cell differentiation, proliferation, and migration [9–11]. Sphingomyelin, the major membrane sphingolipid, is the precursor of these bioactive molecules. Sphingomyelinases (Smae) hydrolyse sphingomyelin to form ceramide, which has been implicated in apoptosis *via* caspase activation [6]. Ceramide can be further metabolized by ceramidases to yield the single fatty-chain sphingosine. Sphingosine in turn can be phosphorylated by SphK, to generate S1P [7–9]. S1P acts as an intracellular “second messenger” as well as an extracellular ligand for specific receptors [10, 11]. This notion that S1P acts as an intracellular second messenger has been supported by a substantial number of studies (reviewed in [6–10]). Firstly, activation of various plasma membrane receptors, such as the antigen receptors FcεRI and FcγRI [2–5, 12, 13], receptors for the chemotactic peptides fMLP and C5a [14–16], and receptors for growth factors and cytokines [17–20], were found to rapidly increase intracellular S1P production through the activation of SphK. Secondly, inhibition of SphK strongly reduced or even blocked cellular events triggered by these receptors, such as gene expression, calcium (Ca^{2+}) mobilization, chemotaxis, and degranulation (reviewed in [7–9]).

S1P released by activated cells, including mast cells and platelets (reviewed in [11]), can be detected in significant amounts in serum [21, 22]. An increasing number of reports show that S1P binds to a family of five G-protein-coupled receptors (the S1P₁, S1P₂, S1P₃, S1P₄, and S1P₅ receptors) [23–28]. 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, migration, and inhibition of apoptosis induced by either ceramide or growth factor withdrawal [10, 11, 27, 28].

SphK

SphK are members of lipid kinases, which include acyl-glycerol kinases and phosphoinositol-kinases (reviewed in [8]). So far, two mammalian SphK have been characterized, encoded by two genes *SPHK1* and *SPHK2* [29–32]. Both SphK1 and SphK2 are capable of phosphorylating erythro-sphingosine, dihydrosphingosine and phytosphingosine; however, no other lipids appear to be significantly phosphorylated by these enzymes [29–32]. SphK are evolutionarily conserved, holding common regions in protozoan, yeast, plant, and mammals (reviewed in [7–9]). Given that three recent elegant reviews address the domain structure, regulation, tissue distribution, substrate specificity, and general functions of Sphk [7–9], these topics will not be covered in this review.

Sphk in mast cell signalling

The first report of SphK signalling in immune cells was in fact based on results derived from mast cells and was published in 1996 [2]. In this report, the authors demonstrated that FcεRI stimulation rapidly induces SphK activity and raises S1P levels in the rat mast cell line RBL. Moreover, an SphK inhibitor (dihydrosphingosine, DHS) inhibited the Ca^{2+} response triggered by FcεRI stimulation [2]. Later on, it was shown that in human bone marrow-derived mast cells, SphK1 is critical for calcium responses and degranulation triggered by FcεRI [3] – a process that is defined by two distinct waves of Ca^{2+} responses. The first one (fast and transient) is dependent on SphK1, and the second, slower Ca^{2+} wave is dependent on phospholipase- $\text{C}\gamma$ 1 and responsible for the influx of external Ca^{2+} . The initial mast cell degranulation in this system is totally and exclusively dependent on SphK1 activity. These findings position SphK1 on par with phospholipase- $\text{C}\gamma$, *i.e.* as an additional major enzyme

creating a “second messenger”, making this part of the sphingolipid pathway equivalent to the diacylglycerol and inositol-1,4,5-trisphosphate generation in the glycerolipid pathway. Furthermore, some proinflammatory molecules secreted by mast cells after FcεRI stimulation, including leukotrien C4 and TNF, are completely inhibited by high intracellular levels of sphingosine, suggesting that SphK not only generates a second messenger but “removes” an inhibitory one. Therefore, it was postulated that a (lipid) rheostat composed of sphingosine and S1P, together with SphK, act as a kind of permissive switch and are involved in the fine tuning of allergic susceptibility of mast cells by FcεRI engagement [33]. Later studies explained how the intracellular activation of SphK and the extracellular functions of S1P cooperate in the activation of mast cells [34]. Activation of SphK, by FcεRI stimulation, leads to S1P secretion, which acts through its receptors S1P₁ and S1P₂ present on human and rodent mast cells, to amplify the responses observed during FcεRI activation [34]. Experimentally, this was demonstrated through inhibition of SphK1, which blocked FcεRI-mediated internalization of S1P₁ and S1P₂ receptors and reduced degranulation and chemotaxis [34]. The authors postulated that the S1P₁ receptor is important for cytoskeletal rearrangements and migration of mast cells toward antigen, but dispensable for degranulation. In contrast, the S1P₂ receptor appeared to be required for degranulation and inhibited migration toward antigen [34]. More recently, it was postulated that both SphK1 and SphK2 are activated by FcεRI in mouse mast cells [5, 35, 36]. Of interest, it was recently shown that the *SPHK1* gene is one of the first genes to be activated during IgE-sensitization of human mast cells, and that SphK1 mRNA is further increased during FcεRI stimulation [37].

