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Sphingosine-1-phosphate synthesis and functions in mast cells

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Abstract

Sphingolipids are not only major lipid components of all eukaryotic cell membranes, but they also comprise an important family of bioactive signaling molecules that regulate a diverse array of biological responses. The sphingolipid metabolite sphingosine-1-phosphate (S1P), is a key regulator of immune responses. Cellular levels of S1P are determined by the balance between its synthesis, involving two sphingosine kinases (SphK1 and SphK2), and its degradation, involving S1P lyase and S1P phosphatases. S1P mainly signals through its cell-surface receptors and may also have intracellular functions. S1P has important functions in mast cells – the major effectors of allergic responses. Antigen triggering of IgE receptors on mast cells activates both SphKs resulting in the production of S1P that is released and regulates and amplifies mast cell functions, including degranulation as well as cytokine and chemokine release.

Keywords

allergy; anaphylaxis; inflammation; mast cells; sphingolipid metabolites; sphingosine-1-phosphate; sphingosine kinase

Over the last decade or so, the work of many investigators has established the importance of the bioactive lipid mediator sphingosine-1-phosphate (S1P) in regulating numerous and diverse cellular processes in various cell types, including proliferation, cell survival, motility and cytoskeletal rearrangements as wells as angiogenesis [1,2]. S1P exerts the majority of its effects

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as an extracellular ligand for a family of five specific G proteincoupled receptors, denoted $S1P_{1–5}$ [1]. These receptors all bind S1P with similar affinity but are able to couple to a variety of G proteins, thus enabling S1P to regulate numerous downstream signaling pathways [2]. In addition, every cell in the body expresses at least one of the S1P receptors, which are differentially expressed from cell to cell, further complicating the understanding of the wide ranging yet distinct actions of S1P. S1P has also demonstrated some actions that are independent of its receptors [1], although its intracellular targets have not yet been unequivocally identified. This review is focused on the emerging importance of S1P functions in mast cells and discusses the potential for targeting sphingosine kinases (SphKs), S1P receptors, as well as S1P itself, to suppress mast cell-mediated inflammation and related pathological conditions.

Sphingolipid metabolism & sphingosine kinases

Intracellular levels of S1P are tightly regulated by the balance between its synthesis, which involves SphK1 and SphK2, and its degradation, which can occur either reversibly by two specific S1P phosphatases or irreversibly by S1P lyase (Figure 1). Therefore, this balance between S1P and its precursors – sphingosine and ceramide – and their overall regulation of opposing signaling pathways is instrumental in determining cell fate and has been termed the ‘sphingo-lipid rheostat’ [3]. SphK1 was the first isozyme discovered and characterized and is, therefore, the most well studied. It is activated by numerous stimuli, including many growth factors and cytokines and crosslinking of immuno globulin receptors [1]. Activation of SphK1, which requires its phosphorylation by ERK1/2 [4,5], is accompanied by its translocation to the plasma membrane where its substrate sphingosine resides [6,7]. Much less is known about the regulation of SphK2. Its subcellular localization is cell-type specific, that is cytosolic in some types and mainly nuclear in others, and it can translocate between these compartments in response to specific stimuli [8]. In rodent mast cells, SphK1 and SphK2 are largely cytosolic under basal conditions and translocate to the plasma membrane following IgE-receptor engagement [6,7].

Interestingly, although SphK1 and SphK2 are highly homologous and utilize the same substrate to produce the same product, they exhibit both functional and experimental differences. In contrast to the growth and survival promoting actions of SphK1, overexpression of SphK2 in many cells induces cell death and growth arrest. Very few studies to date have examined the role of endogenous SphK2. It has recently been demonstrated that SphK2 is also activated by phosphorylation [9], as well as by antigen crosslinking of IgE receptors [7]. Mice with knockouts of either *SphK1* or *SphK2* are viable, have only slightly decreased S1P levels and exhibit no obvious phenotypes [10]. However, knockout of both *SphK1* and *SphK2* results in complete loss of S1P and is embryonically fatal [10], demonstrating the necessity of S1P for life. Together, these observations also suggest that SphK1 and SphK2 may have some redundant, overlapping and/or compensatory functions.

