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Sphingosine 1-phosphate and immune regulation: trafficking and beyond

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Abstract

Sphingosine 1-phosphate (S1P) is a bioactive lipid with important functions in the immune system. S1P levels are regulated by the balance between its synthesis through sphingosine kinases and its degradation by the S1P lyase. S1P signals through plasma membrane G protein-coupled receptors (S1P₁ – S1P₅) or directly acts on intracellular targets. Although the S1P–S1P₁ axis has long been known to mediate T cell egress from lymphoid organs, recent studies have revealed intrinsic functions of S1P and its receptors in both innate and adaptive immune systems that are independent of immune cell trafficking. Here I summarize recent advances in understanding of the roles of S1P and S1P receptors in inflammatory and allergic responses and lymphocyte differentiation, which directly contribute to the regulation of inflammatory and autoimmune diseases. I also describe strategies to target S1P and S1P receptors for immune-mediated diseases, particularly the immunosuppressant FTY720 (fingolimod), which has recently become the first oral therapy for relapsing multiple sclerosis.

Keywords

sphingosine 1-phosphate; inflammation; T cell differentiation; FTY720; multiple sclerosis

Introduction

Sphingosine 1-phosphate (S1P) is a natural bioactive lysophospholipid generated from intracellular sphingosine, a product of the cell membrane component sphingomyelin (Figure 1). S1P is synthesized in most cells by the actions of sphingosine kinases (SphKs), SphK1 and SphK2, which exhibit both overlapping and distinct physiological functions¹. S1P regulates diverse physiological and immunological processes by activating G protein-coupled receptors (GPCRs), S1P₁–S1P₅, to engage downstream pathways including Ras-Erk, PI3K-Akt, and small G proteins Rac and Rho. S1P also functions as a second messenger to act upon intracellular targets. S1P is irreversibly degraded by S1P lyase or reversibly dephosphorylated by S1P phosphatases¹. The constitutive activity of S1P lyase results in low concentrations of S1P in most tissues, including lymphoid organs. Notable exceptions are found in circulation – the plasma and lymph – in which S1P levels are in the sub-to-low micromolar range². Plasma S1P is mainly produced by erythrocytes³, with additional contributions from non-hematopoietic sources such as vascular endothelium^{4, 5}, whereas lymph S1P is mainly non-hematopoietic in origin^{3, 6}. The marked difference in S1P

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concentrations between the circulation and tissues constitutes the “S1P gradient” that drives the trafficking of various immune cells ⁷. As the roles of S1P in immune cell trafficking have been covered in depth by recent excellent reviews ^{1, 2, 8, 9}, I will briefly discuss this topic below. I will then provide more thorough discussions on trafficking-independent roles of S1P and S1P receptors (S1PRs) in immune system function, with a particular focus on recent genetic studies. Finally, I will describe therapeutic targeting of S1P and S1PRs in inflammation and other immune-mediated diseases.

Immune cell trafficking

In 2002, Mandala *et al.* and Brinkmann *et al.* reported that FTY720, a new immunosuppressive drug for transplant rejection, caused lymphopenia and sequestered T cells in lymphoid organs by acting on four of the five S1PRs (excluding S1P₂) ^{10, 11}. Following this seminal discovery, genetic approaches to alter the function of S1P₁ have established that S1P₁ is the main S1P receptor that regulates T cell trafficking: T cells from S1P₁-deficient mice failed to egress from the thymus and peripheral lymphoid organs ^{12, 13}, whereas S1P₁-transgenic T cells preferentially distributed to the blood rather than lymphoid organs ^{14, 15}. S1P₁ facilitates T cell trafficking at multiple stages of T cell development and responses, including thymocyte egress into periphery, egress of mature T cells out of lymph nodes during systemic trafficking as well as after immune activation, and retention of T cells in non-lymphoid tissues ^{12, 13, 16}.

