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Sphingosine Kinase: Role in Regulation of Bioactive Sphingolipid Mediators in Inflammation

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Abstract

Sphingolipids and their synthetic enzymes are emerging as important mediators in inflammatory responses and as regulators of immune cell functions. In particular, sphingosine kinase (SK) and its product sphingosine-1-phosphate (S1P) have been extensively implicated in these processes. SK catalyzes the phosphorylation of sphingosine to S1P and exists as two isoforms, SK1 and SK2. SK1 has been shown to be activated by cytokines including tumor necrosis factor-alpha (TNF-α) and interleukin1-β (IL1-β). The activation of SK1 in this pathway has been shown to be, at least in part, required for mediating TNF-α and IL1-β inflammatory responses in cells, including induction of cyclo-oxygenase 2 (COX-2). In addition to their role in inflammatory signaling, SK and S1P have also been implicated in various immune cell functions including, mast cell degranulation, migration of neutrophils, and migration and maturation of lymphocytes. The involvement of sphingolipids and sphingolipid metabolizing enzymes in inflammatory signaling and immune cell functions has implicated these mediators in numerous inflammatory disease states as well. The contribution of these mediators, specifically SK1 and S1P, to inflammation and disease are discussed in this review.

Keywords

sphingosine-1-phosphate; sphingosine kinase; inflammation; ceramide; tumor necrosis factor alpha

INTRODUCTION

Originally, sphingolipids were thought to serve only as structural components of the plasma membrane [1], but current evidence also suggests sphingolipids are pleiotrophic molecules participating in the regulation of numerous cellular functions [2]. Of recent interest is the role that sphingolipids play in inflammation and inflammatory disease. For example, numerous sphingolipid enzymes are activated by inflammatory cytokines and their downstream lipid mediators regulate inflammatory signaling pathways in addition to immune cell functions. The majority of research has focused on the role of sphingosine kinase (SK) and its product

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sphingosine-1-phosphate (S1P) in these inflammatory processes. Activation of SK has been demonstrated by pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin1-β (IL1-β), leading to production of S1P which has been linked to downstream induction of cyclo-oxygenase (COX2). Additionally, data show that SK and S1P are necessary for migration and maturation of immune cells. This review will discuss the SK/S1P pathway and its involvement in pro-inflammatory signaling and disease.

SPHINGOLIPID METABOLISM

Numerous enzymes are involved in the production and metabolism of sphingolipids, which occur through one of two pathways: *de novo* synthesis or the salvage pathway (Figure 1). The *de novo* synthesis of sphingolipids begins in the endoplasmic reticulum with the condensation of palmitoyl Co-A and serine, catalyzed by serine palmitoyl transferase (SPT) to form 3-ketosphinganine. Very recently, evidence suggests that SPT can utilize alanine to produce 3-keto-1-deoxy-sphinganine, a novel sphingoid base lacking the hydroxyl group at the first carbon [3]. Further examination of SPT may lead to the identification of currently unknown species of sphingolipids and important roles for them. The next step of sphingolipid synthesis is reduction of 3-ketosphinganine by a NADH-dependent reductase to produce dihydrosphingosine. Through the addition of differing lengths of acyl chains by ceramide synthase, dihydroceramide is formed [1] and is subsequently desaturated via dihydroceramide desaturase to ceramide. Ceramide can be phosphorylated by ceramide kinase to ceramide-1-phosphate which has recently been identified as a bioactive sphingolipid [4]. After ceramide formation, the remaining reactions to incorporate ceramide into glycolipids and sphingomyelin occur predominantly in the Golgi apparatus. Sphingolipids can also be recycled and ceramide can be produced by the salvage pathway, whereby glucocerebrosidase and sphingomyelinase breakdown various membrane glycolipids and sphingolipids respectively. Through the action of ceramidases, sphingosine is formed by the removal of the acyl chain from a ceramide substrate. Sphingosine can be recycled back to ceramide via ceramide synthases. Alternatively sphingosine is phosphorylated by SK to S1P [5,6], which can be dephosphorylated by sphingosine-1-phosphate phosphatase (SPP), along with other lipid phosphatases to form sphingosine. Originally viewed as the final step in sphingolipid breakdown, S1P is irreversibly cleaved into ethanolamine phosphate and hexadecenal by S1P lyase.

BIOACTIVE SPHINGOLIPIDS

Ceramide, which forms the backbone of all sphingolipids, has an important role in cellular stress responses such as cell cycle arrest, serum and nutrient deprivation, terminal differentiation, apoptosis, and cellular senescence [6]. In addition to a role in cell death pathways, ceramide has also been implicated in inflammation. Specifically, ceramide has been shown to play a role in skin homeostasis—mice treated topically with a ceramide analog had decreased atopic dermatitis [7]. Similarly, C6-ceramide applied topically in a corneal inflammation model decreased inflammation [8].

Phosphorylation of ceramide by ceramide kinase yields ceramide-1-phosphate (C1P), which may have a role in inflammation through its activation of cytosolic phospholipase A2 (cPLA2) [9]. In addition, C1P has been shown to be required for membrane translocation of cPLA2 and downstream production of PGE2 [10]. In macrophages, a C1P analog prevents the production of pro-inflammatory cytokines such as TNF-α; however neither C1P nor S1P prevented cytokine induction [11,12].