FTY720

While both SphK1 and SphK2 can phosphorylate sphingosine and sphinganine (dihydrosphingosine), only SphK2 can efficiently catalyze the phosphorylation of the immunosuppressive drug FTY720 (Fingolimod) [11] – a sphingosine analog – forming FTY720-phosphate, which is a S1P mimetic capable of binding with high affinity to all of the S1P receptors except $S1P_2$. Its immunosuppresive action as a potent ligand of $S1P_1$ on lymphocytes leads to prolonged downregulation of this receptor [12,13]. Since $S1P_1$ is required for lymphocyte egress from secondary lymph nodes and lymphoid organs, these cells are sequestered in this location by FTY720 administration, resulting in lymphopenia and rendering them incapable of responding to inflammation [14,15].

Sphingosine-1-phosphate receptors

As all mast cells express two of the five S1P receptors ($S1P_1$ and $S1P_2$) [6,16], the following section focuses on these two receptors, and the reader is referred to more comprehensive reviews for information about the other S1P receptors [17,18].

$S1P_1$

$S1P_1$ is widely expressed, with predominant expression found in brain, kidney, spleen, lung and the cardiovascular system [19]. $S1P_1$ was first demonstrated to be important in angiogenesis when mice lacking this receptor were found to have incomplete vascular development and consequently died *in utero* [20]. This receptor is also a key player in the maintenance of vascular integrity, which is important for inflammation and asthmatic lung remodeling [21–23]. Importantly, $S1P_1$ is also critical in lymphocyte egress from the thymus and peripheral lymphoid organs. Indeed, mice lacking expression of $S1P_1$ in hematopoietic cells exhibit lymphopenia since mature T and B cells are unable to exit the thymus [13]. Furthermore, $S1P_1$ signaling is strongly upregulated prior to the exit of T cells from the thymus, suggesting a role in the chemotactic responsiveness of these cells [13].

$S1P_2$

$S1P_2$ is also expressed in a variety of cell types. In contrast to $S1P_1^{-/-}$ mice, mice lacking $S1P_2$ are viable and only display a defect in proper development of auditory and vestibular systems, resulting in complete deafness [24–26]. In mast cells, $S1P_2$ is important for effective degranulation [6]. In the vascular system, activation of $S1P_2$ also increases vascular permeability, similar to $S1P_1$ [23]. Furthermore, $S1P_2$ is considered to be a 'repellant' receptor as binding of S1P to $S1P_2$ decreases motility of many cell types, including mast cells [6].

Roles of SphK1 & SphK2 in mast cells

Mast cells express the high-affinity receptor for IgE – FcεRI – which is an important component of allergic diseases. Its crosslinking by monomeric IgE bound to multivalent antigens initiates an elaborate and complicated cascade of signaling events that leads to the release of preformed granules (degranulation) containing histamine and other mediators of immediate responses as well as the subsequent production and secretion of cytokines and chemokines and lipid mediators, such as eicosanoids (leukotrienes and prostaglandins) and S1P [27,28]. These mast cell mediators promote inflammation by enhancing vascular permeability while initiating the recruitment and activation of other immune cells involved in allergic and inflammatory responses.

Crosslinking of IgE receptors on mast cells results in activation of several key regulators, including Lyn, Fyn and Syk, which are initiators of intricate pathways involving numerous downstream signaling molecules that ultimately co ordinate and control mast cell responsiveness [29]. Loss of Fyn or Lyn in mast cells has widespread effects, impairing degranulation and cytokine production. While Fyn and Lyn tyrosine kinases are associated with SphK1 and SphK2 in murine mast cells, activation of SphK1 requires Fyn but Lyn is partly dispensable [7]. Both Lyn and Fyn contribute to SphK2 translocation to the plasma membrane upon FcεRI triggering. Interestingly, SphK2 was reported to be the major contributor of S1P in murine mast cells derived from embryonic liver progenitors [30]. Mast cells derived from *SphK2*-knockout mice demonstrated impaired degranulation and production of certain cyto kines, primarily due to reductions in intracellular calcium levels and PKC activation. Impairment of degranulation in SphK2-deficient mast cells was partially restored by the addition of exogenous S1P. This confirmed that SphK2 is necessary, but not sufficient, for IgE-mediated responses, at least in murine mast cells [7]. By contrast, in human mast cells,

