Hindawi Publishing Corporation
Journal of Allergy
Volume 2012, Article ID 154174, 14 pages
doi:10.1155/2012/154174

Review Article

Sphingolipids: A Potential Molecular Approach to Treat Allergic Inflammation

Wai Y. Sun[1,2,3] and Claudine S. Bonder[1,2,3,4]

[1] *Centre for Cancer Biology, SA Pathology, Frome Road, Adelaide, SA 5000, Australia*
[2] *School of Medicine, University of Adelaide, Adelaide, SA 5000, Australia*
[3] *Cooperative Research Centre for Biomarker Translation, La Trobe University, Bundoora, VIC 3086, Australia*
[4] *School of Molecular and Biomedical Sciences, University of Adelaide, Adelaide, SA 5000, Australia*

Correspondence should be addressed to Claudine S. Bonder, claudine.bonder@health.sa.gov.au

Received 10 August 2012; Revised 15 October 2012; Accepted 30 October 2012

Academic Editor: Robert J. Bischof

Allergic inflammation is an immune response to foreign antigens, which begins within minutes of exposure to the allergen followed by a late phase leading to chronic inflammation. Prolonged allergic inflammation manifests in diseases such as urticaria and rhinoconjunctivitis, as well as chronic asthma and life-threatening anaphylaxis. The prevalence of allergic diseases is profound with 25% of the worldwide population affected and a rising trend across all ages, gender, and racial groups. The identification and avoidance of allergens can manage this disease, but this is not always possible with triggers being common foods, prevalent air-borne particles and only extremely low levels of allergen exposure required for sensitization. Patients who are sensitive to multiple allergens require prophylactic and symptomatic treatments. Current treatments are often suboptimal and associated with adverse effects, such as the interruption of cognition, sleep cycles, and endocrine homeostasis, all of which affect quality of life and are a financial burden to society. Clearly, a better therapeutic approach for allergic diseases is required. Herein, we review the current knowledge of allergic inflammation and discuss the role of sphingolipids as potential targets to regulate inflammatory development *in vivo* and in humans. We also discuss the benefits and risks of using sphingolipid inhibitors.

1. Introduction

Allergic inflammation can occur rapidly or delayed via the classical inflammatory immune reaction involving the production of specific IgE antibodies as well as the activation of inflammatory cells and the endothelium [1]. Many proinflammatory mediators and cytokines including histamine, leukotriene, and tumor necrosis factor α (TNFα) can activate the vascular endothelial cells (ECs) to cause proinflammatory microvasodilation and mediate leukocyte recruitment from the circulation to the sites of allergic inflammation [2, 3]. Excessive and prolonged leukocyte recruitment can result in extracellular matrix (ECM) remodelling and tissue damage [4]; thus controlling EC activation provides a strategy to minimize allergic inflammation. This review discusses the pathophysiology of vascular ECs during allergic inflammation, current treatments and new therapeutic approaches. We focus on the role of sphingolipids in the regulation of vasculature during the early phase of allergic inflammation, in particular, studies utilizing sphingolipid knockout animals which support their potential as new therapeutic targets.

2. Pathophysiology in Acute Allergic Inflammation

Histamine is a potent proinflammatory mediator primarily released by mast cells and basophils with up to 0.01–1 mol/m^3 found in the periphery during an allergic response [5, 6]. Histamine mediates dendritic cell maturation [7], T lymphocyte differentiation and migration [8–10], and

Table 1: Common antihistamines marketed in Australia.

Some common antihistamines			
First generation	Second generation		Third generation
Systemic	Systemic	Topical	Systemic/topical
Promethazine	Cetirizine	Azelastine	Levocetirizine
Pheniramine	Loratadine	Levocabastine	Desloratadine
Cyproheptadine	Terfenadine		Fexofenadine
Dexchlorpheniramine	Ketotifen		
Trimeprazine	Mizolastine		

endothelial cell proliferation [11] via a family of four G-protein-coupled receptors (H_{1-4}) [12]. Histamine receptors are differentially expressed with only H_1 and H_2 expressed by vascular ECs [13, 14] (Figure 1). Within minutes of histamine exposure and binding to H_1 and H_2, the G-protein subunit αq is recruited to decrease cAMP accumulation and subsequent EC contraction [15]. By contrast, the G protein β and γ subunits are activated to induce the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) [16]. Ligand interaction with the H_1 receptor causes vascular permeability, synthesis of prostacyclin and platelet activating factor, and release of von Willebrand Factor (vWF) and nitric oxide [17, 18]. H_2 receptor stimulation is linked to the Gαs subunit for the activation of adenylate cyclase and formation of cyclic adenosine monophosphate (cAMP), which induces intracellular calcium-mediated vasodilatation at a slower rate of onset than that of H_1 receptor [19, 20]. In addition, the H_2 receptor can negatively regulate the release of histamine by mast cells and basophils [21] and suppress the production of TNFα and IL-12 from inflammatory cells [10, 22, 23].

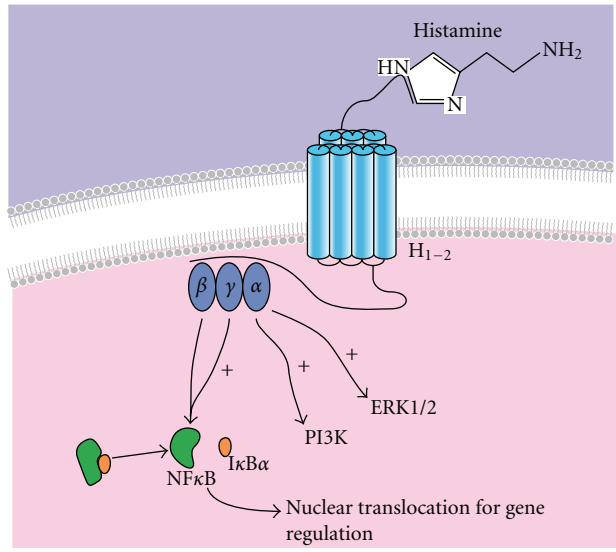


Figure 1: Histamine receptors on ECs. Two histamine receptors (H_1 and H_2) are found on ECs. Within minutes of histamine binding to its receptors, the G-protein subunits are activated to initiate intracellular signalling. The αq subunit of the G-protein contributes to reduced cAMP accumulation, induced ERK1/2, and induced inositol phospholipid (PI3K) signalling. The β and γ subunits contribute to the activation of NFκB and subsequent translocation into the nucleus where transcriptional processes are regulated causing cellular changes, such as vascular contraction and permeability, all of which are important for immune regulation and inflammation.

3. Antihistamines as the Current Mainstay Treatment for Allergic Inflammation

Antihistamines (e.g., diphenhydramine and chlorpheniramine) were first developed in the 1930s as an inverse agonist for the histamine receptors and have been commonly used to treat and prevent allergic symptoms ever since [24] (Table 1). Patients treated with H_1 antihistamines exhibit reduced production of histamine and leukotrienes as well as downregulation of adhesion molecule expression on the vasculature which in turn attenuates allergic symptoms by 40–50% [20, 25–28]. Long term treatment with H_1 antihistamines can retard the progression of respiratory disease by inactivating functions of macrophages and other Th2 cells thus preventing local tissue remodelling and damage [29, 30]. Second- and third-generation antihistamines (e.g., loratadine, fexofenadine, and cetirizine) (Table 1) were generated in the 1980s. These drugs also target the H_1 receptor but, in general, are less lipophilic and therefore exhibit reduced ability to penetrate the blood-brain barrier resulting in a less sedating effect than the first generation counterparts [28, 31]. Notably, 2–5 times higher dose of these second-generation antihistamines are required to control mild seasonal allergic symptoms when compared to the first-generation medications [32]. Using H_1 antihistamines at a high dose remains controversial as (i) animal studies have shown that mice treated with high doses of fexofenadine during the allergen challenge exhibited reduced lung inflammation, reduced Th2 responses, and reduced the secretion of IL-4, -5, and -13 [7, 29], (ii) a recent human clinical study demonstrated that high-doses of desloratadine only marginally improved allergic symptoms in patients without an increase in adverse effects when compared to the standard doses [33] and (iii) long-term high-dose use of antihistamines in patients with chronic urticaria retained adverse effects, such as rapid eye movement, sleep disturbance, and negative impacted on learning and performance [34]. Clearly, other effective clinical approaches are needed to combat allergic inflammation.

4. Antiselectin Therapy for Inflammatory Diseases

Another approach is to target the expression of adhesion molecules on ECs, such as selectins, which are known to initiate the early capturing and rolling of leukocytes from the circulation. Antagonism of the selectins is recognized to be a therapeutic approach to prevent and minimize inflammatory reactions. Evidence for this comes from P-selectin-deficient mice which, when challenged with the inflammatory irritant thioglycollate, exhibit attenuated leukocyte rolling in the blood vessels for up to 4 hours [35]. They also exhibit a significant reduction in leukocyte infiltration at the inflammatory hindlimb by ischemia on postoperative day 14 when compared to wildtype (WT) controls [36]. In humans, the recruitment of activated neutrophils to the local inflamed tissue is largely dependent on adhesion molecules as evidenced by patients with leukocyte adhesion deficiency (LAD II) whose neutrophils lack functional sialyl Lewis X expression (a fucose-containing glycoconjugate ligand for P-, E-, and L-selectin), exhibit reduced rolling and firm adhesion on the endothelium [37]. Together, these show that controlling expression of adhesion molecules can influence the early phase as well as the chronic phase of inflammatory reactions.

Selectin antagonists have been examined in preclinical studies, including cutaneous inflammation, allergy and ischemia-reperfusion injury [38, 39]. The first selectin antagonist CY1503 (Cylexin), an analogue of sialyl Lewis X which inhibits E-, P-, and L-selectins, has demonstrated a reduction in the degree of myocardial infarct size associated with a canine model of coronary artery ischemia and reperfusion, and reduced leukocyte accumulation at 4.5 hours after operation [40]. However, the effects of CY1503 remain controversial as a second similar study failed to consistently reduce myocardial injury and neutrophil accumulation at 48 hours post-operation [41]. Treatment with CY1503 also failed to attenuate the "no-reflow" phenomenon of leukocytes and could not limit the myocardial infarct size in the rabbit [42]. More recently, the oral P-selectin blocking agent, Pentosan Polysulfate Sodium (PPS), has been examined in a Phase I clinical study, wherein a single dose of PPS showed improvement of microvascular blood flow in patients with sickle cell disease [43]. However, no study to date has examined the efficacy of PPS in controlling leukocyte recruitment during allergic inflammation.