The role of each specific SphK isoform in mediating mast cell responses and signalling via FcεRI stimulation remains controversial. Initially, it was shown that SphK1 is essential for calcium signals and degranulation in human and rat mast cells [3, 4]; however, a recent study suggested that SphK1 was not required for degranulation or for cytokine or eicosanoid production in mast cells from SphK1^{−/−} mice [35]. In fact, it was SphK2 that was determined to be responsible for *in vitro* activation of mast cell responses [35]. In contrast, another recent study [36] demonstrated that SphK1 but not SphK2 is critical for FcεRI-induced degranulation, migration, and CCL2 secretion. Interestingly, in the same study, both isoenzymes were required for efficient TNF-α secretion, suggesting that SphK1 and SphK2 may have distinct signalling cascades triggered by FcεRI/Ag in mast cells. Consistent with this idea, recent work has shown that in mouse mast cells, FcεRI stimulation primarily activates SphK1, which is downstream of PLD1 and PKCα [38]. Moreover, blocking this pathway at various points, including blockade of SphK1, inhibited the FcεRI-triggered initial rise in cytosolic calcium, degranulation, NF-κB activation, and the production of various cytokines and eicosanoids [38]. Figure 2 shows a schematic representation of the FcεRI-triggered pathways described in this paragraph.

Role of SphK in allergic anaphylaxis

IgE-mediated anaphylaxis is considered to be primarily a mast cell-mediated response [39]. A recent report by Olivera *et al.* [35], using mice with *SPHK1* or *SPHK2* gene deficiency, showed a further role for both SphK1 and SphK2 in murine mast cell activation; however, the study suggests that SphK2 is more important for mast cell mediated degranulation, and cytokine and eicosanoid production *in vitro*. In contrast, SphK1 was suggested to be more important in anaphylactic response *in vivo* [35], which raises obvious questions. An explanation for these potential discrepancies is the possibility that mice lacking the SphK1 gene during embryonic development adopt compensatory mechanisms to overcome the lack of SphK1 and use other pathways or another SphK, as has been shown for other intracellular signalling molecules [40]. An interesting observation of the knockout studies is that the total amount of S1P in the serum of both knockout mice is around half of that found in wild-type mice, raising the question, which of the two enzymes, SphK1 or SphK2, is more important for their physiological roles during cellular activation? Data from my laboratory, using short-interfering RNA gene-silencing *in vivo*, suggest that SphK1 is indeed required for mast cell degranulation, cytokine and eicosanoid production both *in vitro* and *in vivo* in models of passive cutaneous and systemic anaphylaxis (manuscript submitted for publication).

Targeting SphK in allergic asthma

Allergic diseases, and especially asthma, are a major health problem that has demonstrated an alarming increase in prevalence, particularly in Western countries during the past two decades [41]. Mast cells of asthmatic patients micro-localize within airway smooth muscle (ASM) cells [42], the airway mucous glands [43], and the bronchial epithelium [44]. The disordered airway physiology and wall remodelling features of asthma are consequences of inflammation and bronchial hyper-responsiveness. Furthermore, abnormal ASM function is fundamentally important in the pathophysiology of asthma and there is a positive correlation between ASM mast cell numbers and bronchial hyper-responsiveness [42], evoking a functional interaction between these two cell types (reviewed in [45]). Interestingly, elevated levels of S1P in the “broncho-alveolar lavage” (BAL) fluid were recovered from allergic asthma patients after ragweed Ag challenge have been observed, which has been suggested to play a role in both acute broncho-constriction and airway remodelling through its direct action on ASM cells [46, 47]. Furthermore, it has recently been shown that the specific blockade of SphK1 *in vivo* protects mice from allergic asthma induced by OVA immunization [48, 49]. In a murine model of allergic asthma using mice previously sensitized to OVA, specific silencing of SphK1 with siRNA significantly reduced the total inflammatory cell infiltrate, eosinophilia, and the concentration of