SphK1 but not SphK2 is critical for antigen-induced degranulation, chemokine secretion and migration, while both isozymes are important for cytokine secretion [31]. Furthermore, downregulation of SphK1 reduced the rapid and transient increase in intracellular calcium induced by FcεRI crosslinking, which is necessary for mast cell degranulation [32]. In addition to the engagement of FcεRI, several other stimuli are capable of triggering secretion of inflammatory mediators from activated mast cells, including the anaphylatoxin C5a [33]. With regard to the actions of C5a, SphK1 expression is required for its ability to trigger calcium release, chemotaxis, degranulation and cytokine release from human macrophages [34]. However, neutrophils isolated from *SphK1*-knockout mice showed normal responses to C5a [35].

Extracellular functions of S1P in mast cells

Inside-out signaling, whereby S1P generated intracellularly by activation of SphKs is secreted and activates $S1P_1$ and $S1P_2$ receptors on the same or nearby mast cells, plays important roles in mast cell responses [36]. For example, activation of $S1P_1$ is critical for migration of mast cells toward antigens and might be involved in the movement of mast cells up an antigen gradient to sites of inflammation [6]. Furthermore, expression of the motility-inhibiting $S1P_2$ receptor in mast cells is upregulated by crosslinking of FcεRI by antigens [6], suggesting that mast cells are attracted to an inflammation site by a $S1P_1$-dependent motility process and halt upon reaching their destination owing to upregulation of $S1P_2$. Here, activation by inside-out signaling also enhances their degranulation. Thus, there appears to be an exquisite interplay of S1P controlled responses following FcεRI activation in mast cells.

Secretion of S1P from mast cells

The mechanism by which intracellularly produced S1P can exit from cells to interact with its receptors located on the extracellular leaflet of the plasma membrane has been a long standing mystery. It has been proposed that SphK1 may be secreted from cells and catalyzes the conversion of sphingosine to S1P in the extracellular milieu [37,38], although no evidence has been found for this in mast cells [6]. A partial answer has now been provided by our recent discovery that the ATP-binding cassette transporter ABCC1 promotes the export of S1P across the plasma membrane of activated rodent and human mast cells independent of their degranulation [39]. It is possible that other ABC transporters may also participate in export of S1P.

Blood levels of S1P

The concentration of S1P in blood is maintained at high levels. Plasma levels range from 0.1 to 0.6 μM, while serum levels range from 0.4 to 1.1 μM [40,41]. S1P mainly circulates as a complex with albumin and lipoproteins. Platelets that produce, store and secrete large amounts of S1P, were long considered to be the major source of circulating S1P. However, recent studies suggest that erythrocytes may be the major source of S1P in blood [42,43]. The vascular endothelium, in addition to the hematopoietic system, has also been suggested to be an important contributor of plasma S1P [44].

Levels of S1P in tissues are significantly lower than in blood, possibly owing to the presence of S1P phosphatase and S1P lyase, which are absent or low in platelets and erythrocytes [45]. This leads to the establishment of a concentration gradient of S1P between blood and tissues, which is important for lymphocyte trafficking. Intriguingly, deletion of either isoform of SphK in mice does not abolish this blood–tissue gradient of S1P [30], while loss of S1P lyase activity does so [45]. Similarly, secretion of S1P by mast cells may also serve to establish a gradient that aids in the recruitment of other immune cells whose chemotactic motility is stimulated by

S1P. However, susceptibility to *in vivo* anaphylaxis correlated with circulating S1P generated by SphK1 that was predominantly from a non-mast cell source(s) [30].

Intracellular actions of S1P in mast cells

Although intracellular targets of S1P have yet to be identified, S1P has intracellular second messenger actions that regulate calcium levels in mast cells independently of phosphatidylinositol (3,4,5)-trisphosphate ($InsP_3$) [46]. This calcium mobilization was recently demonstrated to be dependent on clathrin [47]. It has also been suggested that both $InsP_3$ and S1P contribute to FcεRI-induced calcium release from the endoplasmic reticulum and that production of $InsP_3$ is necessary for S1P to cause calcium mobilization from the endoplasmic reticulum [48].