To date, four classes of selectin blocking agents have been developed: (i) carbohydrate based inhibitors targeting all P-, E-, and L-selectins [44], (ii) antihuman selectin antibodies [45], (iii) a recombinant truncated form of PSGL-1 immunoglobulin fusion protein [46], and (iv) small-molecule inhibitors of selectins [47]. Notably, most of the selectin blocking agents have failed in phase II/III clinical trials or the clinical studies were ceased due to their unfavorable pharmacokinetic properties and high cost [39]. Animal models also suggest that the timing and potency of selectin blockade are crucial to preventing the development of allergic inflammation with a greater than 90% reduction in leukocyte rolling required for firm adhesion events to be significantly attenuated [48, 49]. Given that the direct selectin blockade by the current compounds remains unsuccessful to regulate allergic inflammation, new therapeutic approaches which target the regulation and expression of adhesion molecules are warranted.

5. Sphingomyelin Pathway

The lipid enzyme, sphingosine kinase (SK), was originally identified for its role in the sphingomyelin degradation pathway but is increasingly being recognized as an important signalling molecule (Figure 2). There are excellent reviews focusing on the roles of SK/S1P in diseases, such as cancer [50], immunity [51], asthma [52], multiple sclerosis [53], rheumatoid arthritis [54], and pancreatic islet transplantation [55]. Herein, we discuss how SK can be used as a new therapeutic target to combat allergic inflammation, referencing animal models and human trials, together with the benefits and adverse effects of manipulating SK using inhibitors.

6. Sphingosine Kinase

Two isoforms of SK (i.e., SK-1 and SK-2) have been cloned and characterized in mammalian cells, which both catalyze the phosphorylation of sphingosine to form sphingosine-1-phosphate (S1P) [56, 57]. SK-1 has been shown to be the primary contributor to serum S1P levels with *SphK1*−/− mice exhibiting a ∼50% reduction in serum S1P when compared to wildtype (WT) mice [58] and the *SphK2*−/− mice serum S1P levels exhibiting no reduction. In fact, Zemann et al. showed an increase in serum S1P of *SphK2*−/− mice [59]. Notably, S1P was undetectable in plasma and lymph of the conditional double knockout mice [60].

The polypeptide sequences of SK-1 and SK-2 contain 80% similarity, which supports compensatory effects when one isoform of SK is knocked down [56, 57]. Interestingly, the localization of SK-1 and SK-2 differs with SK-1 being predominantly found in the cytoplasm and at the plasma membrane leading to prosurvival effects [61, 62], and SK-2 being predominantly found in the nucleus and at the endoplasmic reticulum (ER) promoting proapoptotic effects [63, 64] (Figure 3). Three splice isoforms of SK-1 have been identified (i.e., SK-1a, SK-1b, and SK-1c) that differ at their N-termini with additional 14 and 86 amino acids in SK-1b and SK-1c, respectively [65]. Two variants of SK-2 have also been identified (i.e., SK-2 and SK-2 long (SK2L)) arising from alternate start sites [57]. The specific physiological role for each SK variant is yet to be further elucidated.

SK has intrinsic activity and can be further activated by many biological stimuli, including histamine [66], cross-linking of immunoglobulin receptors [11], TNFα [67], vascular endothelial growth factor (VEGF), interleukins, complement C5a [68], and bradykinin [11]. Upon stimulation, the catalytic activity of SK-1 increases via the phosphorylation of extracellular signal regulated kinase (ERK)-1/2 at Ser225 which results in the translocation to the inner plasma membrane [69]. The binding of SK-1 to lipid phosphatidylserine can enhance SK-1 activity and plasma

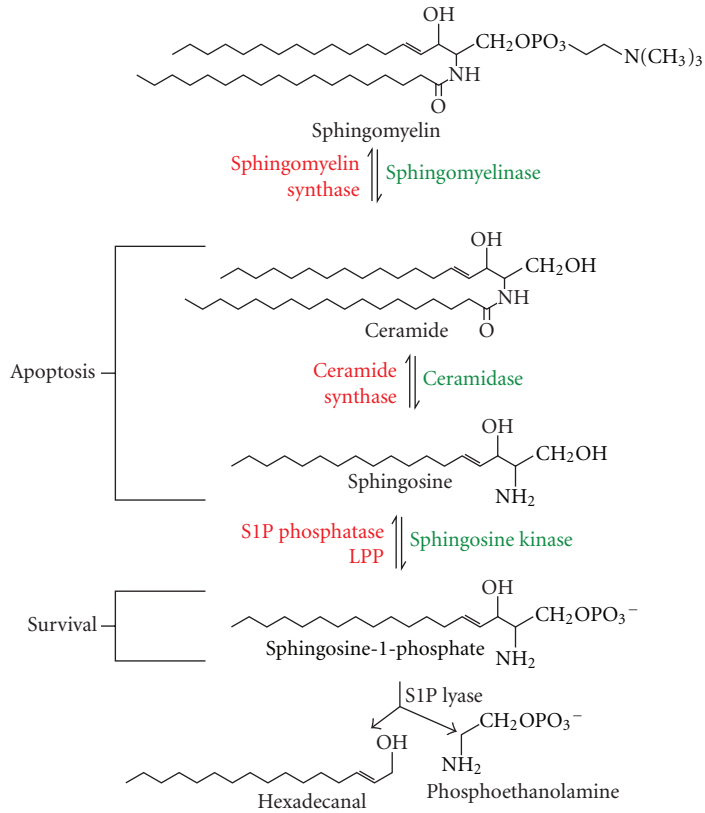


Figure 2: Sphingomyelin pathway. Sphingomylein is hydrolysed to ceramide, which is then metabolized to sphingosine and sphingosine-1 phosphate (S1P) by different kinases (green). This process is reversible via the activities of different synthases and phosphatases (red). The levels of the biological product, S1P, are regulated by S1P lyase which degrades it into hexadecanal and phosphoethanolamine. Although the structures of each sphingolipid are similar, they have divergent cellular functions with ceramide and sphingoine being pro-apoptotic, and S1P being prosurvival.

membrane translocation [70]. More recently, calcium- and integrin-binding protein (CIB)-1 protein has been identified to translocate SK-1 to the plasma membrane [71]. Conversely, dephosphorylation at Ser225 causes deactivation of basal and TNFα-induced SK-1, a process shown to be regulated by protein phosphatase 2A (PP2A) [72, 73]. In contrast, SK-2 does not possess the Ser225 phosphorylation site but its activation, also via the ERK pathway, is suggested to occur by phosphorylation at Ser351 and Thr578, which induces translocation from the nucleus to endoplasmic reticulum [57, 74].

7. Sphingosine-1-Phosphate

S1P is the biological product of SKs and is predominantly formed in the cytoplasm. S1P can be retained intracellularly or released by platelets, neutrophils, leukocytes, ECs, and mast cells via the transporters, ATP-binding cassette (ABC) transporter ABCC1, ABCA1 and ABCG1 [89–92]. S1P is bound to high-density lipoproteins (HDL) and plasma proteins, such as albumin, which stabilizes S1P in the circulation [93]. Platelets secrete the highest levels of S1P but ECs also upregulate their release of S1P in response to activation and shear stress [94]. The concentration of S1P ranges from 4×10^{-4} to 1.2×10^{-3} mol/m^3 in serum, 2×10^{-4} to 5×10^{-4} mol/m^3 in plasma, and 5×10^{-7} to 7.5×10^{-6} mol/m^3 in tissue [93, 95–97]. Interestingly, S1P can also be formed outside the cell as SK-1 has been shown to be secreted by human umbilical vein ECs (HUVEC) and macrophages [98, 99].

Increasing evidence supports intracellular targets for S1P signalling with S1P binding to histone deacetylases (HDAC)-1 and -2 to regulate histone acetylation [100], TNF receptor-associated factor 2 (TRAF2) to regulate inflammation, anti-apoptotic and immune responses via the NFκB pathway [101], and prohibitin 2 (PHB2) for regulation of mitochondrial assembly and function [102]. By contrast, extracellular S1P-mediated signalling has been well described with five S1P receptors ($S1P_{1,2,3,4,5}$) coupled with various Gα proteins (e.g., Gα_i, Gα_q, and G$\alpha_{12/13}$) which activate different downstream targets, such as PI3 K/Akt, Bcl2, adenylyl cyclase, ERK, phospholipase C, and p53 for cellular responses in both an autocrine and paracrine manner [103–107]. Briefly, $S1P_1$ is important to regulate the egress of lymphocytes into the blood stream [108], and $S1P_2$ is involved in mast cell degranulation and recovery from anaphylaxis *in vivo* [109, 110], $S1P_3$ is involved in vascular

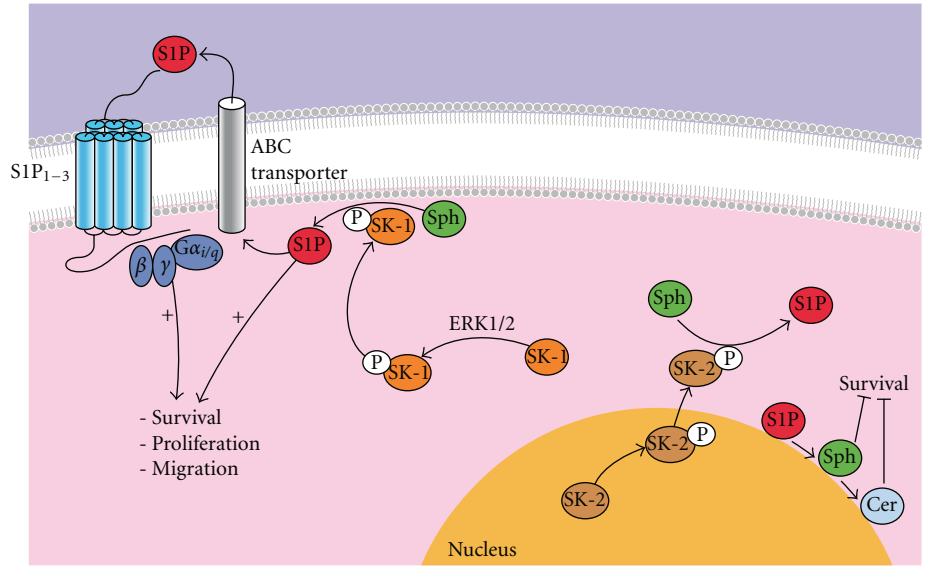


Figure 3: Intracellular SK-1 and SK-2 activity. The activation of SK-1 and SK-2 occurs via ERK1/2 phosphorylation in response to pro-inflammatory mediators, such as histamine and TNFα. Upon the activation, SK-1 is translocated from the cytoplasm to plasma membrane where it catalyses sphingosine to form S1P. S1P can then be transported outside the cell and then act back on its receptors to induce the activation of G-proteins for subsequent cellular changes, such as survival, proliferation, and migration. In contrast, SK-2 activity is associated primarily with the nuclear membrane, where it is phosphorylated prior to being translocated out of the nucleus. At the nuclear membrane and endoplasmic reticulum, S1P can be dephosphorylated to sphingosine and ceramide via the sphingolipid salvage pathway where many enzymes including sphingomyelinases, cerebrosidases, ceramides, and ceramide synthases are involved to induce apoptosis.

development in the embryo [111]. $S1P_4$ and $S1P_5$ are not well studied but have been shown to be expressed by dendritic cells and lymphocytes, respectively [112, 113].