Through the action of ceramidases, ceramides are degraded to sphingosine, which is rapidly phosphorylated to form S1P, which then binds to G-protein coupled receptors; namely, S1P receptors [13]. Upon binding to one of the five known cell surface receptors, S1P initiates signal transduction leading to various cellular responses.

In the last fifteen years S1P has been implicated in many important cell signaling pathways and physiological processes such as, angiogenesis, cell migration and movement, cell survival and proliferation, cellular architecture, cellular contacts and adhesions, heart development, vascular development, atherogenesis, acute lung injury and acute respiratory distress, tumorogenicity and metastasis, and inflammation and immunity [14,15]. Given the wide variety of cellular and physiological processes in which S1P is involved, elucidating mechanisms behind SK1/S1P pathway regulation is warranted. In summary, sphingolipids possess important structural and signaling duties with ceramide, sphingosine, and S1P having the most established cellular signaling roles.

SPHINGOSINE KINASE

A key step in the sphingolipid pathway is the formation of S1P. Two known isoforms of SK, sphingosine kinase 1 and 2 (SK1 and SK2), are responsible for the production of S1P from sphingosine. SK cloning from yeast has dramatically facilitated the study of the enzyme. In humans, three splice variants of SK1 (SK1a, SK1b, and SK1c) have been identified [16], and a high degree of homology exists between human and mouse SK1 enzyme variants [14]. There is a conserved diacylglycerol kinase (DAG) catalytic domain; also homologous between variants is glycine 181 that is necessary for catalytic activity [17], and aspartate 278 which is required for sphingosine binding [18]. ERK phosphorylation sites [19], phosphatidylserine binding residues [20], ATP binding sites [21], Ca^{2+}/calmodulin [22] and TNF receptor associated factor 2 (TRAF2) binding sites [23] are among other homologous regions of the SK1 enzyme. SK1 resides mostly in the cytosol but can translocate to the plasma membrane upon activation by various stimuli [24]. Cellular localization of SK1 is pivotal in determining the effects of S1P production: when formed near the plasma membrane S1P can be exported before coming into contact with S1P lyase or S1P phosphatase which are ER-localized [25, 26]. There is some evidence that SK1 is secreted from HUVEC cells [16] as well as macrophages [27], allowing for the extracellular formation of S1P; however, further exploration in other cellular systems is necessary.

SK1 is known to be regulated by a multitude of growth factors and cytokines including: platelet derived growth factor (PDGF) [28,29], vascular endothelial growth factor (VEGF) [30], nerve growth factor (NGF) [31], insulin-like growth factor (IGF) [32], IGF binding protein 3 (IGFBP3) [33], lysophosphatidic acid (LPA) [34], lipopolysaccharide (LPS) [35], compliment 5a (C5a) [36], TNFα [37,38], and IL-1β [38].

While numerous growth factors and cytokines activate SK, there is only a small body of literature devoted to mechanism(s) by which SK1 is regulated. A 2002 study by Shu *et al.* probes the mechanism by which VEGF mediates activation of SK1: upon VEGF binding its receptor, PKC becomes activated leading to SK1 activation and S1P generation [30]. IGF was shown to activate SK1 and cause S1P production followed by GFP-$S1P_1$ receptor internalization in HEK-293 cells [32]. As for TNF-α, which was shown to activate SK1 in HUVEC, L929 fibroblasts and monocytes [37,39,40], its mechanism of activation has been shown to occur by ERK1/2-mediated phosphorylation at serine residue 225 as well as through association with TRAF2 [19,23]. Moreover, Chow *et al*. propose that TNF-α induces the phosphorylation of serine 225 of SK1 and that this phosphorylation causes conformational or electrostatic changes that allow SK1 to remain at the plasma membrane, enhancing the chance of finding its substrate [20]. SK1 has been shown to selectively bind phosphatidylserine at the plasma membrane; moreover, phosphorylation, calmodulin binding, and PKC have also been shown to be required for plasma membrane localization of SK1 [20,22,24]. Recently, this SK1 translocation to the plasma membrane has been shown to be facilitated by calcium and integrin binding protein 1 (CIB1) [41 to be inserted]. In yet another study, TNF-α was shown to induce

SK1 membrane translocation and activation via a PLD-dependent mechanism in monocytes [40].

A number of SK1-interacting proteins that affect its activity have recently emerged, including Δ-catenin/NRRAP [42], aminocyclase 1 [43], erkaryotic elongation factor 1A [44], filamin A [45], sphingosine kinase 1-interacting protein (SKIP) [46], and platelet endothelial adhesion molecule-1 (PECAM1) [47]. Protein phosphatase 2A (PP2A) has been implicated in the dephosphorylation and deactivation of SK1, whereas cytosolic chaperonin containing TCP-1 (CCT) has been shown to mediate proper folding of the enzyme [48,49].

Another mechanism of regulation of SK1 is at the transcriptional level whereby the SK1 promoter was shown to be up regulated in response to LPS in RAW macrophages leading to possible protection form apoptosis [27]. In addition the hypoxia-inducible factor 2 α (HIF-2 α) has also been shown to transcriptionally uregulate SK1 but not SK2 expression in glioma cells thus leading to S1P secretion and enhancement of transcellular angiogenesis [50]. It was very recently demonstrated that IL1-β could transcriptionally upregulate SK1 but not SK2 expression in glioblastoma cells, thus also implicating this pathway in the invasivness of these tumors [51].

