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The Role of Sphingosine-1-Phosphate and its Receptors in Asthma

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Summary

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that plays important roles in allergic responses, including asthma and anaphylaxis, the incidence of which is rising worldwide especially in industrialized urban populations. In this review, we will discuss how S1P is formed and released, and how it acts at many cellular levels, including mast cells, the airway epithelium, airway smooth muscle and many immune cells. Since the actions of S1P on all of these cells could exacerbate allergic responses, the proteins that synthesize, release and respond to S1P offer plausible targets for a new generation of antiinflammatory therapeutics.

Asthma is characterized by structural and functional abnormalities of the bronchial epithelium, accumulation of inflammatory cells in the bronchial mucosa and remodeling of the airway tissue structure. Reversible airway obstruction in asthma is typically initiated by an immunologic trigger. While many mediators have been identified that play significant roles in the initiation and progression of the disease, this review will focus on sphingosine-1-phosphate (S1P), which is emerging as an important player at many levels in asthma. The initial clue that S1P plays a role in asthma came from the finding that S1P levels were elevated in the bronchoalveolar lavage fluid of asthmatics, but not healthy controls, after antigen challenge.

¹ Indeed, the levels of S1P in the lavage fluid directly correlated with eosinophil numbers, another hallmark of asthma. We will discuss how S1P is formed and released, and how it acts at many cellular levels, including the airway epithelium, airway smooth muscle (ASM) and many immune cells. Since the actions of S1P on all of these cells could exacerbate asthmatic symptoms, the proteins that synthesize, release and respond to S1P offer novel targets for clinical intervention.

S1P receptors

S1P regulates numerous cellular responses including motility and cytoskeletal rearrangements, formation of adherens junctions, proliferation, survival, angiogenesis and the trafficking of immune cells.^{2–4} These myriad effects are partly elicited by binding of S1P to a family of five G protein-coupled receptors (S1PRs), termed S1P_{1–5}. S1PRs couple to overlapping but distinct subsets of heterotrimeric G proteins, allowing divergent signaling effects.^{2,5,6} However, while the S1PR family orchestrates the majority of the known S1P-mediated cellular responses, the effects of S1P on cell growth and survival as well as on calcium mobilization appear to be independent of S1PRs,^{3,7,8} acting through as yet unknown intracellular effectors. Recently, many specific S1P receptor agonists and antagonists have been developed (Table I) which have

proved very useful for elucidating the functions of these receptors in regulating diverse cellular processes important for inflammatory responses.

Formation and release of S1P

Sphingolipids are ubiquitous components of the lipid bilayer of eukaryotic cells. Like glycerolipids, numerous agonists regulate sphingolipid metabolism to generate signaling molecules derived from them, including ceramide, sphingosine and S1P (reviewed in ²). Ceramide (*N*-acyl sphingosine), the backbone of sphingolipids, is produced by de novo synthesis and by turnover of sphingolipids. After removal of sphingolipid head groups during catabolism, deacylation of ceramide by ceramidases yields sphingosine (reviewed in ⁹). Sphingosine is phosphorylated by sphingosine kinase types 1 and 2 (SphK1 and SphK2) to form S1P. S1P can be degraded either by reversible dephosphorylation to sphingosine by a variety of phosphohydrolases or by irreversible cleavage to ethanolamine phosphate and hexadecenal by S1P lyase (Fig. 1).²

Plasma concentrations of S1P range from 0.1–0.6 μ M, a level sufficient to activate all of the S1P receptors.¹⁰ However, tissue levels of S1P are much lower, varying from 0.5–75 pmol/mg.^{11,12} Plasma S1P is predominantly derived from hematopoietic cells, though the precise cellular source is still controversial.^{13–15} Additionally, upon activation, many cells of the immune system can secrete S1P, including platelets, neutrophils, mast cells and mononuclear cells. Non-immune cells have also been shown to secrete S1P.¹⁶ Indeed, many agonists, including growth factors, cytokines and numerous G protein-coupled receptor agonists, induce activation of SphK and increased production and release of S1P, which then acts in an autocrine and/or paracrine fashion on its cell surface receptors. This process is referred to as “inside-out signaling”, and occurs often in immune cell signaling. For example, in mast cells, crosslinking of the high affinity immunoglobulin E (IgE) receptor Fc ϵ RI induces activation of SphK1, production of S1P and subsequent activation of the S1P₁ and S1P₂ receptors.¹⁷ Activation of S1P₂ is required for mast cell degranulation while S1P₁ activation is important for their migration to sites of inflammation. At least in mast cells, S1P does not appear to be released by degranulation of stored mediators, rather de novo formed S1P is secreted, at least in part, through ABCC1, a member of the ATP-binding cassette transporter family.¹⁸