8. Genetic Manipulation of SK/S1P *In Vivo*

To investigate the physiological roles of SK/S1P *in vivo* and whether their manipulation can regulate disease development, genetically modified mice with depletion of either SK-1 or SK-2 gene (*Sphk1* or *Sphk2*) have been generated and no phenotypical abnormalities have been identified under normal conditions [58, 77]. By contrast, the depletion of both *Sphk1* and *Sphk2* is embryonic lethal by day 13.5 due to the severe defects in vasculogenesis and neurogenesis involved in CNS development [114]. More recently, the *Sphk1* and *Sphk2* heterozygous-knockout mice (i.e., *Sphk1*−/−*Sphk2*+/−) have been generated [115]. Although *Sphk1*−/−*Sphk2*+/− mice have not been studied extensively, the female mice exhibit a significant breakage of blood vessels in the uterine causing early pregnancy loss, which suggests that a basal level of SK is required for blood vessel integrity or stability [115]. To investigate the inhibitory effects of both SKs, administration of specific SK inhibitors serves as an alternative approach to attain the double knockdown effects, for example, administration of ABC294640 (SK-2 specific inhibitor) to *SphK1*−/− mice and administration of CB5468139 (SK-1 specific inhibitor) to *SphK2*−/− mice. However, studies using this alternative approach are lacking, which are likely due to the complicated pharmacokinetics and pharmacodynamic of the SK inhibitory agents *in vivo*.

9. SK/S1P in Allergic Inflammation

SK and S1P are involved in multiple cellular functions, such as survival, differentiation, activation and migration (reviewed in [107]). Notably, these cellular properties are involved in many disease developments, including allergic inflammation. To better understand the role of SK/S1P in allergic inflammation, a number of studies have examined the specific roles of each SK isoform and S1P receptors via genetically modified mice. For example, both *Sphk1*−/− and *Sphk2*−/− mice have been shown to exhibit a reduction in ovalbumin (OVA)-induced IgE and IgG production via an inability to increase mast cell protease 1 in response to OVA, an enzyme required for IgE-induced anaphylaxis [116]. Our recent work has shown that *Sphk1*−/− mice but not *Sphk2*−/− mice exhibit an attenuated histamine-induced P-selectin expression and neutrophil recruitment [66]. This is in agreement with a study by Baker et al. who generated hTNF/*Sphk1*−/− mice (i.e., *Sphk1*−/− mice carrying the human modified copy of TNFα) and showed that only hTNF/*Sphk1*−/− mice but not hTNF/*Sphk1*+/+, hTNF/*Sphk1*−/+, or hTNF/*Sphk2*−/− mice exhibited a reduction in paw inflammation and bone deformity [117, 118]. Moreover, this was determined to be due to decreased articular COX2 protein and Th17 cell contribution to inflammation [117]. In terms of recovery from allergic inflammation, *Sphk1*−/− and $S1P_2$−/− mice were observed to have increased vasodilation, poor recovery from anaphylaxis and delayed clearance of histamine. This was not observed in the *Sphk2*−/− mice [109]. Administration of S1P to *Sphk1*−/− mice can rescue these

phenomena, which suggests that SK-1 activity aids in the recovery from anaphylaxis [109].

In humans, increasing evidence suggests that SK and S1P are involved in the pathophysiology of inflammatory diseases, such as asthma [119], chronic obstructive pulmonary disease (COPD) [120], microbial-induced sepsis [121], acute pancreatitis [122], and rheumatoid arthritis [123]. Studies have shown that the SK-1 protein and activity are upregulated markedly in peripheral immune cells including neutrophils, lymphocytes, and macrophages during the early phase of these diseases, which allow for their activation and release of the proinflammatory cytokines TNFα, IL-1β and IL-6 [121, 122]. Not surprisingly, high levels of S1P were detected in the synovial fluid of arthritic patients, which enhances COX-2 expression and prostaglandin E(2) production via the $S1P_1$ receptor [123]. Blockade of SK-1 in tissue samples extracted from these patients exhibited a decrease in proinflammatory cytokine expression [121], which suggests that the regulation of SK-1/S1P pathway is a potential therapy for inflammatory diseases.

10. Pharmacological Manipulation of SK/S1P

There are a number of SK and S1P receptor inhibitors that have been generated and studied in the last few decades (Table 2) (reviewed in [124, 125]). Blockade of SK-1 by inhibitors can attenuate prostate cancer [65], melanoma [126], inflammation in rheumatoid arthritis [123] and asthma [127] *in vivo*. Of all of the SK/S1P inhibitors, only a few have proceeded to clinical trials and been approved for human use based on their pharmacokinetics, target specificity, efficacy, adverse effects, and safety profile. The best example to date is FTY720 (Fingolimod), which was the first oral prodrug to be approved by the Food and Drug Administration (FDA) and Therapeutic Goods Administration(TGA) for the clinical treatment of multiple sclerosis (MS) [128]. The first described mechanism of FTY720 is predominantly phosphorylated by SK-2 to form FTY720-P, which is then able to bind to S1P receptors ($S1P_{1,3,4,5}$) [77, 129]. In MS, FTY720-P blocks S1P signalling largely by the internalization of the $S1P_1$ on lymphocytes causing lymphocyte egress from the lymphoid organs and lymphopenia in the periphery [108].

Interestingly, later studies have shown that FTY720 without phosphorylation can potently inhibit SK-1 by competing with sphingosine as a substrate for SKs and thereby preventing subsequent S1P formation [129–131]. Furthermore, the analogues of FTY720 (i.e., (S)- and (R)- FTY720-vinylphosphonate) bind to an allosteric site of SK-1 to induce proteasomal degradation in cells in a noncompetitive manner [132]. As FTY720 itself can inhibit SK-1, studies have also examined whether high concentrations (larger than the recent clinical dose of 0.5 mg once daily) and multiple dosing of FTY720 can be a potential therapy for cancer and renal transplantation [133, 134]. Unfortunately, results showed that FTY720 does not improve the prognosis for postrenal transplantation when compared to the current protocols [134, 135], likely due to the multiple inhibitory effects of FTY720 on S1P receptors, SK-1, autotoxin, protein phosphatase 2A, ceramide synthases, S1P lysase, protein kinase C and cytosolic phospholipase A [reviewed in [136]]. Clearly, new and specific SK/S1P inhibitors are required. To this end, Schnute et al. recently generated a specific and potent SK-1 inhibitor, PF-543, which inhibits SK-1 by competing with sphingosine and resulting in rapid reduction of S1P formation [79]. The inhibitory effect of SK-1 by PF-543 is over 1000-fold more potent than other SK inhibitors such as N,N-dimethylsphingosine (DMS) and SKI-II. However, the efficacy of PF-543 *in vivo* remains to be examined. In addition, Kharel et al. reported that their two new amidine-based SK-1 inhibitors (1a and 1b) can selectively inhibit SK-1 at high potency for rapid reduction in S1P levels without toxicity *in vitro* and *in vivo* [81].

Although SK-2 is less well studied than SK-1, a role for SK-2 (via the administration of the SK-2 inhibitor, ABC294640) has been described in tumor development [82, 137], Crohn's disease [138], hepatic ischemia-perfusion [139], and osteoarthritis [140]. However, this SK-2 inhibitor also binds to oestrogen receptor [141], which suggests that administration of this compound may result in additional off-target effects. Interestingly, a new selective SK-2 inhibitor, SLR080811, has been shown to inhibit SK-2 at a higher potency than ABC294640 *in vitro* and drive an SK-1-dependent increase in blood S1P in WT mice [83]. Whether this small molecule is suitable for the clinic still requires long-term efficacy and safety data development.

Notably, pharmacological manipulation of SK/S1P does not always lead to the same results as observed for genetic manipulation *in vivo*. As mentioned above, the hTNF/*Sphk2*−/− mice exhibited no significant difference in arthritic inflammation when compared to controls [118]. However, the hTNF mice treated with ABC294640 exhibited severe arthritic inflammation in the same study, which may suggest that high dose of the agent and acute inhibition of SK-2 contribute to this phenomenon [118]. Moreover, other animal models include that thioglycollate-induced peritonitis and collagen-induced arthritis (CIA) have shown that the recruitment of neutrophils and lymphocytes to sites of inflammation in *Sphk1*−/− mice did not differ from that of WT mice [142]. By contrast, Lai et al. have shown that knockdown of either SK-1 protein or gene in mice by DMS and small interfering (si)RNA, respectively, exhibit reduced CIA severity [123, 143]. These different observations may be due to the different time period of stimulus challenge, animal strains and models for susceptibility. Nevertheless, taken together these studies clearly indicate that SK and S1P are involved in the development of allergic inflammation.

11. Adverse Effects of SK Inhibition

The inhibition of SK/S1P pathway may be an effective therapeutic approach to control allergic diseases as shown by the *in vivo* studies discussed above. However, excessive or prolonged blockade of SK/S1P may lead to profound adverse effects as evidenced by $S1P_1$−/− and double knockout of *Sphk1*−/− *Sphk2*−/− animals being embryonic lethal [106, 114] as well as $S1P_2$−/− mice being deaf [144] and

Table 2: Synthetic inhibitors of SK and S1P receptors.