Another emerging important mechanism of SK1 regulation is its downregulation by proteolysis after prolonged exposure to DNA damaging agents [52] and to TNF-α [53], thus leading to leading to loss of S1P, accumulation of ceramide, and cell death. This SK1 proteolysis was shown to be dependent on p53 activation in Molt-4 leukemia cells [52], and cathepsin-B was implicated in SK1 degradation in MCF-7 breast cancer cells [53]. SK1 activity and protein levels were also shown to decrease in response to reactive oxygen species (ROS) in cardiac cells, again leading to ceramide accumulation and apoptosis [54]. Moreover, knockdown of SK1 using siRNA indeed led to ceramide accumulation, apoptosis, and autophagy [55]. Taken together these studies indicate that perhaps inhibition and or loss of SK1 maybe important for induction of apoptosis not only due to a decrease in the pro-survival lipid S1P but also due to accumulation of the pro-death lipid ceramide. These studies, therefore, underscore an important role for SK1 in ceramide metabolism.

While SK1 has recently been the subject of intense investigation, SK2 has remained the less well-characterized isoform of the enzyme that produces S1P. In addition to being implicated in the induction of apoptosis, SK2 has been shown to have somewhat opposing functions to SK1 [56–58]. Most recently, SK2 has been reported to have a role in the epigenetic regulation of gene expression via modulation of histone acetylation, whereby, nuclear S1P, produced by SK2 activity, was shown to prevent the removal of acetyl groups contained within histone tails via direct interaction with and inhibition of histone deacetylases 1 and 2 (HDAC1 and HDAC2) [59], representing the first direct, intracellular target that has been identified for S1P.

SK1 and SK2 are located on separate genes and are highly homologous, with the exception of an extended N-terminal tail possessed by SK2 [14,58]. In contrast to SK1, SK2 is thought to be localized to the ER and the nucleus [60]. There is some functional redundancy between SK1 and SK2, evidenced by a lack of phenotype in SK1 single knockout and SK2 single knockout mice [61]; however, SK1/SK2 double knockouts are embryonic lethal due to inadequate blood vessel formation, providing compelling evidence for the necessity of SK in development [62].

SPHINGOSINE-1-PHOSPHATE

SK1 and SK2, along with other sphingolipid metabolizing enzymes, control the fine balance among lipid mediators ceramide, sphingosine, and S1P [15]. S1P can be either dephosphorylated by SPPs or irreversibly degraded into hexadecanal and

phosphoethanolamine by S1P lyase [63]. The discovery of five G protein-coupled S1P cell surface receptors (S1PR1-S1PR5) was the catalyst for studies implicating S1P as a multifaceted, bioactive signaling lipid-molecule [4] Understanding the regulation of this lipid-molecule is vital to address the numerous cellular processes and pathologies in which it is involved.

S1P is implicated in both extracellular and intracellular-mediated signaling; however, as yet there is only one, recently identified, direct intracellular S1P target [51,59]. S1P has been shown to promote growth and survival independent of its G protein-coupled receptors in mouse embryonic fibroblasts devoid of S1P receptors [64]. There is also evidence supporting a role for intracellular S1P in calcium mobilization [65]. To date, most S1P functions have been attributed to receptor-mediated signaling. The five S1P receptors (S1PRs) couple to various α subunits of heterotrimeric G proteins: G_i, G_q and $G_{12/13}$. S1PR1 signaling is known to be essential for embryonic blood vessel development as the murine knockout is embryonic lethal as a result of hemorrhage [66]. In addition, plasma S1P has been shown to elicit egress of lymphocytes into the blood in an S1PR1-dependent manner [67] and to regulate basal and inflammation-induced vascular leak *in vivo* [68]. S1PR2 and S1PR3 activate phospholipase-C and Rho, and knockout of both receptors in mice decreases litter size and survival rates [69]. S1P4 and S1P5 are the least studied receptors; although, it is known that S1PR4 is expressed primarily in lymphocytes and is involved in T cell proliferation [70], while S1PR5 is expressed on dendritic and natural killer cells [71]. The five known S1PRs can lead to activation of different downstream targets, such as Rac, ERK, PI_3K, adenylyl cyclase, phospholipase C, Rho or JNK, resulting in the aforementioned cellular responses [64]. The pathways mediated by each specific receptor are common; however, because there have been agonists and antagonists that exhibit receptor specificity, it is probable that the S1P receptors are not totally redundant [72]

S1PRs can undergo activation by signaling molecules other than S1P, such as growth factors. Such receptor transactivation is also known as “receptor crosstalk” [73]. For example, PDGF has been shown to activate SK1, the enzyme responsible for catalyzing the formation of S1P from sphingosine, thus activating the S1P receptor [28]. PDGF has been shown to bind its receptor and activate SK1; this specific example results in the transactivation of S1PR1, leading to Rac-mediated cell motility in HEK-293 cells [29]. Given the multiple molecules capable of activating SK1, resulting in S1P production and ultimately S1P receptor transactivation, this could be a common mechanism by which G protein-dependent signals are elicited by non-G protein-coupled receptors.