Intriguingly, fetal liver-derived mast cells from mice lacking SphK2 display impaired calcium mobilization upon IgE-receptor activation, even when S1P is added exogenously [30]. Additionally, exogenous S1P only partially restored degranulation to mast cells isolated from mice lacking Fyn kinase [7]. Collectively, these data suggest that S1P may be a *bona fide* second messenger in mast cells, although acting in a manner that still requires clarification.

Anaphylaxis

Anaphylaxis is a severe and potentially fatal immediate systemic allergic reaction that occurs suddenly after contact with an allergy-causing substance and is triggered by rapid, IgE-mediated immune release of potent mediators from tissue mast cells and peripheral basophils [49]. Mast cells reside at mucosal, submucosal and perivascular locations in close proximity to epithelial surfaces, near blood vessels, nerves and glands, where they are able to detect invading pathogens and changes in their environment [28]. In humans, mast cell-derived mediators contribute to the pathophysiology of allergic diseases, inducing tissue edema, broncho constriction, increased vascular permeability, influx of inflammatory cells and mucus secretion. In addition, mast cells express numerous receptors for cytokines, chemokines and eicosanoids, as well as Toll-like receptors, which enable them to recognize diverse allergic stimuli. The diversity in cellular location, as well as the repertoire of receptors expressed and mediators released, permits mast cells to be key regulators of innate and adaptive immunity.

Murine and human immune systems are reasonably similar and so animal models of anaphylaxis may provide information that is potentially relevant to human anaphylaxis. Systemic anaphylaxis in the mouse can be mediated via two independent mechanisms; a classical pathway mediated by IgE, FcεRI, mast cells, histamine and platelet-activating factor (PAF), and an alternative pathway mediated by IgG, FcγRIII, macrophages and PAF [50]. Most human systemic anaphylaxis is probably IgE-dependent, although there is some evidence for IgE-independent anaphylaxis [51]. Some potent food allergens, particularly peanuts and tree nuts, can stimulate an anaphylactic-like, non-IgE-mediated response, thereby synergizing with IgE-induced mast cell activation to exacerbate anaphylaxis.

Role of S1P in anaphylaxis

Recent studies indicate that SphKs are also determinants of anaphylaxis. SphK2 is the main isoform required for generation of S1P, calcium influx and degranulation of rodent mast cells [7]. However, susceptibility to anaphylaxis in mice was correlated with circulating S1P generated by SphK1, predominantly from a non-mast cell source [30]. Mast cells do not contribute to basal circulating levels of S1P as mast cell-deficient mice have similar levels of plasma S1P compared with their counterparts engrafted with normal mast cells [30]. Mice deficient in SphK1 have reduced levels of circulating S1P and are resistant to anaphylaxis. They also have impaired histamine responses despite normal mast cell function. However,

mice deficient in SphK2 have enhanced levels of S1P in the blood and respond normally to anaphylactic challenge with normal histamine release [30].

Intestinal anaphylaxis (allergic diarrhea) is almost totally IgE- and mast cell-dependent, but is mediated predominantly by PAF and serotonin. In a murine intestinal anaphylaxis model, $S1P_1$ expression was preferentially associated with pathogenic $CD4^+$ T cells induced by allergen challenge in the large intestine. FTY720 prevented allergic diarrhea by inhibiting the migration of these cells and decreased mast cell infiltration into the large intestine, but did not affect eosinophil infiltration or serum IgE production [52].

Asthma

In asthma, mast cells infiltrate the bronchial epithelium and, upon activation, release inflammatory mediators that influence bronchial epithelial function. Mast cell numbers are greater in the mucosal epithelium of patients with asthma and allergic diseases compared with disease-free controls, with no substantial change in the numbers of mast cells in the adjacent connective tissues [53]. Abnormal airway smooth muscle function is a key feature in the pathophysiology of asthma, with a positive correlation between mast cell numbers and bronchial hyper-responsiveness [54].