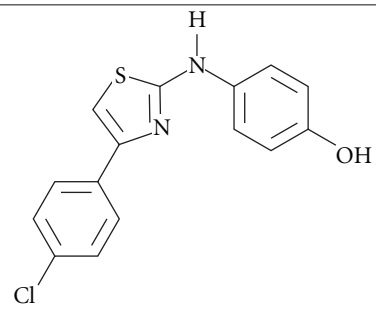
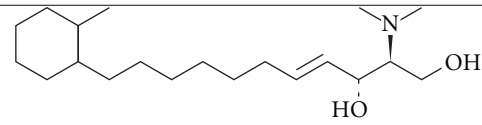
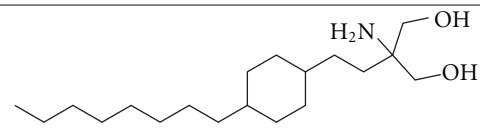
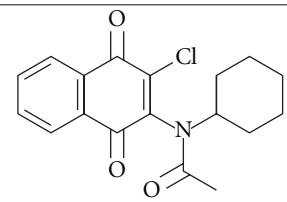
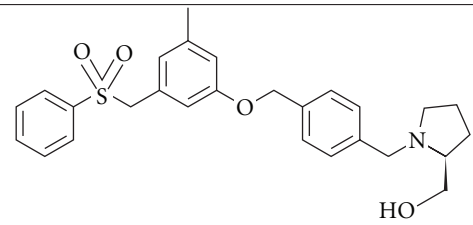
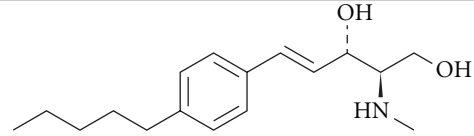
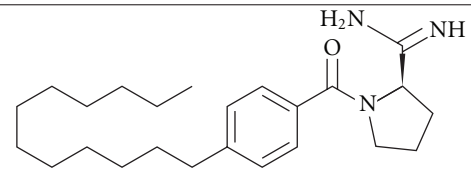
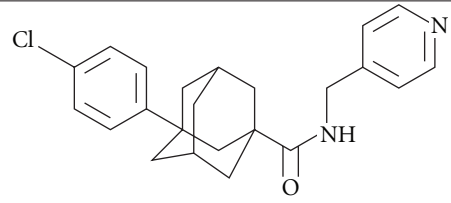
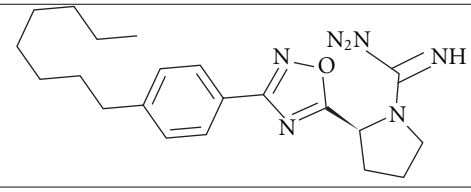
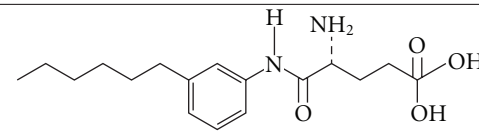
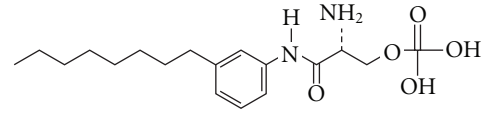
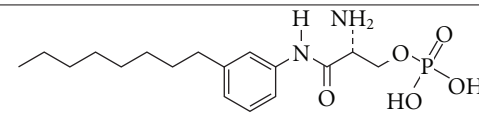
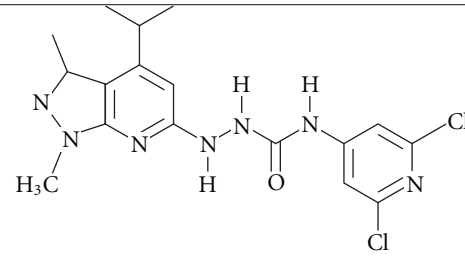
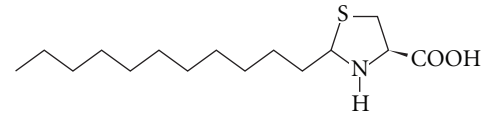
Compound	Inhibitory target(s)	Structure	Ref.
SKI-II	SK-1 SK-2		[75]
DMS	SK-1 SK-2		[76]
FTY720	SK-1 $S1P_{1,3,4,5}$		[77]
CB5468139	SK-1		[78]
PF543	SK-1		[79]
SK1-I	SK-1		[80]
Compound 1a	SK-1		[81]
ABC294640	SK-2		[82]
SLR080811	SK-2		[83]

Table 2: Continued.

Compound	Inhibitory target(s)	Structure	Ref.
W146	$S1P_1$		[84]
VPC44116	$S1P_{1\,\&\,3}$		[85]
VPC23019	$S1P_{1\,\&\,3}$		[86]
JTE013	$S1P_2$		[87]
CAY10444	$S1P_3$		[88]

experiencing occasional seizures [145]. The "side effects" of small molecule therapy that modulate the SK/S1P pathway may also raise concerns. For example, FTY720 at the clinical dose has been reported to cause transient bradycardia, atrioventricular block, macula oedema, hypertension, dyspnea, and elevated liver enzymes [146]. These symptoms are infrequent and manageable; however, compliance of this treatment can be discouraged by patients. In addition, treatment with FTY720 is also thought to increase the risk of infections as *Sphk1*−/− mice are more susceptible for endotoxin-induced lung inflammation than WT controls [147]. However, human preclinical data showed that FTY720-treated patients have no increased risk of infections in 2-year treatment when compared to the placebo group, except a small increased risk of lower respiratory tract and lung infections [128]. Notably, although the regulation of SK/S1P looks promising for controlling disease development, high specificity and potency of the pharmacological agents are preferable to avoid the undesirable off-target effects.

12. Strategy for Targeting Sphingolipids as a Therapeutic Approach

An effective approach to target sphingolipids for allergic inflammation diseases and avoid adverse effects is to better understand "when" and "where" such that specific SK/S1P inhibitors can be administrated appropriately. In ECs, we and others have demonstrated that the SK/S1P pathway regulates the expression of adhesion molecules to control neutrophil recruitment *in vitro* and *in vivo* (Figure 4). For example, during the early phase of allergic inflammation, histamine-induced SK-1 activity (but not SK-2 activity) rapidly exocytoses P-selectin to the surface of ECs to initiate neutrophil rolling in the postcapillary venules of WT mice, a process shown to be S1P receptor independent [66]. As expected, this histamine-induced neutrophil recruitment does not occur in *Sphk1*−/− mice [66]. Furthermore, TNFα-induced SK-1 activates $\alpha_5\beta_1$ integrin on human umbilical vein ECs (HUVEC) to promote the adhesion of neutrophils under shear stress, again the events appear to be S1P receptor independent and can be inhibited by FTY720 [148].

By contrast in the late phase of allergic inflammation (>4 hours), S1P receptor-activated pathways promote vascular adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, and E-selectin gene and protein expression on HUVEC in response to TNFα [67], globular adiponectin [149], or histamine [150]. Exposure of ECs to S1P can also increase Weibel Palade body (WPB) exocytosis of vWF in a PLC-γ-induced calcium-dependent manner. However, prolonged exposure of S1P enhances PI3K-induced nitric oxide production resulting in reduced WPB exocytosis by ECs [151]. Taken together, these studies suggest that increased SK-1 activity is predominantly involved in the early phase of allergic inflammation whilst S1P/S1P receptors are primarily involved in more delayed immune responses.

$S1P_{1–5}$ are distributed in different tissues with $S1P_{1–3}$ being widely expressed and at high levels in brain, lung, spleen, heart, liver, skeletal muscle, and kidney with addition

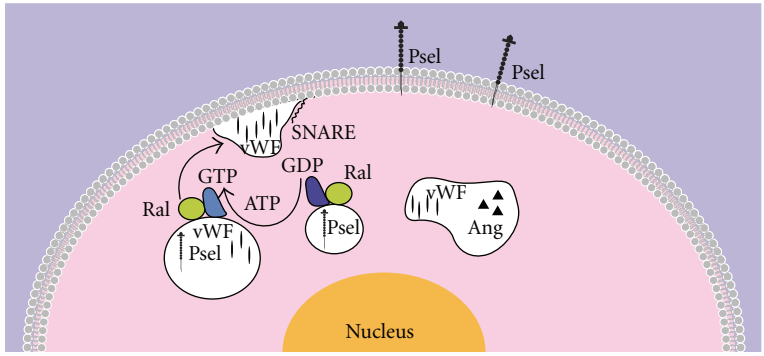


Figure 4: . Exocytosis of P-selectin by ECs. P-selectin is preformed and stored in Weibel Palade bodies (WPBs). It is found to be solely present or co-stored in WPBs with von Willebrand Factor (vWF) or angiopoietins (Ang). Upon extracellular stimulation, WPBs exocytose to the cell surface via the activation of Ral-GTP from Ral-GDP. WPB-containing vWF is also driven and translocated to the plasma membrane by SNARE. The rapid surface expression of P-selectin mediates the initial recruitment of leukocytes to ECs by rolling and tethering, which is important during the early development of allergic inflammation.

of $S1P_1$ in lymphoid and $S1P_3$ in testis; $S1P_4$ is restricted to lymphoid and lung tissue and $S1P_5$ is only expressed in brain, skin, and spleen (reviewed in [152]). These divergent tissue distributions of S1P receptors may provide some insight into which specific S1P receptor inhibitors should be administered in relation to the development of inflammation and disease. Notablty, FTY720-P binds to $S1P_{1,3,4,5}$ and may result in multiple side effects; thus other selective $S1P_1$ inhibitors (ONO-4641 and CS-0777) have been generated and undergone Phase 1 and 2 clinical trials for MS and psoriasis (reviewed in [125, 153]). Different methods of administration can be used to deliver the inhibitors/drugs for local inhibitory effects as evident by *in vivo* studies where the inhalation of SK inhibitor can attenuate airway inflammation [127], the administration of FTY720 in the eyes can prolong corneal graft survival [154], and nanoparticle-mediated delivery of drugs can enhance the therapeutic outcomes in hindlimb ischemic mice [155]. However, many questions remain to be answered, such as whether this nanotechnology is effective enough to deliver SK/S1P inhibitors to specific sites of the body and whether it is safe to be used in humans.

13. Conclusion and Future Perspectives

Early allergic reactions and recruitment of inflammatory cells are key to allergic disease formation and progression. An effectual therapeutic approach is lacking amongst the current treatment options, and most treatments (e.g., H_1 antagonists) are ineffective in their regulation of the early phase of allergic inflammation. Thus a better therapeutic strategy is urged for a rapid control of allergic symptoms to prevent tissue damage and development of severe conditions. The SK/S1P pathway has been shown to be important in cell survival, migration, differentiation, and immune responses. Herein, we discuss its role in allergic inflammation, both the early and late phases as well as chronic inflammation. Further studies involving the manipulation of SK/S1P pathway and its impact on a variety of diseases as well as the early phase of allergic inflammation will culminate to provide better insight into how we can translate animal studies into a new clinical treatment for human allergic inflammation.