Role of S1P in mast cell functions and allergic responses

Mast cells (MC) are tissue resident effector cells best known for their role in atopic disease. However, these cells can alter both innate and acquired immune responses to various stimuli, by releasing a plethora of vasoactive mediators, proteases and chemokines and cytokines that collectively enhance vascular permeability, elicit bronchoconstriction and incite recruitment and function of leukocytes. The end effects range from local to systemic inflammation.¹⁹

MC develop in peripheral tissues from bone marrow-derived, circulating committed progenitors, with two subpopulations that vary in the composition of their intragranular proteases: those expressing tryptase only are designated MC_T and predominate in lung; while those that also contain chymase, carboxypeptidase and cathepsin G are termed MC_{TC} and predominate in skin. The concentration of MC_{TC} cells in ASM in asthmatics increases dramatically and correlates with bronchial hyperreactivity.²⁰ The presence of IgE antibodies to innocuous antigens (Ag) causes dysregulation of this normally protective response, resulting in allergic inflammatory disease.

Crosslinking of Fc ϵ RI on MC activates many downstream signaling molecules, including Src family tyrosine kinases, the Syk tyrosine kinase, calcium flux, protein kinase C (PKC), and the Ras-MAPK pathway (reviewed in ²¹). The result is a biphasic response characterized by degranulation with release of histamine and other preformed mediators, and the subsequent de

novo synthesis and secretion of cytokines and chemokines as well as the arachidonic acid (AA) metabolites, leukotrienes and prostaglandins.

An addition to the recognized repertoire of FcεRI-mediated signaling events is the activation of SphK, which has been implicated in calcium mobilization from intracellular stores in MC independently of both phospholipase C (PLC) and the generation of inositol trisphosphate (IP3) that is necessary for MC degranulation (Fig. 2).^{22,23} It has been suggested that the decisive balance between sphingosine and S1P is a determinant of the allergic responsiveness of MC.²⁴ Whereas sphingosine inhibits Ag-mediated leukotriene synthesis and cytokine production by preventing activation of ERK1/2, S1P activates ERK1/2, stimulating degranulation and cytokine production.²⁴ Thus, SphK plays a pivotal role in MC activation by modulating the balance of these counterregulatory sphingolipid signaling molecules. Moreover, expression of MCP-1, MIP1-α, MIP1-β, MIP2 (belonging to the CC-chemokine family), and MIF, all important modulators of monocyte and eosinophil recruitment and inflammation, were significantly increased by S1P in MC.¹⁷ Thus, secretion of S1P by MC can also promote inflammation by activating and recruiting other immune cells involved in allergic and inflammatory responses, including eosinophils (25) and Th2 lymphocytes (Fig. 2).²⁶ S1P also profoundly affects endothelial cell functions,^{27–30} induces contraction and proliferation of ASM cells and stimulates interleukin-6 (IL-6) production,¹ and shifts maturing dendritic cell-induced polarization of T cells into a Th2 phenotype.³¹ In recent years, it has become apparent that S1P also mediates many cytokine and chemokine functions and a central finding is that activation of SphK by a variety of cytokines and concomitant formation of S1P are important for various inflammatory responses.^{2,32–34} Collectively, S1P production by mast cells appears to augment the Th2 response and could promote the establishment of allergic disease.