Role of S1P in asthma

Previous studies demonstrated that S1P was elevated in the airways of asthmatic individuals after antigen challenge and that S1P modulates human airway smooth muscle cell functions that promote inflammation and airway remodeling in asthma [55] and can induce contraction of airway smooth muscle [56]. S1P can also amplify and enhance mast cell functions and may regulate their arrival to sites of inflammation [6,57]. Rodent models in which asthma-like symptoms are introduced by sensitization and challenge with antigen in an adjuvant are characterized by airway eosinophilia, which contributes to the observed airway hyper-responsiveness (AHR). It has recently been demonstrated that S1P induces dose-dependent contraction of bronchi and increases AHR in ovalbumin (OVA)-sensitized mice [58]. These events were associated with increased expression of SphK1 and SphK2, as well as $S1P_2$ and $S1P_3$ receptors. Local administration of S1P caused inflammation and eosinophil recruitment in a rat-paw inflammation model [59]. Furthermore, S1P and the kinases that produce it play important roles in many types of immune cells involved in allergic responses and asthma (Table 1), implicating S1P as a pleiotropic lipid mediator important in the inflammatory and allergic reactions and asthma.

FTY720 is highly effective in reducing the severity of autoimmune diseases in several animal models [60]. Neither FTY720 nor FTY720-phosphate, despite its similarity to S1P, affect mast cell degranulation, yet both significantly reduce antigen-induced secretion of prosta glandin D_2 and cysteinyl leukotrienes [61]. FTY720 was suggested to be a direct inhibitor of cytosolic phospholipase A_2, the rate-limiting enzyme in the production of all eicosanoids [61]. Indeed, oral treatment of mice with FTY720 inhibits AHR induced by adoptive transfer of Th1 and Th2 cells and asthma induced by active immunization and challenge with OVA [62]. In addition, inhalation administration of FTY720 prior to, or during, ongoing allergen challenge suppressed Th2-dependent eosinophilic airway inflammation and bronchial AHR by inhibition of migration of lung dendritic cells to the mediastinal lymph nodes, thus preventing the formation of allergen-specific Th2 cells in the lymph nodes [63].

SphK inhibitors: new targeted antiallergic therapies?

Current therapeutic strategies for these unfortunately most common diseases are often of poor efficacy and associated with undesirable side effects. Complete allergen avoidance is very

difficult to achieve. Desensitizing immunotherapy has been performed for many years, with doubtful efficacy in many cases and has occasionally even been hazardous. Thus, new approaches for the development of novel inhibitors of allergic diseases have great potential. Modulating the production of S1P and/or specifically targeting its receptors are attractive novel approaches for the modulation of mast cell-mediated allergic diseases. Promising observations in murine models of allergic disease provide proof of concept for the importance of SphKs and production of S1P as targets in inflammatory responses. For example, increased levels of proinflammatory cytokines in the peritoneal cavity of mice administered C5a were substantially decreased by treatment with the pan SphK inhibitor, *N,N*-dimethylsphingosine (DMS) [64]. This was accompanied by suppression of C5a-induced neutropenic responses, as well as by increased vascular permeability [64]. Moreover, C5a activates SphK in human neutrophils and the SphK inhibitor DMS largely blocked C5a-stimulated calcium mobilization, chemotaxis and cytokine production [34]. However, although an *in vivo* model of bacterial lung infection revealed an accelerated p rogression of disease in *SphK2* but not *SphK1*-knockout mice, effector functions of SphK1- or SphK2-deficient neutrophils and their capacity to kill bacteria were normal [35].

A recent study by Lai *et al*. utilized the pan-SphK inhibitor DMS and downregulation of SphK1 expression to demonstrate that they both effectively suppressed airway eosinophilia, pulmonary inflammation and secretion of Th2 cytokines and chemokines, and markedly attenuated OVA-induced AHR in sensitized mice [65]. Serum levels of OVA-specific IgE were reduced by SphK1 siRNA, suggesting that production of S1P may regulate B-cell trafficking and IgE production. In another study, OVA inhalation caused S1P release into bronchial alveolar lavage (BAL) and expression of SphK1 around bronchial epithelial walls. Inhalation of pan-SphK inhibitors decreased S1P in BAL, accompanied by decreased eosinophil infiltration and eotaxin expression. Furthermore, bronchial hyper-responsiveness to inhaled methacholine and goblet cell hyperplasia were improved by SphK inhibitors [66].

Executive summary

Sphingolipid metabolism & sphingosine kinases

- Sphingosine kinases (SphK) 1 and 2 are highly homologous, but have distinct functions.
- Antigens and many other external stimuli activate SphK1, inducing its phosphorylation and translocation to the plasma membrane.
- Although antigen also stimulates SphK2 in rodent mast cells, much less is known about the regulation of SphK2.