Based on these *in vitro* and *in vivo* studies, sphingolipids are clearly involved in the regulation of adhesion molecule expression on the vasculature and as such may be a biological marker for attenuating leukocyte recruitment and subsequent allergic inflammatory reactions. The next step is to translate these animal models into human clinical studies with the ultimate goal of developing new treatments to tackle allergic diseases. Herein we propose that the current sphingolipid compounds may be effective in attenuation of allergic inflammation. For example, FTY720 or new small molecular inhibitors could be further investigated for their drug adverse effect profile to then determine their suitability for long-term use as prophylaxes.

Acknowledgment

W. Y. Sun holds a Ph.D. Scholarship with the Cooperative Research Centre for Biomarker Translation, C. S. Bonder (Ph.D.) is a Heart Foundation Fellow of Australia and holds NHMRC project grants to fund this work.

References

[1] P. Jiang, J. Liu, X. B. Yan, and R. Y. Liu, "Several interleukin-4 and interleukin-13 gene single nucleotide polymorphisms among Chinese asthmatic patients.," *Allergy and Asthma Proceedings*, vol. 30, no. 4, pp. 413–418, 2009.

[2] A. B. Kay, "Allergy and allergic diseases. First of two parts," *New England Journal of Medicine*, vol. 344, no. 1, pp. 30–37, 2001.

[3] K. Hakim-Rad, M. Metz, and M. Maurer, "Mast cells: makers and breakers of allergic inflammation," *Current Opinion in Allergy and Clinical Immunology*, vol. 9, no. 5, pp. 427–430, 2009.

[4] M. A. Grimbaldeston, M. Metz, M. Yu, M. Tsai, and S. J. Galli, "Effector and potential immunoregulatory roles of mast cells in IgE-associated acquired immune responses," *Current Opinion in Immunology*, vol. 18, no. 6, pp. 751–760, 2006.

[5] N. Iriyoshi, K. Takeuchi, A. Yuta, K. Ukai, and Y. Sakakura, "Increased expression of histamine H1 receptor mRNA in allergic rhinitis," *Clinical and Experimental Allergy*, vol. 26, no. 4, pp. 379–385, 1996.

[6] B. L. Jones and G. L. Kearns, "Histamine: new thoughts about a familiar mediator," *Clinical Pharmacology and Therapeutics*, vol. 89, no. 2, pp. 189–197, 2011.

[7] A. McIlroy, G. Caron, S. Blanchard et al., "Histamine and prostaglandin E2 up-regulate the production of Th2-attracting chemokines (CCL17 and CCL22) and down-regulate IFN-γ-induced CXCL10 production by immature human dendritic cells," *Immunology*, vol. 117, no. 4, pp. 507–516, 2006.

[8] M. Jutel, T. Watanabe, M. Akdis, K. Blaser, and C. A. Akdis, "Immune regulation by histamine opinion," *Current Opinion in Immunology*, vol. 14, no. 6, pp. 735–740, 2002.

[9] T. C. T. M. van der Pouw Kraan, A. Snijders, L. C. M. Boeije et al., "Histamine inhibits the production of interleukin-12 through interaction with H2 receptors," *Journal of Clinical Investigation*, vol. 102, no. 10, pp. 1866–1873, 1998.

[10] M. Dy and E. Schneider, "Histamine-cytokine connection in immunity and hematopoiesis," *Cytokine and Growth Factor Reviews*, vol. 15, no. 5, pp. 393–410, 2004.

[11] A. Huwiler, F. Döll, S. Ren et al., "Histamine increases sphingosine kinase-1 expression and activity in the human arterial endothelial cell line EA.hy 926 by a PKC-α-dependent mechanism," *Biochimica et Biophysica Acta*, vol. 1761, no. 3, pp. 367–376, 2006.

[12] M. Jutel, M. Akdis, and C. A. Akdis, "Histamine, histamine receptors and their role in immune pathology," *Clinical and Experimental Allergy*, vol. 39, no. 12, pp. 1786–1800, 2009.

[13] D. MacGlashan Jr, "Histamine: a mediator of inflammation," *Journal of Allergy and Clinical Immunology*, vol. 112, supplement 4, pp. S53–S59, 2003.

[14] R. Torres, C. Decastellarnau, L. L. Ferrer, A. Puigdemont, L. F. Santamaría, and F. De Mora, "Mast cells induce upregulation of P-selectin and intercellular adhesion molecule 1 on carotid endothelial cells in a new *in vitro* model of mast cell to endothelial cell communication," *Immunology and Cell Biology*, vol. 80, no. 2, pp. 170–177, 2002.

[15] T. Maruko, T. Nakahara, K. Sakamoto et al., "Involvement of the $\beta\gamma$ subunits of G proteins in the cAMP response induced by stimulation of the histamine H1 receptor," *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 372, no. 2, pp. 153–159, 2005.

[16] R. A. Bakker, S. B. J. Schoonus, M. J. Smit, H. Timmerman, and R. Leurs, "Histamine H1-receptor activation of nuclear factor-κB: roles for G$\beta\gamma$- and Gαq/11-subunits in constitutive and agonist-mediated signaling," *Molecular Pharmacology*, vol. 60, no. 5, pp. 1133–1142, 2001.

[17] M. J. Smit, M. Hoffmann, H. Timmerman, and R. Leurs, "Molecular properties and signalling pathways of the histamine H1 receptor," *Clinical and Experimental Allergy, Supplement*, vol. 29, supplement 3, pp. 19–28, 1999.

[18] R. Leurs, M. K. Church, and M. Taglialatela, "H1-antihistamines: inverse agonism, anti-inflammatory actions and cardiac effects," *Clinical and Experimental Allergy*, vol. 32, no. 4, pp. 489–498, 2002.

[19] C. Shayo, N. Fernandez, B. L. Legnazzi et al., "Histamine H2 receptor desensitization: involvement of a select array of G protein-coupled receptor kinases," *Molecular Pharmacology*, vol. 60, no. 5, pp. 1049–1056, 2001.

[20] M. S. Repka-Ramirez, "New concepts of histamine receptors and actions," *Current Allergy and Asthma Reports*, vol. 3, no. 3, pp. 227–231, 2003.

[21] L. M. Lichtenstein and E. Gillespie, "The effects of the H1 and H2 antihistamines on "allergic" histamine release and its inhibition by histamine," *Journal of Pharmacology and Experimental Therapeutics*, vol. 192, no. 2, pp. 441–450, 1975.

[22] M. R. Emerson, D. M. Orentas, S. G. Lynch, and S. M. LeVine, "Activation of histamine H2 receptors ameliorates experimental allergic encephalomyelitis," *NeuroReport*, vol. 13, no. 11, pp. 1407–1410, 2002.

[23] J. D. Del Valle and I. Gantz, "Novel insights into histamine H2 receptor biology," *American Journal of Physiology*, vol. 273, no. 5, pp. G987–G996, 1997.

[24] M. B. Emanuel, "Histamine and the antiallergic antihistamines: a history of their discoveries," *Clinical and Experimental Allergy, Supplement*, vol. 29, supplement 3, pp. 1–11, 1999.

[25] P. J. Bryce, C. B. Mathias, K. L. Harrison, T. Watanabe, R. S. Geha, and H. C. Oettgen, "The H1 histamine receptor regulates allergic lung responses," *Journal of Clinical Investigation*, vol. 116, no. 6, pp. 1624–1632, 2006.

[26] A. R. Qasem, C. Bucolo, M. Baiula et al., "Contribution of $\alpha 4\beta 1$ integrin to the antiallergic effect of levocabastine," *Biochemical Pharmacology*, vol. 76, no. 6, pp. 751–762, 2008.

[27] Y. J. Jang, J. H. Wang, J. S. Kim, H. J. Kwon, N. K. Yeo, and B. J. Lee, "Levocetirizine inhibits rhinovirus-induced ICAM-1 and cytokine expression and viral replication in airway epithelial cells," *Antiviral Research*, vol. 81, no. 3, pp. 226–233, 2009.

[28] D. Axelrod and L. Bielory, "Fexofenadine hydrochloride in the treatment of allergic disease: a review," *Journal of Asthma and Allergy*, no. 1, pp. 19–29, 2008.

[29] E. W. Gelfand, Z. H. Cui, K. Takeda, A. Kanehiro, and A. Joetham, "Fexofenadine modulates T-cell function, preventing allergen-induced airway inflammation and hyperresponsiveness," *Journal of Allergy and Clinical Immunology*, vol. 110, no. 1, pp. 85–95, 2002.

[30] J. O. Warner, "A double-blinded, randomized, placebo-controlled trial of cetirizine in preventing the onset of asthma in children with atopic dermatitis: 18 months' treatment and 18 months' posttreatment follow-up," *Journal of Allergy and Clinical Immunology*, vol. 108, no. 6, pp. 929–937, 2001.

[31] G. Ciprandi, M. A. Tosca, C. Cosentino, A. M. Riccio, G. Passalacqua, and G. W. Canonica, "Effects of fexofenadine and other antihistamines on components of the allergic response: adhesion molecules," *Journal of Allergy and Clinical Immunology*, vol. 112, supplement 4, pp. S78–S82, 2003.

[32] S. L. Spector, C. F. Nicodemus, J. Corren et al., "Comparison of the bronchodilatory effects of cetirizine, albuterol, and both together versus placebo in patients with mild-to-moderate asthma," *Journal of Allergy and Clinical Immunology*, vol. 96, no. 2, pp. 174–181, 1995.

[33] F. Siebenhaar, F. Degener, T. Zuberbier, P. Martus, and M. Maurer, "High-dose desloratadine decreases wheal volume and improves cold provocation thresholds compared with standard-dose treatment in patients with acquired cold urticaria: a randomized, placebo-controlled, crossover study," *Journal of Allergy and Clinical Immunology*, vol. 123, no. 3, pp. 672–679, 2009.

[34] T. Zuberbier, "Pharmacological rationale for the treatment of chronic urticaria with second-generation non-sedating

antihistamines at higher-than-standard doses," *Journal of the European Academy of Dermatology and Venereology*, vol. 26, no. 1, pp. 9–18, 2012.

[35] R. C. Johnson, T. N. Mayadas, P. S. Frenette et al., "Blood cell dynamics in P-selectin-deficient mice," *Blood*, vol. 86, no. 3, pp. 1106–1114, 1995.

[36] K. Egami, T. Murohara, M. Aoki, and T. Matsuishi, "Ischemia-induced angiogenesis: role of inflammatory response mediated by P-selectin," *Journal of Leukocyte Biology*, vol. 79, no. 5, pp. 971–976, 2006.

[37] A. Etzioni, "Defects in the leukocyte adhesion cascade," *Clinical Reviews in Allergy and Immunology*, vol. 38, no. 1, pp. 54–60, 2010.