Abundant evidence indicates that S1P has direct effects on mast cells acting in an autocrine manner (Fig. 2). Binding of S1P to S1P₁ induced cytoskeletal rearrangements, important for regulation of movement of MC towards an Ag gradient, whereas activation of S1P₂ enhanced degranulation.¹⁷ Thus, low Ag concentrations could attract MC to the site of action via S1P₁, and, as they approach higher concentrations of Ag, a shift in the expression of S1P receptors (enhanced S1P₂ expression) would resolve migration while promoting degranulation, a process known to require a stronger stimulus.¹⁷

The roles of SphK1 and SphK2 in mast cell functions

Although there are two SphK isoenzymes that have been cloned and characterized,^{35,36} the majority of published studies to date on the role of intracellular production of S1P in MC have been focused on SphK1.^{17,23,37,38} Downregulation of SphK1 but not SphK2 inhibited calcium mobilization, degranulation and migration of MC induced by Ag.^{17,23,37} In human and murine MC, SphK1 translocates to the plasma membrane within minutes of Fcε clustering.^{17,23} SphK1 also selectively interacts with the Src kinase family members Lyn and Fyn, but not with Src or other tyrosine kinases such as Syk. Following interaction with Lyn, SphK1 is recruited to membrane rafts where conformational changes may occur allowing for increased activity of both kinases.³⁸

However, recent studies have shown that both SphK1 and SphK2 are activated upon FcεRI engagement in murine MC and immature human MC, and this requires Fyn kinase in bone marrow-derived MC.³⁹ Moreover, this signaling pathway may be critical to mast cell function. While Fyn-deficient bone marrow-derived MC exhibit deficiencies in IgE-mediated migration and degranulation, both of these responses can be restored by exogenous addition of S1P with IgE/Ag stimulation.³⁹ The importance of S1P to mast cell function has made the understanding of the activity and functions of SphK1 and SphK2 in mast cells even more relevant. Using fetal liver-derived MC from mice null for SphK1 or SphK2, it was recently shown that deletion of

Sphk2, but not Sphk1, suppressed IgE-mediated generation of S1P, as well as calcium influx, PKC activation, degranulation and cytokine secretion.⁴⁰ Deletion of both SphK1 and SphK2 had no further effect, arguing that SphK2 may be the critical determinant of S1P levels in these murine MC. These results emphasize an appreciation for the potential divergent effects of the SphK1 and SphK2 in MC-related disorders in humans.

S1P in acute mast cell responses

The results of several studies in murine asthma and anaphylaxis models suggest that targeting S1P generation may be a productive means of treating acute disease involving MC. It has recently been suggested that S1P is involved in mouse airway hyperresponsiveness as *in vivo* inhibition of SphK with D,L-threo-dihydrosphingosine did not significantly affect normal physiological bronchial tone, yet markedly inhibited bronchial hyperresponsiveness in ovalbumin-sensitized animals.⁴¹ Hence, SphK does not seem to play a role in normal airway physiological processes, while it may become critical in pathological conditions.⁴¹ In this regard, an initially counterintuitive report suggested that loss of SphK1, but not SphK2, can ablate IgE-mediated anaphylactic shock in mice. Although SphK2 appeared to be most critical for MC-derived S1P production, non-MC sources of S1P generated by SphK1 appeared to be the source of circulating levels of S1P that are required for full MC activation and the *in vivo* responses of anaphylaxis.⁴⁰ Hence, targeting S1P production could also yield progress in treating IgE-related disease.

Apart from their activation by FcεRI, MC also respond in an IgE-independent manner to a number of secretagogues, such as bee venom, polyamines and the anaphylatoxins (complement activation products C3a and C5a). In this peptidergic triggering, heterotrimeric G proteins are activated either via classical G protein-coupled receptor signaling or directly.^{42,43} This pathway is particularly important for skin MC, as they possess abundant levels of G proteins,⁴⁴ and has particular relevance to S1P actions through G protein-dependent S1PRs. Significant amounts of C5a in the blood can lead to shock and adult respiratory distress syndrome, and also stimulate release of mediators of both acute and chronic inflammation. Interestingly, C5a activates SphK1⁴⁵ and intravenous administration of the SphK inhibitor, N,N-dimethylsphingosine, suppresses C5a-induced neutropenia, peritonitis, and cytokine (tumor necrosis factor [TNF]-α and IL-6) production *in vivo*.⁴⁶ It is hoped that development of potent and isoenzyme-specific SphK inhibitors that can effectively inhibit MC functions *in vivo* will reveal new therapeutic interventions in mast cell-related disorders, be they IgE- or non-IgE-mediated.