S1P–S1P₁-dependent T cell egress is mainly regulated at three levels. The first is ligand availability. Because of the essential role of the S1P gradient, disruption of this gradient by eliminating S1P lyase activity causes altered distribution and aberrant development of T cells ^{7, 17, 18}. Recent studies have revealed that lymphatic endothelial cells are an *in vivo* source of S1P that is required for lymphocyte egress from lymph nodes and Peyer's patches ⁶. In contrast, neural crest-derived perivascular cells, a specialized type of vessel-ensheathing cells, provide S1P to promote thymic egress ⁵. The second is the receptor surface expression. S1P₁ can be internalized by its natural ligand S1P and upregulated in its absence, and this cyclical ligand-induced modulation of S1P₁ on circulating lymphocytes has been proposed to contribute to establishing the lymphoid organ transit time ⁸. Consistently, when the endogenous S1P₁ gene was replaced with a mutant form resistant to internalization, the T cells exhibited significantly delayed lymphopenia after S1P₁ agonist administration or disruption of the S1P gradient, indicating that surface residency of S1P₁ is a primary determinant of lymphocyte egress kinetics ¹⁹. Also, S1P₁ can interact with the transmembrane C-type lectin CD69 in a mutually antagonizing manner ²⁰. The third is transcriptional regulation of S1P₁ expression, with the transcription factor KLF2 serving as the primary factor to drive S1P₁ transcription in T cells ²¹.

Although the role of S1P₁ in T cell trafficking was initially revealed by the use of FTY720 ^{10, 11}, it remains controversial whether FTY720, after being phosphorylated into FTY720-P *in vivo*, acts as an agonist or a functional antagonist or both to regulate lymphocyte trafficking. Also, whether the critical effect of the drug is on lymphocytes themselves or endothelial cells is under debate ^{12, 22}. Moreover, despite rapid internalization, S1P₁ receptors treated by FTY720 retain persistent signaling activity for hours ²³. Finally, FTY720 possesses immunomodulatory activities independent of S1P receptors ²⁴. Whereas S1P might regulate T cell trafficking by acting on both lymphocytes and endothelial cells, an intrinsic requirement for S1P₁ in T cells is undisputable from the genetic evidence ^{12, 13}. S1P₁ also facilitates migration of B cells ^{25–27} and osteoclasts ²⁸. By contrast, trafficking of natural killer cells and dendritic cells (DCs) requires S1P₅ and S1P₃ respectively ^{29–31}. S1P₂ negative regulates macrophage migration and recruitment to sites of inflammation ³². These

studies illustrate that S1P has key roles in immune cell trafficking, a function that appears to be mediated exclusively by signaling through GPCRs.

Inflammatory and allergic responses mediated by the innate immune system

The innate and adaptive immune systems are two integral components of protective immunity. The innate immune system provides the first line of defense against invading pathogens. Innate immune responses are generally initiated by the engagement of pattern recognition receptors (PRRs) that bind highly conserved structures expressed by microorganisms, and can be further shaped by other receptor systems such as cytokine receptors. However, excessive or prolonged activation of the innate immune system can cause inflammation and severe immunopathology. Recent studies have identified essential functions of S1P and S1PRs in inflammation via modulating signaling of certain innate receptors (Figure 2).

Toll-like receptor (TLR) signaling and proinflammatory responses

Activation of TLRs, a prominent group of PRRs, results in a potent inflammatory response characterized by the release of proinflammatory cytokines, including tumor necrosis factor (TNF)- α , interleukin (IL)-1 and IL-6. Whereas this response is important for clearance of infectious organisms, exuberant production of these cytokines can lead to sepsis and death from septic shock. SphK1 was upregulated in stimulated human phagocytes and in peritoneal phagocytes of patients with severe sepsis³³. Blockade of SphK1 inhibited TLR-induced NF- κ B activation and production of proinflammatory cytokines. More importantly, mice treated with a specific SphK1 inhibitor were protected from sepsis. TLR-dependent nuclear factor (NF)- κ B activation required protein kinase C (PKC) δ , whose activation was enhanced by S1P, thus placing sequential activation of SphK1 and PKC δ as important steps for NF- κ B activation. Although the molecular detail of how SphK1 and S1P activate PKC remains to be established, these results have demonstrated a critical role for SphK1 in TLR signaling and established SphK1 as a new therapeutic target for sepsis³³.