[38] T. M. Zollner, K. Asadullah, and M. P. Schön, "Targeting leukocyte trafficking to inflamed skin-still an attractive therapeutic approach?" *Experimental Dermatology*, vol. 16, no. 1, pp. 1–12, 2007.

[39] B. Rossi and G. Constantin, "Anti-selectin therapy for the treatment of inflammatory diseases," *Inflammation and Allergy-Drug Targets*, vol. 7, no. 2, pp. 85–93, 2008.

[40] D. J. Lefer, D. M. Flynn, M. L. Phillips, M. Ratcliffe, and A. J. Buda, "A novel sialyl Lewis(x) analog attenuates neutrophil accumulation and myocardial necrosis after ischemia and reperfusion," *Circulation*, vol. 90, no. 5, pp. 2390–2401, 1994.

[41] E. A. Gill, Y. Kong, and L. D. Horwitz, "An oligosaccharide sialyl-Lewis(x) analogue does not reduce myocardial infarct size after ischemia and reperfusion in dogs," *Circulation*, vol. 94, no. 3, pp. 542–546, 1996.

[42] Y. Birnbaum, M. Patterson, and R. A. Kloner, "The effect of CY1503, a sialyl lewis(x) analog blocker of the selectin adhesion molecules, on infarct size and "no-reflow" in the rabbit model of acute myocardial infarction/reperfusion," *Journal of Molecular and Cellular Cardiology*, vol. 29, no. 8, pp. 2013–2025, 1997.

[43] A. Kutlar, K. I. Ataga, L. McMahon et al., "A potent oral P-selectin blocking agent improves microcirculatory blood flow and a marker of endothelial cell injury in patients with sickle cell disease," *American Journal of Hematology*, vol. 87, no. 5, pp. 536–539, 2012.

[44] R. Anaya-Prado, J. R. Ramos-Kelly, L. H. Toledo-Pereyra, J. Walsh, and P. A. Ward, "Multiple selectin blockade with a small-molecule selectin inhibitor does not affect survival after a second inflammatory challenge with nonlethal LPS," *Journal of Investigative Surgery*, vol. 15, no. 3, pp. 171–180, 2002.

[45] M. S. Co, N. F. Landolfi, J. O. Nagy et al., "Properties and pharmacokinetics of two humanized antibodies specific for L-selectin," *Immunotechnology*, vol. 4, no. 3-4, pp. 253–266, 1999.

[46] K. Wang, X. Zhou, Z. Zhou et al., "Recombinant soluble P-selectin glycoprotein ligand-Ig (rPSGL-Ig) attenuates infarct size and myeloperoxidase activity in a canine model of ischemia-reperfusion," *Thrombosis and Haemostasis*, vol. 88, no. 1, pp. 149–154, 2002.

[47] K. Ley, "The role of selectins in inflammation and disease," *Trends in Molecular Medicine*, vol. 9, no. 6, pp. 263–268, 2003.

[48] P. Kubes and S. M. Kerfoot, "Leukocyte recruitment in the microcirculation: the rolling paradigm revisited," *News in Physiological Sciences*, vol. 16, no. 2, pp. 76–80, 2001.

[49] M. D. Catalina, P. Estess, and M. H. Siegelman, "Selective requirements for leukocyte adhesion molecules in models of acute and chronic cutaneous inflammation: participation of E- and P- but not L-selectin," *Blood*, vol. 93, no. 2, pp. 580–589, 1999.

[50] S. M. Pitson, J. A. Powell, and C. S. Bonder, "Regulation of sphingosine kinase in hematological malignancies and other cancers," *Anti-Cancer Agents in Medicinal Chemistry*, vol. 11, no. 9, pp. 799–809, 2011.

[51] A. J. Melendez, "Sphingosine kinase signalling in immune cells: potential as novel therapeutic targets," *Biochimica et Biophysica Acta*, vol. 1784, no. 1, pp. 66–75, 2008.

[52] W. Q. Lai, W. S. F. Wong, and B. P. Leung, "Sphingosine kinase and sphingosine 1-phosphate in asthma," *Bioscience Reports*, vol. 31, no. 2, pp. 145–150, 2011.

[53] M. Podbielska, H. Krotkiewski, and E. L. Hogan, "Signaling and regulatory functions of bioactive sphingolipids as therapeutic targets in multiple sclerosis," *Neurochemical Research*, vol. 37, no. 6, pp. 1154–1169, 2012.

[54] P. F. Hu, Y. Chen, P. F. Cai, L. F. Jiang, and L. D. Wu, "Sphingosine-1-phosphate: a potential therapeutic target for rheumatoid arthritis," *Molecular Biology Reports*, vol. 38, no. 6, pp. 4225–4230, 2011.

[55] C. F. Jessup, C. S. Bonder, S. M. Pitson, and P. T. Coates, "The sphingolipid rheostat: a potential target for improving pancreatic islet survival and function," *Endocrine, Metabolic & Immune Disorders-Drug Targets*, vol. 11, no. 4, pp. 262–272, 2011.

[56] S. M. Pitson, R. J. D'Andrea, L. Vandeleur et al., "Human sphingosine kinase: purification, molecular cloning and characterization of the native and recombinant enzymes," *Biochemical Journal*, vol. 350, no. 2, pp. 429–441, 2000.

[57] H. Liu, M. Sugiura, V. E. Nava et al., "Molecular cloning and functional characterization of a novel mammalian sphingosine kinase type 2 isoform," *Journal of Biological Chemistry*, vol. 275, no. 26, pp. 19513–19520, 2000.

[58] M. L. Allende, T. Sasaki, H. Kawai et al., "Mice deficient in sphingosine kinase 1 are rendered lymphopenic by FTY720," *Journal of Biological Chemistry*, vol. 279, no. 50, pp. 52487–52492, 2004.

[59] B. Zemann, B. Kinzel, M. Müller et al., "Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720," *Blood*, vol. 107, no. 4, pp. 1454–1458, 2006.

[60] R. Pappu, S. R. Schwab, I. Cornelissen et al., "Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate," *Science*, vol. 316, no. 5822, pp. 295–298, 2007.

[61] A. Olivera, T. Kohama, L. Edsall et al., "Sphingosine kinase expression increases intracellular sphingosine-1- phosphate and promotes cell growth and survival," *Journal of Cell Biology*, vol. 147, no. 3, pp. 545–557, 1999.

[62] J. R. Gamble, W. Y. Sun, X. Li et al., "Sphingosine kinase-1 associates with integrin $\alpha V\beta$ 3 to mediate endothelial cell survival," *American Journal of Pathology*, vol. 175, no. 5, pp. 2217–2225, 2009.

[63] S. M. Pitson, "Regulation of sphingosine kinase and sphingolipid signaling," *Trends in Biochemical Sciences*, vol. 36, no. 2, pp. 97–107, 2011.

[64] M. Maceyka, H. Sankala, N. C. Hait et al., "SphK1 and SphK2, sphingosine kinase isoenzymes with opposing functions in sphingolipid metabolism," *Journal of Biological Chemistry*, vol. 280, no. 44, pp. 37118–37129, 2005.

[65] K. G. Lim, F. Tonelli, E. Berdyshev et al., "Inhibition kinetics and regulation of sphingosine kinase 1 expression in prostate cancer cells: functional differences between sphingosine kinase 1a and 1b," *International Journal of Biochemistry & Cell Biology*, vol. 44, no. 9, pp. 1457–1464, 2012.

[66] W. Y. Sun, L. D. Abeynaike, S. Escarbe et al., "Rapid histamine-induced neutrophil recruitment is sphingosine kinase-1 dependent," *American Journal of Pathology*, vol. 180, no. 4, pp. 1740–1750, 2012.

[67] P. Xia, J. R. Gamble, K. A. Rye et al., "Tumor necrosis factor-α induces adhesion molecule expression through the sphingosine kinase pathway," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 24, pp. 14196–14201, 1998.

[68] A. J. Melendez and F. B. M. Ibrahim, "Antisense knockdown of sphingosine kinase 1 in human macrophages inhibits C5a receptor-dependent signal transduction, Ca2+ signals, enzyme release, cytokine production, and chemotaxis," *Journal of Immunology*, vol. 173, no. 3, pp. 1596–1603, 2004.

[69] S. M. Pitson, P. A. B. Moretti, J. R. Zebol et al., "Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation," *EMBO Journal*, vol. 22, no. 20, pp. 5491–5500, 2003.

[70] R. V. Stahelin, J. H. Hwang, J. H. Kim et al., "The mechanism of membrane targeting of human sphingosine kinase 1," *Journal of Biological Chemistry*, vol. 280, no. 52, pp. 43030–43038, 2005.

[71] K. E. Jarman, P. A. B. Moretti, J. R. Zebol, and S. M. Pitson, "Translocation of sphingosine kinase 1 to the plasma membrane is mediated by calcium- and integrin-binding protein 1," *Journal of Biological Chemistry*, vol. 285, no. 1, pp. 483–492, 2010.

[72] R. K. Barr, H. E. Lynn, P. A. B. Moretti, Y. Khew-Goodall, and S. M. Pitson, "Deactivation of sphingosine kinase 1 by protein phosphatase 2A," *Journal of Biological Chemistry*, vol. 283, no. 50, pp. 34994–35002, 2008.

[73] M. R. Pitman, R. K. Barr, B. L. Gliddon, A. M. Magarey, P. A. B. Moretti, and S. M. Pitson, "A critical role for the protein phosphatase 2A B$'\alpha$ regulatory subunit in dephosphorylation of sphingosine kinase 1," *International Journal of Biochemistry and Cell Biology*, vol. 43, no. 3, pp. 342–347, 2011.

[74] N. C. Hait, A. Bellamy, S. Milstien, T. Kordula, and S. Spiegel, "Sphingosine kinase type 2 activation by ERK-mediated phosphorylation," *Journal of Biological Chemistry*, vol. 282, no. 16, pp. 12058–12065, 2007.

[75] K. J. French, R. S. Schrecengost, B. D. Lee et al., "Discovery and evaluation of inhibitors of human sphingosine kinase," *Cancer Research*, vol. 63, no. 18, pp. 5962–5969, 2003.

[76] L. C. Edsall, J. R. Van Brocklyn, O. Cuvillier, B. Kleuser, and S. Spiegel, "N,N-dimethylsphingosine is a potent competitive inhibitor of sphingosine kinase but not of protein kinase C: modulation of cellular levels of sphingosine 1-phosphate and ceramide," *Biochemistry*, vol. 37, no. 37, pp. 12892–12898, 1998.

[77] Y. Kharel, S. Lee, A. H. Snyder et al., "Sphingosine kinase 2 is required for modulation of lymphocyte traffic by FTY720," *Journal of Biological Chemistry*, vol. 280, no. 44, pp. 36865–36872, 2005.