S1P, airway smooth muscle and airway remodeling

One of the defining features of an asthmatic attack is the contraction of ASM. S1P stimulates contraction of human ASM in a Rho-dependent manner, likely through the activation of S1P₂.⁴⁷ S1P also plays a role in airway hyperresponsiveness in the ovalbumin-sensitized mouse model of asthma. S1P induced a dose-dependent contraction in isolated bronchi from sensitized but not from unsensitized mice.⁴¹ Similarly, S1P induced airway resistance in whole lungs only from the sensitized mice. S1P has also been shown to sensitize guinea pig tracheal smooth muscle strips to methacholine-induced contraction.⁴⁸ In these preparations, S1P-mediated contraction was associated with Rho-dependent inhibition of myosin phosphatase. Together, these studies suggest that targeting S1P could alleviate bronchial hyperresponsiveness, ameliorating asthmatic attacks.

Another important hallmark of chronic asthma is airway remodeling, characterized in part by increased smooth muscle mass, extracellular matrix deposition and vasculogenesis. S1P is likely involved in all of these processes. First, S1P promotes the growth of both human ASM cells¹ and fibroblasts.^{49,50} Moreover, S1P produced by SphK1 has recently been shown to

be involved in fibroblast differentiation into myofibroblasts,⁵¹ cells thought to be responsible for thickening of the lamina reticularis. transforming growth factor (TGF)- β and S1P, which are both increased in asthmatic lung, can induce α -smooth muscle actin expression in lung fibroblasts, a hallmark of their differentiation into myofibroblasts.⁵¹ Subsequently, it was shown that TGF- β stimulates SphK1, which in turn releases S1P that activates S1P₂ and S1P₃ in an autocrine manner, leading to α -smooth muscle actin expression.⁵² Consistent with this observation, expression of both S1P₂ and S1P₃ receptors, in addition to SphK1 and SphK2, are upregulated in the ovalbumin-sensitized mouse asthma model.⁴¹

S1P and eicosanoid production

Eicosanoids are well-established mediators of inflammatory responses that play important roles in the pathogenesis of asthma. The formation of AA via activation of phospholipase A₂ (PLA₂) is the initial rate-limiting step in eicosanoid biosynthesis. Inflammatory agonists and Fc ϵ RI cross-linking induce activation and translocation of group IVA cytosolic PLA₂ (cPLA₂ α).^{53,54} Cyclooxygenase-1 (COX-2) and lipoxygenase (LO) then utilize the AA released by cPLA₂ to initiate prostaglandin and leukotriene synthesis, respectively,⁵³ and both are increased in airways of asthma patients.⁵⁵ Classically described activities of the prostaglandin PGD₂ include vasodilatation and increased capillary permeability leading to edema formation, increased mucus production, bronchoconstriction and eosinophil recruitment.^{56,57} Leukotrienes activate myeloid leukocytes, such as neutrophils, monocytes and eosinophils. Secreted leukotriene C₄ (LTC₄) is rapidly metabolized to LTD₄ and LTE₄, collectively referred to as cysteinyl leukotrienes (CysLTs), which act as potent mediators of asthma, hypersensitivity reactions and anaphylaxis by inducing bronchoconstriction, microvascular permeability and vasoconstriction.^{56,57}