The majority of S1P effects are thought to be attributed to intracellular production, export to extracellular space, and finally activation of the S1P receptors (Figure 2); therefore, it is important to determine the factors which regulate these processes. To further confound S1P signaling, S1P autocrine or paracrine activation of S1PRs are know to activate sphingosine kinase, leading to further production of S1P [74]. The crossing of S1PR pathways with other GPCR and receptor tyrosine kinase (RTK) signaling pathways, along with the activation of SK by numerous agonists or stimuli and “inside-out” signaling capabilities of S1P clearly demonstrate the complexity involved in the SK/S1P signaling pathway. Elucidating various S1P-mediated signals has enormous potential for eliciting possible specific therapeutic targets.

SK1 AND S1P IN INFLAMMATION AND DISEASE

The SK1/S1P pathway has been implicated in inflammation mediated by TNF-α. This TNF-α signaling enhances the expression of adhesion molecules, such as vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM) [37]. SK1 was shown to be activated by TNF-α in a dose-dependent manner, an event required for TNF–α-mediated

adhesion molecule expression in HUVEC cells [37]. Subsequently, Pettus *et al.* demonstrated a role for sphingolipid metabolism in the inflammatory response in L929 fibroblasts whereby, it was shown that SK1 and S1P are necessary for TNF-α-induced COX2 and PGE2 production [39]. Billich *et al.* found increased SK1 mRNA synthesis under inflammatory conditions, again, implicating sphingolipids in inflammation [38]. These studies, along with many others have firmly established that TNF-α signaling leads to activation of the SK1/S1P pathway (Table 1). Other inflammatory signaling molecules such as IL-1β, IFN-γ, IgE and C5a [75], have also been shown to activate SK1, further suggesting the importance of the SK1/S1P pathway in the inflammatory response.

In addition to a role for SK1/S1P in TNF-α-mediated signaling, SK1/S1P and the S1PRs have also been shown to regulate numerous types of immune cells involved in inflammatory diseases. For example, SK1 is necessary for TNF-α-mediated responses in human primary monocytes [76], and it has been suggested, using dimethylsphingosine (DMS), that SK1 is required for catestatin-stimulated migration of monocytes [77]. Macrophages stimulated with LPS increase SK1 message and activity, resulting in generation of S1P and induction of COX2 [35]. In a more recent study, SK1 message and protein have been shown to be increased in LPS-activated microglia [78], perhaps implicating a possible role for the SK1/S1P pathway in neuroinflammation. Also in macrophages, SK1 has been implicated in cytokine production and chemotaxis in response to C5a [36]. Exogenous addition of low concentrations of sphingosine to C5a-primed neturophils stimulated an oxidative burst, which was attributed to activation of SK and generation of S1P [79]. In the same study, neutrophil migration in response to C5a was inhibited by DMS, suggesting a role for SK in neutrophil chemotaxis. S1PR transactivation by FcεRI is necessary for mast cell degranulation and migration [80], whereas mast cell degranulation and cytokine production require SK [81].

In addition to the growing literature implicating SK and S1P in inflammation and immune cell functions, the emergence of FTY720 and other S1PR modulators were instrumental to the discovery that S1PRs are essential for migration of lymphocytes [82,83]. FTY720 decreases the number of mature circulating lymphocytes and prevents the egress of lymphocytes from thymus and secondary lymphoid tissues through its agonistic effects on and subsequent downregulation of S1PR1 [84–86]. This immunesuppression has prompted the examination of FTY720 treatment in numerous disease states where SK1/S1P has been implicated (Table 2) including colitis [87], arthritis [88], and asthma [89] in addition to Phase III clinical trials for multiple sclerosis.

Further insights into the physiological functions of SK and S1P were anticipated from studies using SK1 and SK2 knockout mice ($SK1^{-/-}$ and $SK2^{-/-}$). However, this model initially did not provide much information about the *in vivo* role of these lipid kinases because, unexpectedly, $SK1^{-/-}$ and $SK2^{-/-}$ mice appeared morphologically and functionally normal [90], whereas, the double knockout mice were found to be embryonic lethal due to defects in both neurogenesis and angiogenesis [62]. On closer examination the $SK1^{-/-}$ mice had decreased tissue SK activity and a 50% decrease in serum S1P, but no significant difference in tissue S1P under normal conditions. These mice were rendered lymphopenic in response to the immunosuppressant FTY720 [83], whereas $SK2^{-/-}$ mice were not [91]. In other studies, $SK1^{-/-}$ and $SK2^{+/-}$ mice were crossed to render $SK1^{-/-}SK2^{+/-}$ mice and these animals had defective lymphocyte egress due to low blood S1P generation [67].

Taken altogether the above studies have begun to establish a key role for the SK1/S1P pathway in inflammatory processes which warrants closer examination of this pathway in specific inflammatory diseases as follows.

ASTHMA

Allergic asthma is characterized by constriction of the smooth muscle cells in the airway and influx of inflammatory cells into the lungs. The onset of this type of asthma is commonly due to an inhaled or ingested allergen. Asthma is typically controlled with inhaled corticosteroids and other medications that prevent or decrease symptom severity. Numerous processes involved in the progression of an asthmatic attack are regulated by or involve SK and S1P. Ingested antigen binding to the FcεR causes mast cell migration and degranluation which is dependent on transactivation of the S1PR2 [80]. S1P induces airway smooth muscle contraction [92] and can influence the migration of inflammatory cells, such as eosinophils [93]. Elevated bronchiolar S1P is found in human ragweed-allergic patients when they are challenged with allergen, compared to normal non-allergic patients [94].