FTY720

- Only SphK2 catalyzes the phosphorylation of the immunosuppressive drug FTY720 (Fingolimod) producing a mimetic of sphingosine-1-phosphate (S1P) that induces lymphopenia.

S1P receptors

- The $S1P_1$ receptor regulates migration of mast cells toward antigen and sites of inflammation and is necessary for T- and B-lymphocyte egress from thymus and peripheral lymphoid organs.
- The $S1P_2$ receptor is important for proper mast cell degranulation and the resolution of their migration at sites of antigen challenge.

Roles of SphK1 & SphK2 in mast cells

- S1P generated by activation of SphK1 and SphK2 in mast cells promotes inflammation by stimulating calcium influx, degranulation and cytokine production. This increases vascular permeability, inducing airway smooth muscle contraction and triggering airway hyper-responsiveness, while simultaneously signaling the recruitment and activation of other immune cells involved in allergic and inflammatory responses.
- SphK1 is required for optimal histamine release and plays a key role in allergic responses.
- SphK2 is important for degranulation, calcium influx and production of certain cytokines in murine mast cells.

Extracellular functions of S1P in mast cells

- Inside-out signaling, whereby S1P generated intracellularly by activation of SphKs in response to antigen is secreted and activates $S1P_1$ and $S1P_2$ receptors on the same or nearby mast cells, plays important roles in amplification of mast cell responses.

Secretion of S1P from mast cells

- The ABC transporter ABCC1 exports S1P in addition to cysteinyl leukotriene from mast cells.

Blood levels of S1P

- Major sources of blood S1P are platelets and erythrocytes, and possibly endothelial cells.

Intracellular actions of S1P in mast cells

- S1P generated intracellularly may have unique functions in mast cells acting by mechanisms that are not fully understood.

Role of S1P in anaphylaxis

- Susceptibility to anaphylaxis in mice correlates with circulating S1P generated by SphK1, predominantly from a non-mast cell sources.

Role of S1P in asthma

- S1P is elevated in the airways of asthmatic individuals after antigen challenge.
- S1P and SphKs are involved in murine models of airway hyper-responsiveness.
- Local application of FTY720 to the lung abrogates experimental asthma by altering dendritic cell function.

SphK inhibitors: new targeted antiallergic therapies?

- Activation of SphK1 and SphK2, and subsequent production of S1P, exacerbate the symptoms of allergic responses, asthma and anaphylaxis. SphKs, as well as S1P and its receptors, are attractive targets for the development of therapeutics for treatment of these human disorders.
- Promising observations in murine models of allergic disease provide proof-of-concept for the importance of SphKs and production of S1P as targets in inflammatory responses.

Conclusion & future perspective

Although S1P has been implicated as an important component of the regulation of immune responses, there are still many questions waiting to be answered. The lack of SphK isozyme-specific inhibitors has made it challenging to assign specific functions to SphK1 and SphK2. However, the recent development and availability of specific S1P receptor agonists and antagonists will likely encourage more preclinical and clinical trials to target effects mediated by S1P. In conclusion, the relevance of mast cells, S1P, SphKs and S1P receptors for the maintenance of normal physiology, or in disease states, constitutes an outstanding and intricate combination of players important for immune responses at the cellular, signaling and molecular levels. The need for specific SphK inhibitors is driving the development of new compounds by many pharmaceutical companies. Combining knowledge gained from molecular strategies and conditional gene knockouts to interfere with expression of enzymes that regulate S1P levels with pharmacological approaches will surely aid in this quest.