These potent inflammatory activities have made AA metabolites a common and effective target in asthma therapy. LT modifiers or 5-LO inhibitors and CysLT₁ receptor antagonists (montelukast) are used clinically in long-term maintenance of asthma control.⁵⁷ Inhibition of cPLA₂ α by a small-molecule inhibitor⁵⁸ or its deletion by gene targeting^{59,60} reduced both the immediate and the delayed phases of eicosanoid generation and alleviated airway hyperresponsiveness in the ovalbumin-sensitized mouse model and markedly reduced anaphylactic responses. Agents such as aspirin that block prostaglandin synthesis by inhibition of COX can actually exacerbate asthma, an effect thought to be caused by inhibition of COX-1-dependent synthesis of PGE₂, which is an important bronchodilator and an endogenous inhibitor of LT production and histamine release.⁶¹

S1P has also been shown to play a role in eicosanoid production, inducing COX-2 expression, both directly by exogenous S1P acting through S1PRs⁶² and indirectly by “inside-out signaling” after activation of SphK by TNF- α ⁶³ and IL-1 β .⁶⁴ Quantitation of changes in sphingolipid levels occurring in response to cytokine treatment by tandem mass spectrometry revealed a significant increase in levels of S1P within 10 minutes after stimulation with TNF- α . Down-regulation of SphK1, but not SphK2, blocked TNF-induced S1P formation and induction of COX-2, as well as PGE₂ production. Conversely, decreasing expression of the enzymes that metabolize S1P, S1P lyase and S1P phosphatase, which should increase S1P levels, enhanced TNF- α -induced COX-2 and PGE₂ production. Moreover, similar results were also found in macrophages, suggesting that SphK1 and S1P signaling are indeed important physiological regulators of inflammatory cytokine-mediated induction of COX-2 and PGE₂ production. However, it is still not clear whether S1P also regulates COX-2 *in vivo*.

The immunosuppressant drug FTY-720 inhibits airway hyperresponsiveness

Interest in the functions of S1P in the immune system has increased immensely due to the discovery that the immunosuppressive drug FTY-720, a sphingosine analogue, potently

inhibits a variety of experimental autoimmune disorders, such as type 1 diabetes⁶⁵ and arthritis,⁶⁶ but clinically, its greatest potential for humans appears to be in treatment of multiple sclerosis where it is currently in phase III clinical trials.⁶⁷ The immunosuppressive properties of FTY-720 stem from its ability to prevent egress of T cells from secondary lymphoid organs back into circulation, sequestering them away from the transplanted grafts or sites of inflammation.^{4,68-71} We and others have found that FTY-720 is more effectively phosphorylated by SphK2 than SphK1,⁷²⁻⁷⁴ and a large body of evidence indicates that the phosphorylated drug (FTY-720-P), an analogue of S1P, is the biologically active form. FTY-720-P can bind to all of the known S1P receptors, except S1P₂.^{4,68,75,76} Importantly, however, FTY-720-P binds to S1P₁ on lymphocytes and potently downregulates its cell surface expression.⁷⁷ This results in lymphopenia due to their sequestration in secondary lymphoid organs, as this S1PR is required for proper lymphocyte trafficking and their ability to respond to antigenic stimuli.^{4,71,76,78,79} Adaptive immunity depends on circulation of T and B cells between secondary lymphoid organs to monitor antigens. Although it is not completely understood how S1P gradients regulate lymphocyte trafficking, it is clear that S1P is an important chemoattractant in the immune system, modulating lymphocyte responses to chemokines,^{32,71} and also recruitment of MC¹⁷ and eosinophils²⁵ to sites of inflammation.