[78] P. Gao, Y. K. Peterson, R. A. Smith, and C. D. Smith, "Characterization of isoenzyme-selective inhibitors of human sphingosine kinases," *PLoS One*, vol. 7, no. 9, Article ID e44543, 2012.

[79] M. E. Schnute, M. D. McReynolds, T. Kasten et al., "Modulation of cellular S1P levels with a novel, potent and specific inhibitor of sphingosine kinase-1," *Biochemical Journal*, vol. 444, no. 1, pp. 79–88, 2012.

[80] M. M. Price, C. A. Oskeritzian, Y. T. Falanga et al., "A specific sphingosine kinase 1 inhibitor attenuates airway hyperresponsiveness and inflammation in a mast cell-dependent murine model of allergic asthma," *Journal of Allergy and Clinical Immunology*. In press.

[81] Y. Kharel, T. P. Mathews, A. M. Gellett et al., "Sphingosine kinase type 1 inhibition reveals rapid turnover of circulating sphingosine 1-phosphate," *Biochemical Journal*, vol. 440, no. 3, pp. 345–353, 2011.

[82] K. J. French, Y. Zhuang, L. W. Maines et al., "Pharmacology and antitumor activity of ABC294640, a selective inhibitor of sphingosine kinase-2," *Journal of Pharmacology and Experimental Therapeutics*, vol. 333, no. 1, pp. 129–139, 2010.

[83] Y. Kharel, M. Raje, M. Gao et al., "Sphingosine kinase type 2 inhibition elevates circulating sphingosine 1-phosphate," *Biochemical Journal*, vol. 447, no. 1, pp. 149–157, 2012.

[84] M. G. Sanna, S. K. Wang, P. J. Gonzalez-Cabrera et al., "Enhancement of capillary leakage and restoration of lymphocyte egress by a chiral S1P1 antagonist *in vivo*," *Nature Chemical Biology*, vol. 2, no. 8, pp. 434–441, 2006.

[85] F. W. Foss Jr, A. H. Snyder, M. D. Davis et al., "Synthesis and biological evaluation of γ-aminophosphonates as potent, subtype-selective sphingosine 1-phosphate receptor agonists and antagonists," *Bioorganic and Medicinal Chemistry*, vol. 15, no. 2, pp. 663–677, 2007.

[86] M. D. Davis, J. J. Clemens, T. L. Macdonald, and K. R. Lynch, "Sphingosine 1-phosphate analogs as receptor antagonists," *Journal of Biological Chemistry*, vol. 280, no. 11, pp. 9833–9841, 2005.

[87] M. Osada, Y. Yatomi, T. Ohmori, H. Ikeda, and Y. Ozaki, "Enhancement of sphingosine 1-phosphate-induced migration of vascular endothelial cells and smooth muscle cells by an EDG-5 antagonist," *Biochemical and Biophysical Research Communications*, vol. 299, no. 3, pp. 483–487, 2002.

[88] R. Tao, H. E. Hoover, J. Zhang, N. Honbo, C. C. Alano, and J. S. Karliner, "Cardiomyocyte S1P1 receptor-mediated extracellular signal-related kinase signaling and desensitization," *Journal of Cardiovascular Pharmacology*, vol. 53, no. 6, pp. 486–494, 2009.

[89] A. J. Snider, K. Alexa Orr Gandy, and L. M. Obeid, "Sphingosine kinase: role in regulation of bioactive sphingolipid mediators in inflammation," *Biochimie*, vol. 92, no. 6, pp. 707–715, 2010.

[90] Z. Tanfin, M. Serrano-Sanchez, and D. Leiber, "ATP-binding cassette ABCC1 is involved in the release of sphingosine 1-phosphate from rat uterine leiomyoma ELT3 cells and late pregnant rat myometrium," *Cellular Signalling*, 2011.

[91] Y. Yatomi, Y. Ozaki, T. Ohmori, and Y. Igarashi, "Sphingosine 1-phosphate: synthesis and release," *Prostaglandins and Other Lipid Mediators*, vol. 64, no. 1–4, pp. 107–122, 2001.

[92] P. Mitra, C. A. Oskeritzian, S. G. Payne, M. A. Beaven, S. Milstien, and S. Spiegel, "Role of ABCC1 in export of sphingosine-1-phosphate from mast cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 44, pp. 16394–16399, 2006.

[93] N. Murata, K. Sato, J. Kon et al., "Interaction of sphingosine 1-phosphate with plasma components, including lipoproteins, regulates the lipid receptor-mediated actions," *Biochemical Journal*, vol. 352, no. 3, pp. 809–815, 2000.

[94] S. Aoki, M. Osada, M. Kaneko, Y. Ozaki, and Y. Yatomi, "Fluid shear stress enhances the sphingosine 1-phosphate responses in cell-cell interactions between platelets and endothelial cells," *Biochemical and Biophysical Research Communications*, vol. 358, no. 4, pp. 1054–1057, 2007.

[95] A. Olivera and S. Spiegel, "Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens," *Nature*, vol. 365, no. 6446, pp. 557–560, 1993.

[96] Y. Yatomi, Y. Igarashi, L. Yang et al., "Sphingosine 1-phosphate, a bioactive sphingolipid abundantly stored in platelets, is a normal constituent of human plasma and serum," *Journal of Biochemistry*, vol. 121, no. 5, pp. 969–973, 1997.

[97] S. R. Schwab, J. P. Pereira, M. Matloubian, Y. Xu, Y. Huang, and J. G. Cyster, "Immunology: lymphocyte sequestration through S1P lyase inhibition and disruption of S1P gradients," *Science*, vol. 309, no. 5741, pp. 1735–1739, 2005.

[98] K. Venkataraman, S. Thangada, J. Michaud et al., "Extracellular export of sphingosine kinase-1a contributes to the vascular S1P gradient," *Biochemical Journal*, vol. 397, no. 3, pp. 461–471, 2006.

[99] S. M. Hammad, T. A. Taha, A. Nareika, K. R. Johnson, M. F. Lopes-Virella, and L. M. Obeid, "Oxidized LDL immune complexes induce release of sphingosine kinase in human U937 monocytic cells," *Prostaglandins and Other Lipid Mediators*, vol. 79, no. 1-2, pp. 126–140, 2006.

[100] N. C. Hait, J. Allegood, M. Maceyka et al., "Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate," *Science*, vol. 325, no. 5945, pp. 1254–1257, 2009.

[101] S. E. Alvarez, K. B. Harikumar, N. C. Hait et al., "Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2," *Nature*, vol. 465, no. 7301, pp. 1084–1088, 2010.

[102] G. M. Strub, M. Paillard, J. Liang et al., "Sphingosine-1-phosphate produced by sphingosine kinase 2 in mitochondria interacts with prohibitin 2 to regulate complex IV assembly and respiration," *FASEB Journal*, vol. 25, no. 2, pp. 600–612, 2011.

[103] V. Limaye, X. Li, C. Hahn et al., "Sphingosine kinase-1 enhances endothelial cell survival through a PECAM-1-dependent activation of PI-3K/Akt and regulation of Bcl-2 family members," *Blood*, vol. 105, no. 8, pp. 3169–3177, 2005.

[104] B. Oskouian, P. Soonyakumaran, A. D. Borowsky et al., "Sphingosine-1-phosphate lyase potentiates apoptosis via p53- and p38-dependent pathways and is down-regulated in colon cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 46, pp. 17384–17389, 2006.

[105] S. Colié, P. P. Van Veldhoven, B. Kedjouar et al., "Disruption of sphingosine 1-phosphate lyase confers resistance to chemotherapy and promotes oncogenesis through Bcl-2/Bcl-xL upregulation," *Cancer Research*, vol. 69, no. 24, pp. 9346–9353, 2009.

[106] Y. Liu, R. Wada, T. Yamashita et al., "Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation," *Journal of Clinical Investigation*, vol. 106, no. 8, pp. 951–961, 2000.

[107] S. M. Pitson, "Regulation of sphingosine kinase and sphingolipid signaling," *Trends in Biochemical Sciences*, vol. 36, no. 2, pp. 97–107, 2011.

[108] M. Matloubian, C. G. Lo, G. Cinamon et al., "Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1," *Nature*, vol. 427, no. 6972, pp. 355–360, 2004.

[109] A. Olivera, C. Eisner, Y. Kitamura et al., "Sphingosine kinase 1 and sphingosine-1-phosphate receptor 2 are vital to recovery from anaphylactic shock in mice," *Journal of Clinical Investigation*, vol. 120, no. 5, pp. 1429–1440, 2010.

[110] P. S. Jolly, M. Bektas, A. Olivera et al., "Transactivation of sphingosine-1-phosphate receptors by fcεRI triggering is required for normal mast cell degranulation and chemotaxis," *Journal of Experimental Medicine*, vol. 199, no. 7, pp. 959–970, 2004.

[111] M. Kono, Y. Mi, Y. Liu et al., "The sphingosine-1-phosphate receptors S1P1, S1P2, and S1P3 function coordinately during embryonic angiogenesis," *Journal of Biological Chemistry*, vol. 279, no. 28, pp. 29367–29373, 2004.

[112] Y. Y. Lan, A. De Creus, B. L. Colvin et al., "The sphingosine-1-phosphate receptor agonist FTY720 modulates dendritic cell trafficking *in vivo*," *American Journal of Transplantation*, vol. 5, no. 11, pp. 2649–2659, 2005.

[113] W. Wang, M. H. Graeler, and E. J. Goetzl, "Type 4 sphingosine 1-phosphate G protein-coupled receptor (S1P4) transduces S1P effects on T cell proliferation and cytokine secretion without signaling migration," *FASEB Journal*, vol. 19, no. 12, pp. 1731–1733, 2005.

[114] K. Mizugishi, T. Yamashita, A. Olivera, G. F. Miller, S. Spiegel, and R. L. Proia, "Essential role for sphingosine kinases in neural and vascular development," *Molecular and Cellular Biology*, vol. 25, no. 24, pp. 11113–11121, 2005.

[115] K. Mizugishi, C. Li, A. Olivera et al., "Maternal disturbance in activated sphingolipid metabolism causes pregnancy loss in mice," *Journal of Clinical Investigation*, vol. 117, no. 10, pp. 2993–3006, 2007.

[116] S. C. Diesner, A. Olivera, S. Dillahunt et al., "Sphingosine-kinase 1 and 2 contribute to oral sensitization and effector phase in a mouse model of food allergy," *Immunology Letters*, vol. 141, no. 2, pp. 210–219, 2012.