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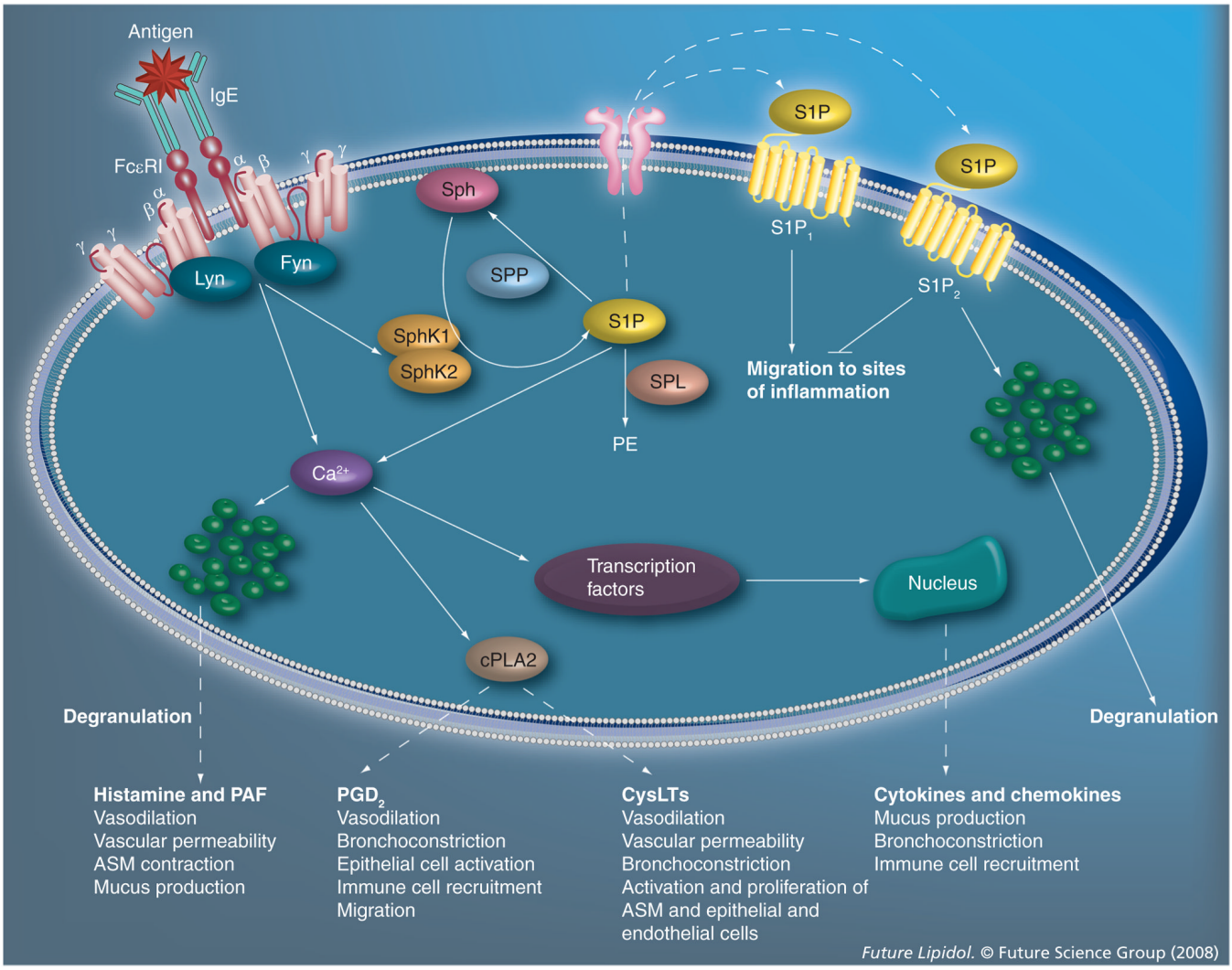

Figure 1. The role of S1P in mast cell-mediated allergic responses and anaphylaxis

Antigens induce allergic responses via crosslinking of IgE-bound FcεRI receptors on the surface of mast cells, triggering elaborate signaling cascades that result in degranulation and immediate release of a plethora of inflammatory mediators. One such pathway is the activation of SphK1 and SphK2 and the subsequent production of S1P. S1P can be degraded by SPP or SPL, leading to the production of PE. Intracellular S1P can then be secreted from mast cells via the ABCC1 transporter to bind its receptors extracellularly, or may act intracellularly to induce calcium release independent of phosphatidylinositol (3,4,5)-trisphosphate. Extracellular signaling through $S1P_1$ is important for migration of mast cells to sites of inflammation, while $S1P_2$ inhibits motility, probably to resolve cellular movement upon arrival at target sites and also aids in degranulation. Calcium influx is necessary for processes that are critical to the induction of allergic responses and anaphylaxis, such as degranulation and activation of cPLA2, and various transcription factors. Histamine and PAF induce vasodilation, ASM contraction, and increase vascular permeability and mucus production. Activation of cPLA2 is the rate-limiting step in the production of all eicosanoids, including PGD_2 and CysLTs, which themselves induce vasodilation, bronchoconstriction vascular permeability, epithelial and endothelial cell activation and proliferation, immune cell recruitment, migration and ASM activation and proliferation. Release of immediate mediators of inflammation from mast cells is followed by increased transcription of various cytokine and chemokine factors