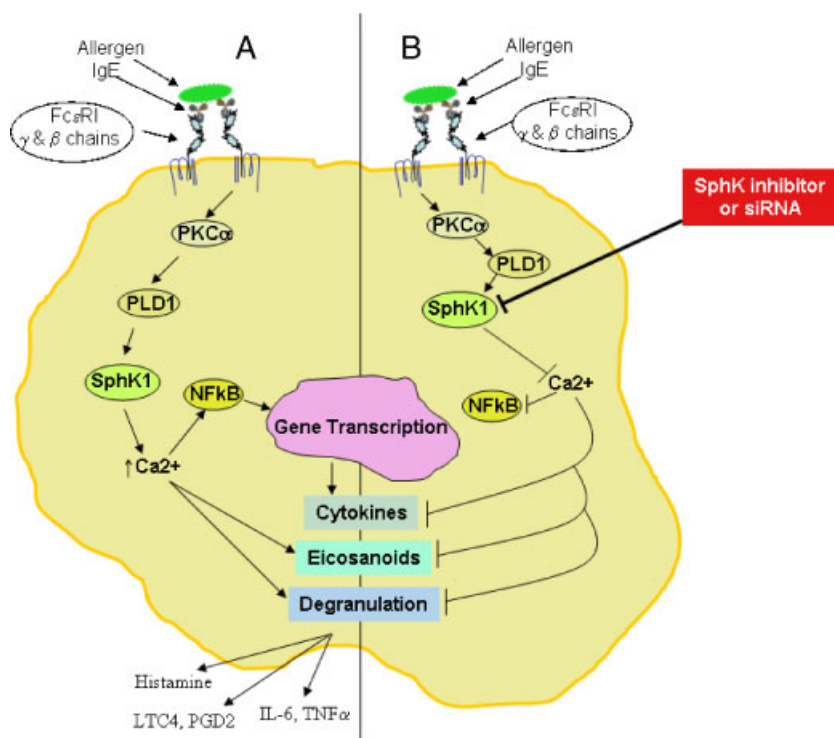


Figure 2. Blockade of SphK1 inhibits FcεRI-mediated mast cell responses. (A) Normally, FcεRI couples to PKCα, PLD1 and SphK1 to trigger the initial raise in cytosolic calcium responsible for triggering degranulation, NF-κB activation, and cytokine and eicosanoids secretion. (B) Blockade of SphK1 by either siRNA-gene silencing or pharmacological inhibitors, blocked the FcεRI-triggered SphK1-dependent calcium signal, and the key mast cell responses are blocked.

IL-4, IL-5, and eotaxin in BAL fluid in response to inhaled OVA challenge [48]. In another recent study, Nishiuma *et al.* [49] showed that, using a similar model with an SphK1 inhibitor [SK-I; 2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl) thiazole], resulted in a decrease in S1P concentration in BAL fluid to basal levels, accompanied by decreased eosinophil infiltration and peroxidase activity. Moreover, bronchial hyperresponsiveness to inhaled methacholine and goblet cell hyperplasia were improved in both studies [49].

Conclusion

The considerable progress achieved in a few years of research on the roles of SphK in mast cell responses, strongly suggests that SphK are key signalling molecules of mast cell activation, and are essential for the FcεRI-triggered mast cell-mediated responses. Moreover, by generating S1P, SphK further amplify the inflammatory response as S1P is, perhaps, a unique signalling molecule in that it can act both as an intracellular second messenger, and as an extracellular ligand for several G-protein-coupled receptors [3, 4]. Interestingly, S1P levels are elevated in the BAL fluid of asthmatic patients after Ag challenge, suggesting that S1P is relevant in allergic responses [46, 47]. Interestingly, S1P has recently been found to play an important role in Th17 development [50].

Many questions remain to be answered; there is still controversy over the different roles of SphK1 and SphK2 in mast cell responses. Due to a lack of specific SphK inhibitors, it has been difficult to properly validate the specific role(s) played by each

isoform in physiology and/or pathophysiology; however, using siRNA and/or novel inhibitors to validate SphK *in vivo* [48, 49], a clearer picture is starting to emerge, suggesting that SphK may indeed be potential novel therapeutic targets for asthma and other allergic diseases.

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Abbreviations: ASM: airway smooth muscle cells · BAL: broncho-alveolar lavage · FcεRI: high-affinity IgE-receptor · S1P: sphingosine-1-phosphate · SphK: sphingosine kinase

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