Allergic asthma is a chronic inflammatory disease of the airways, characterized by reversible airway obstruction and airway hyperresponsiveness. The inflammatory response in asthma is associated with a characteristic infiltration of eosinophils and T lymphocytes into the airway submucosa. There is strong evidence based on experimental animal models as well as clinical studies that allergenspecific Th2 lymphocytes and their products are the central mediators of asthma.⁸⁰ Th2 cytokines can account directly or indirectly for the majority of pathophysiological manifestations. An elegant study clearly demonstrated that FTY-720 not only inhibits the Th2 cell-mediated eosinophilic inflammation but is also active in a relevant disease model using active sensitization and challenge to allergen, which besides allergen-specific Th2 cells also involves allergen-specific IgE and subsequent allergen-mediated MC degranulation events.²⁶ Indeed, in a murine asthma model, local application of FTY-720 via inhalation prior to or during ongoing Ag challenge suppressed Th2-dependent eosinophilic airway inflammation and bronchial hyperresponsiveness without causing lymphopenia and T-cell retention in the lymph nodes.⁸¹ It was recently reported that although FTY-720 does not affect Ag-induced MC degranulation, it drastically inhibits secretion of the AA-derived eicosanoids, PGD₂ and CysLT. Importantly, this effect was independent of its phosphorylation and S1P receptor functions. Unexpectedly, this study uncovered a unique action of FTY-720 as a direct inhibitor of cPLA₂ α , the rate-limiting step in eicosanoid formation.⁸² While the accompanying lymphopenia caused by metabolism of FTY-720 to FTY-720-P could be a serious side effect that would preclude its use as an antiasthmatic drug, these results suggest that a nonphosphorylatable FTY-720 analogue that still potently inhibited cPLA₂ might be very useful therapeutically.

Conclusions

We hope the reader has gained an appreciation for the myriad sites of action of S1P in inflammatory diseases, particularly in allergic asthma. Available in the serum as well as from activated inflammatory cells, S1P can act both by receptor and nonreceptor mechanisms to enhance Th2 responses and promote inflammation. Many lines of evidence implicate S1P generation and signaling as potential targets for treating inflammatory disease. The successful use of SphK inhibitors in preclinical models and the recent encouraging results in clinical trials with FTY-720 offers hope for development of a new generation of sphingolipid based antiinflammatory agents.

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Fig. 1.

Sphingolipid metabolism leading to formation of sphingosine-1-phosphate (S1P). Ceramide, the backbone of all sphingolipids, is formed by the *de novo* pathway or by degradation of sphingolipids. Ceramidases (CERase) deacylate ceramide to form sphingosine, which can be phosphorylated to S1P by sphingosine kinase type 1 or 2 (SphK1 or SphK2). S1P is hydrolyzed by phosphatases or irreversibly degraded by a lyase. SPP1/2, S1P phosphatase 1 and 2.

Fig. 2.

Roles of sphingosine-1-phosphate (S1P) in human mast cell functions. Crosslinking of the mast cell high affinity immunoglobulin E receptor (FcεRI) activates sphingosine kinase SphK1 and SphK2, increasing S1P formation. S1P in turn transactivates S1P₁ and S1P₂ receptors, leading to migration and degranulation. S1P also stimulates release of cytokines and chemokines that exacerbate allergic responses. It is still not clear whether S1P regulates cyclooxygenase-2 (COX-2) expression in mast cells or how it regulates cytokine expression. For simplicity, all other known pathways downstream of FcεRI are not shown. In addition to its effects on mast cells, S1P produced by mast cells and other sources has a coordinated action on nearly all cells involved in allergic disease pathobiology. These actions make S1P generation and function a novel and important target for therapeutic intervention. Ag, antigen; DMS, dimethylsphingosine; cPLA₂, cytoplasmic phospholipase A₂; LOX, lipoxygenase; PGD₂, prostaglandin D₂; CysLT, cysteinyl leukotrienes.

TABLE I**Sphingosine-1-Phosphate (S1p) Receptor Agonists and Antagonists**

PHARMACOLOGICAL AGENTS	ACTIONS	REF.
SEW-2871	S1P ₁ selective agonist	83
Fingolomod (FTY-720)/FTY-720-P	Agonist of S1P ₁ , S1P ₃ , S1P ₄ , S1P ₅	68
KRP-203/KRP-203-P	S1P ₁ selective agonist	84
Dihydro-S1P	S1P ₁₋₅ agonist, least potent with S1P ₂	85
AUY954	S1P ₁ selective agonist	86
VPC-23019	S1P ₁ , S1P ₃ antagonist, S1P ₄ agonist, S1P ₅ partial agonist	87
VPC-44116	S1P ₁ antagonist	88
W-123	S1P ₁ antagonist	89
W-146	S1P ₁ selective antagonist	90
JTE-013	S1P ₂ selective antagonist	91