[117] D. A. Baker, J. Barth, R. Chang, L. M. Obeid, and G. S. Gilkeson, "Genetic sphingosine kinase 1 deficiency significantly decreases synovial inflammation and joint erosions in murine TNF-α-induced arthritis," *Journal of Immunology*, vol. 185, no. 4, pp. 2570–2579, 2010.

[118] D. A. Baker, J. Eudaly, C. D. Smith, L. M. Obeid, and G. S. Gilkeson, "Impact of sphingosine kinase 2 deficiency on the development of TNF-alpha-induced inflammatory arthritis," *Rheumatology International*. In press.

[119] A. J. Ammit, A. T. Hastie, L. C. Edsall et al., "Sphingosine 1-phosphate modulates human airway smooth muscle cell functions that promote inflammation and airway remodeling in asthma.," *The FASEB Journal*, vol. 15, no. 7, pp. 1212–1214, 2001.

[120] F. Cordts, S. Pitson, C. Tabeling et al., "Expression profile of the sphingosine kinase signalling system in the lung of patients with chronic obstructive pulmonary disease," *Life Sciences*, vol. 89, no. 21-22, pp. 806–811, 2011.

[121] P. Puneet, C. T. Yap, L. Wong et al., "SphK1 regulates proinflammatory responses associated with endotoxin and polymicrobial sepsis," *Science*, vol. 328, no. 5983, pp. 1290–1294, 2010.

[122] Q. Li, C. Wang, Q. Zhang, C. Tang, N. Li, and J. Li, "The role of sphingosine kinase 1 in patients with severe acute pancreatitis," *Annals of Surgery*, vol. 255, no. 5, pp. 954–962, 2012.

[123] W. Q. Lai, A. W. Irwan, H. H. Goh et al., "Anti-inflammatory effects of sphingosine kinase modulation in inflammatory arthritis.," *Journal of Immunology*, vol. 181, no. 11, pp. 8010–8017, 2008.

[124] M. R. Pitman and S. M. Pitson, "Inhibitors of the sphingosine kinase pathway as potential therapeutics," *Current Cancer Drug Targets*, vol. 10, no. 4, pp. 354–367, 2010.

[125] D. Marsolais and H. Rosen, "Chemical modulators of sphingosine-1-phosphate receptors as barrier-oriented therapeutic molecules," *Nature Reviews Drug Discovery*, vol. 8, no. 4, pp. 297–307, 2009.

[126] S. V. Madhunapantula, J. Hengst, R. Gowda, T. E. Fox, J. K. Yun, and G. P. Robertson, "Targeting sphingosine kinase-1 to

inhibit melanoma," *Pigment Cell & Melanoma Research*, vol. 25, no. 2, pp. 259–274, 2012.

[127] T. Nishiuma, Y. Nishimura, T. Okada et al., "Inhalation of sphingosine kinase inhibitor attenuates airway inflammation in asthmatic mouse model," *American Journal of Physiology*, vol. 294, no. 6, pp. L1085–L1093, 2008.

[128] L. Kappos, E. W. Radue, P. O'Connor et al., "A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis," *New England Journal of Medicine*, vol. 362, no. 5, pp. 387–401, 2010.

[129] S. W. Paugh, S. G. Payne, S. E. Barbour, S. Milstien, and S. Spiegel, "The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2," *FEBS Letters*, vol. 554, no. 1-2, pp. 189–193, 2003.

[130] D. A. Vessey, M. Kelley, J. Zhang, L. Li, R. Tao, and J. S. Karliner, "Dimethylsphingosine and FTY720 inhibit the SK1 form but activate the SK2 form of sphingosine kinase from rat heart," *Journal of Biochemical and Molecular Toxicology*, vol. 21, no. 5, pp. 273–279, 2007.

[131] F. Tonelli, K. G. Lim, C. Loveridge et al., "FTY720 and (S)-FTY720 vinylphosphonate inhibit sphingosine kinase 1 and promote its proteasomal degradation in human pulmonary artery smooth muscle, breast cancer and androgen-independent prostate cancer cells," *Cellular Signalling*, vol. 22, no. 10, pp. 1536–1542, 2010.

[132] K. G. Lim, F. Tonelli, Z. Li et al., "FTY720 analogues as sphingosine kinase 1 inhibitors: enzyme inhibition kinetics, allosterism, proteasomal degradation and actin rearrangement in MCF-7 breast cancer cells," *Journal of Biological Chemistry*, vol. 286, no. 21, pp. 18633–18640, 2011.

[133] D. Pchejetski, T. Bohler, L. Brizuela et al., "FTY720 (fingolimod) sensitizes prostate cancer cells to radiotherapy by inhibition of sphingosine kinase-1," *Cancer Research*, vol. 70, no. 21, pp. 8651–8661, 2010.

[134] H. Tedesco-Silva, M. I. Lorber, C. E. Foster et al., "FTY720 and everolimus in de novo renal transplant patients at risk for delayed graft function: results of an exploratory one-yr multicenter study," *Clinical Transplantation*, vol. 23, no. 5, pp. 589–599, 2009.

[135] A. J. Hoitsma, E. S. Woodle, D. Abramowicz, P. Proot, and Y. Vanrenterghem, "FTY720 combined with tacrolimus in de novo renal transplantation: 1-year, multicenter, open-label randomized study," *Nephrology Dialysis Transplantation*, vol. 26, no. 11, pp. 3802–3805, 2011.

[136] M. R. Pitman, J. M. Woodcock, A. F. Lopez, and S. M. Pitson, "Molecular targets of FTY720 (fingolimod)," *Current Molecular Medicine*. In press.

[137] V. Beljanski, C. S. Lewis, and C. D. Smith, "Antitumor activity of sphingosine kinase 2 inhibitor ABC294640 and sorafenib in hepatocellular carcinoma xenografts," *Cancer Biology and Therapy*, vol. 11, no. 5, pp. 524–534, 2011.

[138] L. W. Maines, L. R. Fitzpatrick, C. L. Green, Y. Zhuang, and C. D. Smith, "Efficacy of a novel sphingosine kinase inhibitor in experimental Crohn's disease," *Inflammopharmacology*, vol. 18, no. 2, pp. 73–85, 2010.

[139] Y. Shi, H. Rehman, V. K. Ramshesh et al., "Sphingosine kinase-2 inhibition improves mitochondrial function and survival after hepatic ischemia-reperfusion," *Journal of Hepatology*, vol. 56, no. 1, pp. 137–145, 2012.

[140] L. R. Fitzpatrick, C. Green, L. W. Maines, and C. D. Smith, "Experimental osteoarthritis in rats is attenuated by ABC294640, a selective inhibitor of sphingosine kinase-2," *Pharmacology*, vol. 87, no. 3-4, pp. 135–143, 2011.

[141] J. W. Antoon, M. D. White, W. D. Meacham et al., "Antiestrogenic effects of the novel sphingosine kinase-2 inhibitor ABC294640," *Endocrinology*, vol. 151, no. 11, pp. 5124–5135, 2010.

[142] J. Michaud, M. Kohno, R. L. Proia, and T. Hla, "Normal acute and chronic inflammatory responses in sphingosine kinase 1 knockout mice," *FEBS Letters*, vol. 580, no. 19, pp. 4607–4612, 2006.

[143] W. Q. Lai, A. W. Irwan, H. H. Goh, A. J. Melendez, I. B. McInnes, and B. P. Leung, "Distinct roles of sphingosine kinase 1 and 2 in murine collagen-induced arthritis," *Journal of Immunology*, vol. 183, no. 3, pp. 2097–2103, 2009.

[144] M. Kono, I. A. Belyantseva, A. Skoura et al., "Deafness and stria vascularis defects in S1P2 receptor-null mice," *Journal of Biological Chemistry*, vol. 282, no. 14, pp. 10690–10696, 2007.

[145] A. J. MacLennan, P. R. Carney, W. J. Zhu et al., "An essential role for the H218/AGR16/Edg-5/LPB2 sphingosine 1-phosphate receptor in neuronal excitability," *European Journal of Neuroscience*, vol. 14, no. 2, pp. 203–209, 2001.

[146] J. A. Cohen and J. Chun, "Mechanisms of fingolimod's efficacy and adverse effects in multiple sclerosis," *Annals of Neurology*, vol. 69, no. 5, pp. 759–777, 2011.

[147] K. Bachmaier, E. Guzman, T. Kawamura, X. Gao, and A. B. Malik, "Sphingosine kinase 1 mediation of expression of the anaphylatoxin receptor C5L2 dampens the inflammatory response to endotoxin," *PLoS One*, vol. 7, no. 2, Article ID e30742, 2012.

[148] W. Y. Sun, S. M. Pitson, and C. S. Bonder, "Tumor necrosis factor-induced neutrophil adhesion occurs via sphingosine kinase-1-dependent activation of endothelial $\alpha 5\beta$ 1 integrin," *American Journal of Pathology*, vol. 177, no. 1, pp. 436–446, 2010.

[149] H. Kase, Y. Hattori, T. Jojima et al., "Globular adiponectin induces adhesion molecule expression through the sphingosine kinase pathway in vascular endothelial cells," *Life Sciences*, vol. 81, no. 11, pp. 939–943, 2007.

[150] K. Shimamura, Y. Takashiro, N. Akiyama, T. Hirabayashi, and T. Murayama, "Expression of adhesion molecules by sphingosine 1-phosphate and histamine in endothelial cells," *European Journal of Pharmacology*, vol. 486, no. 2, pp. 141–150, 2004.

[151] K. Matsushita, C. N. Morrell, and C. J. Lowenstein, "Sphingosine 1-phosphate activates Weibel-Palade body exocytosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 31, pp. 11483–11487, 2004.

[152] H. Rosen, P. J. Gonzalez-Cabrera, M. G. Sanna, and S. Brown, "Sphingosine 1-phosphate receptor signaling," *Annual Review of Biochemistry*, vol. 78, pp. 743–768, 2009.

[153] T. Hla and V. Brinkmann, "Sphingosine 1-phosphate (S1P): physiology and the effects of S1P receptor modulation," *Neurology*, vol. 76, supplement 3, no. 8, pp. S3–S8, 2011.

[154] Y. Liu, J. Jiang, H. Xiao et al., "Topical application of FTY720 and cyclosporin A prolong corneal graft survival in mice," *Molecular Vision*, vol. 18, pp. 624–633, 2012.

[155] R. Nagahama, T. Matoba, K. Nakano, S. Kim-Mitsuyama, K. Sunagawa, and K. Egashira, "Nanoparticle-mediated delivery of pioglitazone enhances therapeutic neovascularization in a murine model of hindlimb ischemia," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 32, no. 10, pp. 2427–2434, 2012.