Ovalbumin (OVA) administration is commonly used in rodents to model allergic asthma. Chemical inhibition of SK has been examined in the OVA model using SK-I or DMS. Mice treated with SK-I or DMS had decreased bronchiolar lavage (BAL) levels of S1P and less peroxidase activity and decreased eosinophil migration [95]. Administration of OVA to guinea pigs increased ceramide levels and ceramide synthase activity in the airway epithelium. Inhibition of this pathway with fumonisin B1 decreased ceramide synthase activity, ceramide levels and improved asthma symptoms [96]. In this study, other sphingolipids or sphingolipid metabolizing enzymes were not investigated; however, future experiments to determine whether ceramide was degraded by ceramidases to provide a substrate for SK are needed—most studies have implicated SK/S1P in allergic asthma.

S1PR modulation with FTY720 has also been studied in mouse models of asthma. Mice sensitized to OVA, but pretreated with FYTY720 30 minutes prior to OVA exposure, had less bronchial constriction and eosinophilia than vehicle-pretreated mice. FTY720 prevented migration of dendritic cells, inhibiting T cell activation and improving disease in this model [89].

S1P has also been implicated in vascular leak after antigen challenge. Interestingly, $SK1^{-/-}$ mice have increased inflammation-induced vascular leak in the lungs. $SK1^{-/-}$ mice treated with either LPS or thrombin had increased vascular permeability in lung tissue [97]. Addition of S1P reversed this effect, indicating a crucial role for SK1 and S1P in lung endothelial cell function and barrier integrity. The recent development of conditional knockouts of both SK1 and SK2 in plasma further confirmed that S1P in the plasma mediates vascular integrity [68]. Whereas inhibition of SK1 in asthma may prevent some disease aspects, S1P obviously plays a vital role in preventing vascular leak in the lungs.

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune disease involving inflammatory cell movement into joint tissue, resulting in tissue destruction and excessive pro-inflammatory cytokine production. Current therapies directed against RA consist of physical therapy, anti-inflammatory medications, and steroids. More recently, therapeutics have been targeted to inhibit increased cytokine production, specifically TNF-α. TNF-α can activate SK1, leading to the production of S1P in synoviocytes from RA patients. The addition of S1P also causes proliferation and cytokine production in these synoviocytes [98]. Furthermore, S1P is elevated in the synovium of patients with RA [99]; also, SK2 protein levels are elevated in synovial fibroblasts from RA patients [100]. These findings suggest that sphingolipid enzyme(s) and their products may play a role in the pathology involved in RA.

In studies utilizing modulation of S1PR function or non-specific inhibition of SK1, RA severity is improved. Modulation of the S1PR1 using FTY720 has been examined in two common

animal models of arthritis. Both collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AA) are well established models for studying T-cell dependent RA. In studies conducted using the immunosuppressant FYT720 in rats with both CIA and AA, hindpaw edema and joint destruction was inhibited, and lymphocyte invasion into the joints was decreased [88,101]. In addition to receptor modulation, non-specific inhibition of SK with DMS has been shown to decrease severity and cytokine production in CIA [99].

Recently, the use of knockout mice and specific knockdown of each SK isoform in RA studies have produced conflicting results. When $SK1^{-/-}$ and $SK2^{-/-}$ mice were utilized in the CIA model, no differences were noted between these strains and wild type (WT) mice [61]. In this study, disease had reached 100% incidence by 40 days post-injection and all mice developed severe joint inflammation [61]. However, in another study, mice were treated intraperitoneally with SK1 siRNA and subjected to CIA, and disease progression was prevented. Mice treated with SK1 siRNA had reduced incidence and severity of disease and decreased cytokine production [102]. In the same study, treatment of mice with SK2 siRNA increased disease incidence and severity suggesting distinct roles for SK1 and SK2 *in vivo*. Such discrepancies between these two studies could be explained by the collagen dose used to induce disease. Michaud *et al.* used 100 μg collagen to induce disease, whereas Lai and co-workers used twice that amount. Study outcome differences could also be explained by the mode of enzyme knockdown. Perhaps knockout animals develop compensatory mechanisms to overcome the complete loss of an SK isoform, whereas mice with acute knockdown of either isoform may more closely resemble what would occur with chemical inhibition of the enzyme.

INFLAMMATORY BOWEL DISEASE AND COLON CARCINOGENESIS

Inflammatory bowel disease encompasses ulcerative colitis (UC) and Crohns Disease (CD). Both forms of IBD are characterized by uncontrolled influx of inflammatory cells into the intestinal tract and overproduction of pro-inflammatory cytokines. CD can present in the entire intestinal tract, whereas UC is limited to the colon. IBD is usually treated by immunesuppression with corticosteroids and more recently, like RA, with anti-TNF-α therapies. TNF-α has been shown to activate SK1 in numerous cell types [38,39] including HT29 colon cancer cells and rat intestinal epithelial cells [103]. This SK1 activation has also been implicated in COX2 induction and PGE2 production [103].