that induce mucus production, bronchoconstriction and immune cell recruitment. Collectively, mast-cell mediators promote inflammation and, furthermore, function to activate and recruit other immune cells, thereby exacerbating the symptoms of allergy and anaphylaxis. ASM: Airway smooth muscle; cPLA: Cytosolic phospholipase A2; CysLT: Cysteinyl leukotriene; PAF: Platelet-activating factor; PE: Phosphatidylethanoloamine; PGD_2: Prostaglandin D2; PG: Prostaglandin; SphK: Sphingosine kinase; S1P: Sphingosine-1-phosphate; SPL: S1P lyase; SPP: Specific phosphatase.

Table 1

Involvement of SphKs in response to stimulation of various types of immune cells.

Cell type	Source	Stimulus	SphK involved	Effects	Ref.
Mast	Rat RBL-2H3	IgE/Ag	SphK1	Initial rise in calcium from internal stores, degranulation, cytokine production and/or smigration towards antigen	[6]
	Murine BMMC	IgE/Ag	SphK2	Production of S1P, calcium influx, PKC activation, degranulation and/or cytokine production	[30]
	Human LAD2/CB-MC	IgE/Ag	SphK1	Degranulation, CCL2 secretion and/or migration towards antigen	[31]
	Human LAD2/CB-MC	IgE/Ag	SphK1/SphK2	TNF secretion	[31]
Macrophages	Human blood	C5a	SphK1	Intracellular calcium signaling, degranulation, cytokine generation (TNF, Il-6 and IL-8) and/or chemotaxis	[34]
	Murine RAW264	LPS	SphK1	ERK1/2 and NF-κB activation	[67]
	Human U937	IFN-γ	SphK1	Vesicular trafficking	[68]
	Murine bone marrow	RANKL	SphK1	Osteoclastogenesis via regulation of p38, ERK, NFATc1 and/or cFos	[69]
	Human blood	Apoptosis Inducers	SphK2	Polarization to M2 phenotype, decreased TNF and IL-12-p70 production, increased IL-8 and IL-10 production and/or decreased NF-κB signaling	[70]
	Murine *in vivo*	C5a	SphK1	Acute peritonitis, systemic inflammation, multiorgan damage and/or release of proinflammatory mediators	[71]
B cells	Human lymphoblasts	S1P	SphK1	Resistance to Fas-mediated cell death	[72]
T cells	Murine $CD4^+$	IL-2	SphK2	Regulation of proliferation, secretion of cytokines and/or STAT5 activation	[73]
	Murine	T-cell receptor	SphK1	Negative regulation of chemokine expression s (IFN-ã, TNF and IL-2)	[74]
	Murine 2D6 clone	IL-12	SphK2	Promotes Th1 differentiation and cell-mediated immune responses	[75]
Neutrophils	Human blood	C5a	SphK1	Calcium release, degranulation, chemotaxis and/or sactivation of NADPH oxidase	[76]
Eosinophils	Murine *in vivo*	OVA	SphKs*	Inflammatory cell infiltration, eosinophilia, increased bronchoalveolar lavage fluid, IL-4, IL-5 and eotaxin production and/or increased serum sIgE levels	[65]

Cell type	Source	Stimulus	SphK involved	Effects	Ref.
	Murine *in vivo*	MeCh	SphKs*	Mucus production and/or airway hyper-responsiveness	[65]

*Isozyme not identified.

BMMC: Bone marrow-derived mast cells; CB-MC: Cord blood-derived mast cells; CCL2: CC chemokine ligand 2; ERK: Extracellular signal-regulated kinase; LPS: Lipopolysaccharide; MeCh: Methacholine; OVA: Ovalbumin; S1P: Sphingosine-1-phosphate; SphK: Sphingosine kinase.