Recent studies with mice deficient in sphingolipid enzymes and experiments with chemical inhibitors have underscored a role for sphingolipids in IBD. Numerous studies depict the efficacy of FTY720 and other S1PR modulators in mouse models of colitis. For example, FTY720 inhibits oxazolone-induced colitis, reducing macro and microscopic aspects of disease, and decreases cytokine production by modulating T-helper type 2 functions [104]. Agonism of S1P1R by KRP-203 also improved colitis in IL-10-deficient mice by decreasing lymphocyte infiltration into the lamina propria [105]. In the CD4+/CD62L+ T cell transfer model of colitis, FTY720 decreased disease by preventing the migration of CD4+ T cells into the colon tissue [87]. These studies, along with others, suggest an important role for S1PR modulation as a potential therapeutic target for IBD.

Chemical inhibition of SK has been shown to improve DSS-induced colitis, both acutely and chronically, improving disease and decreasing cytokine production [106]; however, no iso-enzyme specific inhibitors have been demonstrated *in vivo.* Similarly, we have demonstrated that both blood and colon tissue S1P increases in WT mice but not in $SK1^{-/-}$ mice with DSS-induced colitis. Systemic inflammation is decreased in $SK1^{-/-}$ mice, when compared to WT mice in this model. In addition, local inflammation, specifically neutrophil infiltration, is decreased in response to DSS in $SK1^{-/-}$ mice. Perhaps most interesting in this study is the

absence of COX2 induction in colons of $SK1^{-/-}$ mice, suggesting that SK1 is necessary for induction of COX2 *in vivo* [107].

Given the increasing evidence linking inflammation and carcinogenesis, it is noteworthy that there is increasing evidence linking SK1 expression and activity to the development and progression of cancer [108–110]. Of particular interest is the role of the SK1/S1P pathway in inflammation-induced cancer. Specifically, SK1 has been shown to be overexpressed in many human cancers including lung and colon [103,111]. When crossed with $SK1^{-/-}$ mice, intestinal neoplasia model Apc Min/+ mice had decreased intestinal adenoma size [112]. In addition, SK1 has been shown to be overexpressed in human colon cancer samples and $SK1^{-/-}$ mice develop significantly less colon cancer than WT mice in an inflammation-induced colon carcinogenesis model [113].

Thus, with an obvious link between inflammation and cancer, investigation of the role of SK and S1P is needed, especially in view of the fact that many anti-inflammatory medications currently used to treat diseases have deleterious side effects. A better understanding of how SK and S1P facilitate the processes involved in inflammation and disease should lead to improved therapeutics.

CONCLUSION

Inflammation and recruitment of inflammatory cells are important in disease progression and are at least, in part, mediated by the SK1/S1P pathway. This pathway could be manipulated to shift disease processes from pro-inflammatory to anti-inflammatory by decreasing cytokine production and infiltration of inflammatory cells. Future studies involving the modulation and chemical inhibition of SK and S1P levels, in addition to modulation of S1PRs, may lead to improved therapeutics for inflammatory diseases.

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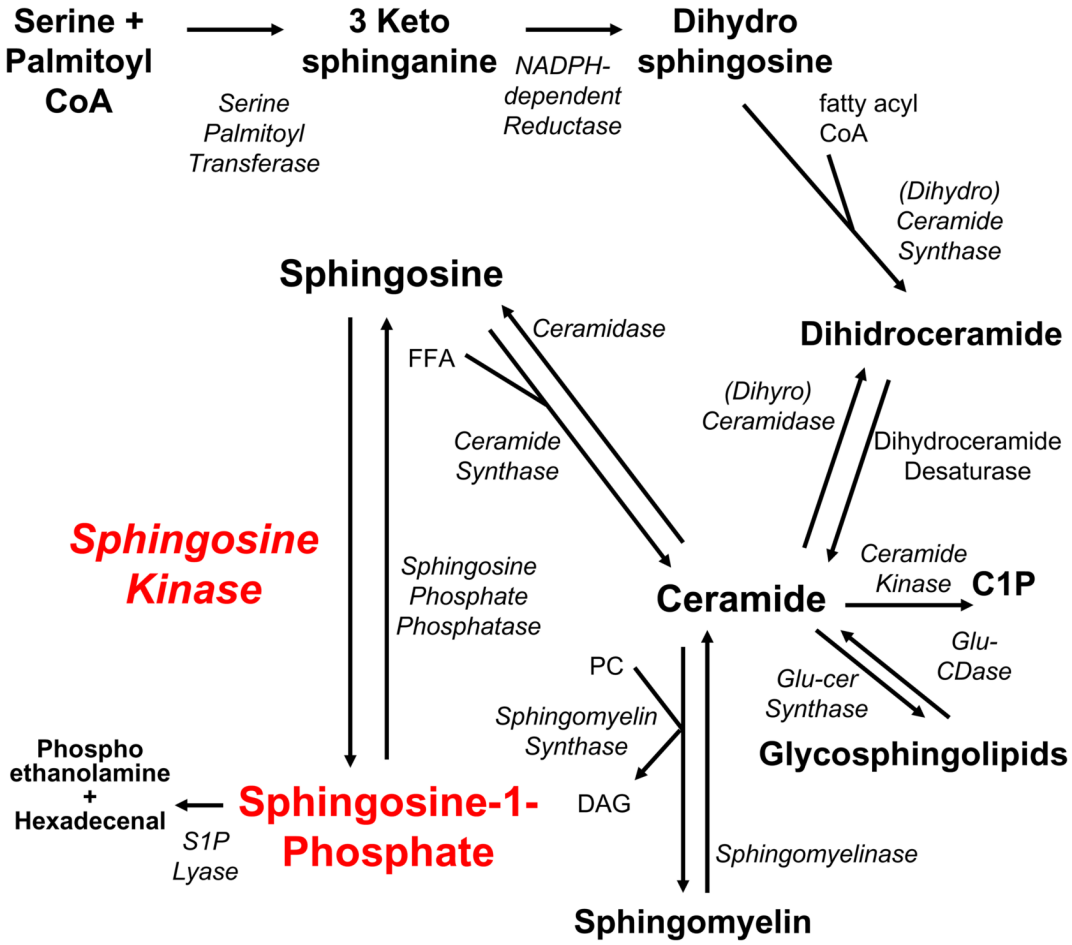

Figure 1. Pathways of Sphingolipid Metabolism

Production of bioactive sphingolipids ceramide, sphingosine, and S1P occurs through *de novo* synthesis or the salvage pathway. *De novo* synthesis involves the condensation of serine and palmitoyl CoA by serine palmitoyl transferase for form 3-keto-sphinganine, which is rapidly reduced to dihydrosphingosine by an NADPH-dependent reductase. Dihydrosphingosine is converted to dihydroceramide with the addition of fatty acyl CoA by dihydroceramide synthase which is desaturated to form ceramide. Ceramide can be converted to glycosphingolipids by glucosylceramide synthase and back to ceramide by glucosylceramidase. Ceramide can also be formed by the salvage pathway through the action of sphinogmyelinases. Ceramide can be phosphorylated to ceramide-1-phosphate or deacylated by ceramidase to form sphingosine. Sphingosine is quickly phosphorylated by sphingosine kinase to form S1P. S1P can be dephosphorylated by S1P phosphatases, forming sphingosine, which can be converted back to ceramide with the addition of free fatty acid by ceramide synthases. S1P can be terminally degraded by S1P lyase to form hexadecanal and phosphoethanolamine. FFA: free fatty acid, Glu-Cer Synthase: glucosylceramide synthase, Glu-CDase: Glucosylceramidase.

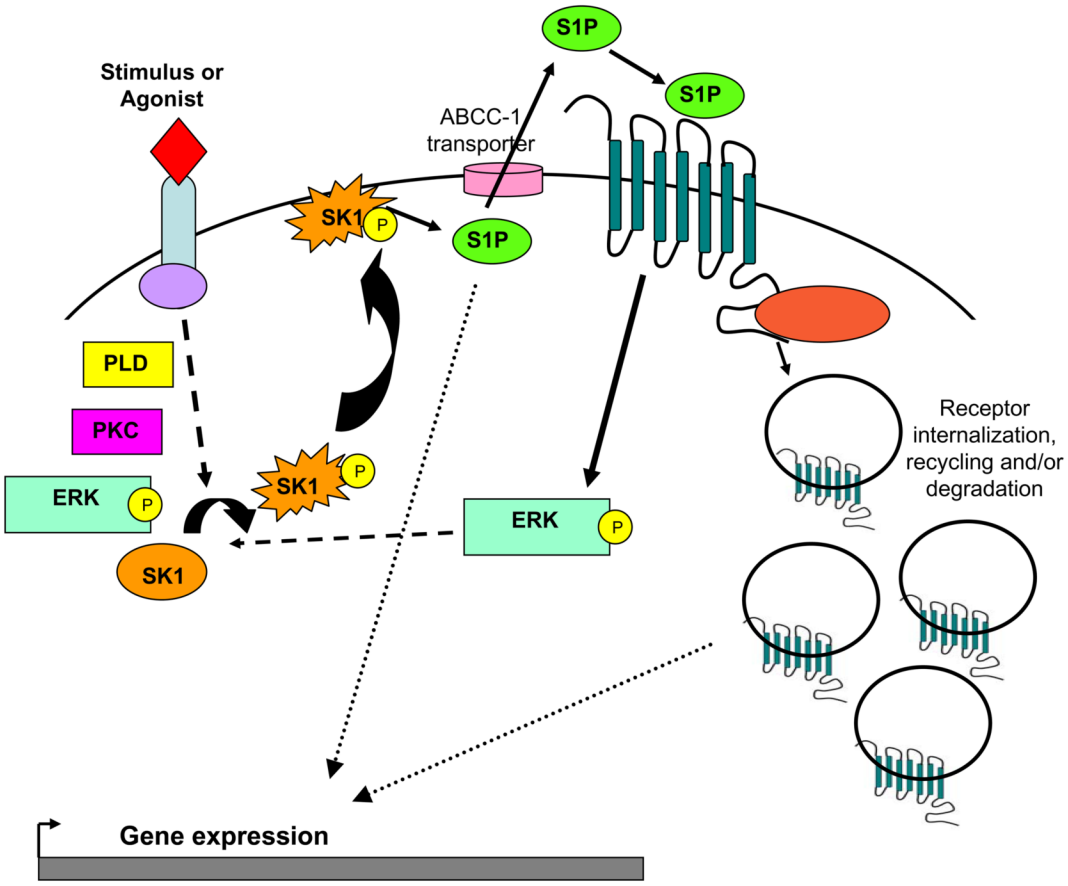

Figure 2. Inside-out S1P Signaling

Various agonists or stimuli bind and activate their respective receptors, leading to the activation of the SK/S1P pathway by both known and unknown mechanisms. Shown are molecules implicated in the phosphorylation (ERK and PKC) and/or the activation (PLD) of SK. Once activated by phosphorylation or by undefined mechanisms, SK is thought to translocate to the plasma membrane where it comes into contact with its substrate, sphingosine. SK then catalyzes the formation of S1P, which can act as an intracellular signaling molecule, or it can be exported via ABCC transporters to act in an autocrine or paracrine manner. Once activated, S1PRs elicit specific G-protein-mediated signals and this is followed by receptor internalization. Receptors are then recycled or degraded. ERK: extracellular regulated kinase; PKC: protein kinase c; PLD: phospholipase D; SK: sphingosine kinase; S1P: sphingosine 1-phosphate; S1PRs: sphingosine 1-phosphate receptors.

TABLE 1

TNF-α-Induced Events Mediated by the SK1/S1P Pathway

Cell Type	Response	Mechanism	Source
HUVEC	Expression of E-selectin and VCAM, ERK and NFkB activation	ND	[37]
HUVEC	eNOS activation	S1PR1 and S1PR3 activation	[114]
HUVEC	ICAM expression	Akt, ERK and NFkB activation	[115]
HAEC	MCP-1 expression and secretion, VCAM expression	p38	[116]
Human neutrophils	Neutrophil priming	ND	[117]
RAW 264.7 macrophages	COX-2 expression, PGE2 production	ND	[35]
U937 human monocytic cells	ERK and NFkB activation	PLD activation	[40]
C6 glioma	Activation of GTP cyclohydrolase I	Intracellular S1P signaling	[118]
1321N1 glioblastoma	Proliferation	Akt and Cyclin D activation	[119]
SKNBE human neuroblastoma	eNOS activation	ND	[120]
H441 lung epithelial	IL-8 expression	ERK, p38 and AP-1 activation	[121]
A549	Cox-2 expression and PGE2 production, IL-6, RANTES, MCP-1 and VCAM expression	NFkB activation	[38]
A549 and L929	COX-2 and PGE2 production	ND	[39]
Fibroblasts	COX-2 expression	ND	[122]
Fibroblasts	ERK activation and MMP1 expression	dihydroS1P via Gαi signaling	[122]
C2C12 myoblasts	Myogenesis	S1PR2	[123]
MC3T3-E1 osteoblast-like	IL-6 production	PLC and PKC	[124]
HEK 293T	NFkB activation	SK:TRAF2 binding	[23]

Shown are cellular or biological responses induced by TNF in various cell types. These responses elicited by TNF are dependent on the SK1/S1P pathway. The mechanism(s) by which TNF activates the pathway or by which the SK1/S1P pathway is involved in TNF-mediated events is listed when known. ND: not determined

TABLE 2

Animal Models of Inflammation and the SK/S1P Pathway

Disease Model	Animal Model	Inhibitor	Response	Source
OVA Albumin Challenge	Mice	DMS/SK-I	Decreased BAL S1P, decreased neutrophil and eosinophil infiltration	[95]
OVA Albumin Challenge	Guinea pig	Fumonisin B1	Decreased CerS activity and ceramide levels in airway epithelium	[96]
OVA Albumin Challenge	Mice	FTY720	Decreased bronchial constriction, eosinophilia, migration of dendritic cells and inhibition of T cell activation	[89]
Antigen Challenge	$SK1^{-/-}$ mice	LPA/Thrombin	Increased vascular permeability in lung tissue	[97]
Collagen/Adjuvant- Induced Arthritis	Mice	FTY720	Decreased hindpaw edema, joint destruction and lymphocyte infiltration	[88,101]
Collagen -Induced Arthritis	Mice	DMS	Decreased cytokine production	[99]
Collagen -Induced Arthritis	Mice	SK1 and SK2 siRNA	SK2 siRNA resulted in increased disease and cytokine production	[102]
Oxazolone-induced colitis	Mice	FTY720	Decreased cytokine production and macro and microscopic aspects of disease	[104]
$IL10^{-/-}$ Mice Colitis	Mice	KRP-203	Decreased lymphocyte infiltration	[105]
CD4+/CD62L+ T cell Transfer Colitis	Mice	FTY720	Prevented migration of CD4+ T	[87]
DSS-Induced Colitis	Mice	ABC294640 and ABC747080	Decreased cytokine production, tissue destruction and lymphocyte infiltration	[106]
DSS-Induced Colitis	$SK1^{-/-}$ mice		Decreased systemic and local inflammatory response	[107]
Intestinal Neoplasia	$SK1^{-/-}$ mice crossed with $Apc^{Min/+}$ mice		Decreased intestinal adenoma size	[112]
AOM/DSS Colon Carcinogenesis	$SK1^{-/-}$ mice		Decreased polyp size and number	[113]

Shown are animal models where the SK1/S1P pathway have been shown to influence disease. These responses are elicited by the inhibition or modulation of the SK1/S1P pathway. are dependent on the SK1/S1P pathway.