TNF receptor (TNFR) dependent NF- κ B activation

TNF- α is a pivotal pro-inflammatory cytokine implicated in a number of inflammatory and autoimmune diseases. Activation of the TNFR results in the assembly of a multi-component signaling complex, comprised of adaptors such as TNFR type 1-associated DEATH domain protein (TRADD), TNFR-associated factor 2 (TRAF2), and receptor-interacting protein 1 (RIP1), that is essential for transducing signals to trigger downstream NF- κ B and MAP kinase pathways^{34, 35}. Activation of NF- κ B by TNF- α requires the assembly of polyubiquitination chains linked by lysine-63 on the substrate RIP1 to recruit the TAK1 and IKK complexes, and by lysine-48 on the substrate I κ B to mediate its proteasomal degradation³⁶. Although TRAF2 can function as a ubiquitin ligase, direct evidence that TRAF2 catalyzes RIP1 ubiquitination had been lacking. A recent study has shown that S1P produced by SphK1 is the missing cofactor for TRAF2 ubiquitin ligase activity³⁷. S1P specifically binds to TRAF2 at the amino-terminal RING domain and stimulates its ligase activity to catalyze lysine-63-linked polyubiquitination of RIP1, which in turn activates downstream NF- κ B signaling. These responses are mediated by intracellular S1P independently of its cell-surface GPCRs, indicating TRAF2 is a novel intracellular target of S1P. This elegant work establishes a new paradigm for the regulation of lysine-63-linked polyubiquitination, and highlights the key roles of SphK1 and its product S1P in the TNFR-dependent NF- κ B pathway³⁷. Considering the roles of SphK1 in both TNF- α production and TNFR signaling^{33, 37}, inhibition of SphK1 is a promising strategy for sepsis and other inflammatory diseases involving TNF- α .

Protease-activated receptor 1 (PAR1) signaling in coagulation and inflammation

Coagulation initiated by the cytokine-receptor family member known as tissue factor is a hallmark of systemic inflammatory response in bacterial sepsis. The mechanism coupling coagulation and inflammation has been recently shown to involve PAR1; PAR1 deficiency attenuated coagulation and inflammation and protected mice from sepsis-induced lethality³⁸. DCs promote systemic coagulation and are the primary cells at which coagulation and inflammation intersect within the lymphatic compartment. In DCs, PAR1 signaling results in the activation of SphK1 and production of S1P, which signals through S1P₃ in an autocrine/paracrine manner, a phenomenon known as “S1P inside-out signaling”³⁹. Loss of PAR1–S1P₃ signaling sequesters DCs and inflammation into draining lymph nodes, and attenuates dissemination of IL-1 β to the lungs. Thus, the SphK1–S1P–S1P₃ axis mediates and amplifies PAR1 signaling in DCs to couple systemic inflammation and coagulation in innate immune responses³⁸.

Fc ϵ RI-dependent allergic responses in mast cells

Mast cells play pivotal roles in immediate-type and inflammatory allergic reactions and are the primary cell type for causing asthma⁴⁰. Mast cells are potent producers of intracellular and secreted S1P via SphK activation, following activation of the high-affinity Fc receptor for IgE (Fc ϵ RI). Although SphK activation and S1P production are clearly required for Fc ϵ RI-dependent allergic responses in mast cells, studies using mice deficient in SphK1 or SphK2 have yielded conflicting conclusions on the importance of the selective SphK isoenzyme^{40, 41}. Whereas one study has demonstrated that SphK2 is required for Fc ϵ RI-mediated responses⁴⁰, another study has found normal mast cell responses in either SphK1^{-/-} or SphK2^{-/-} mice⁴¹. By contrast, results from siRNA-dependent silencing approaches have shown that SphK1 appears to have a dominant role in the generation of S1P in mast cells^{41, 42}. It is possible that both isoenzymes contribute to mast cell functions, and the overlap between them accounts for the differential phenotypes developed as a compensatory mechanism in genetically deficient mice⁴¹.

Mast cells express S1P₁ and S1P₂ receptors, and export of S1P after Fc ϵ RI stimulation results in the rapid transactivation of S1P₁ and S1P₂, which is another example of “S1P inside-out signaling”^{2, 39}. Although S1P₁ activation is important for cytoskeletal rearrangements and migration of mast cells, S1P₁ is dispensable for Fc ϵ RI-triggered degranulation. Instead, S1P₂, whose expression is upregulated by Fc ϵ RI cross-linking, is required for degranulation *in vitro*^{2, 39, 43}. In models of anaphylaxis (a severe allergic reaction mediated by mast cells), the S1P₂ antagonist JTE-013 or S1P₂ deficiency attenuated elevation of circulating histamine and the associated pulmonary edema in mice⁴³. However, in a separate study, S1P₂-deficient mice showed a delay in plasma histamine clearance and a poor recovery from anaphylaxis, which was ascribed to a mast cell-independent function for S1P₂⁴⁴. Therefore, although SphKs and S1P₂ are important regulators of mast cell responses, more defined systems such as tissue-specific or inducible knockout approaches are required to unambiguously determine their functions *in vivo*.

The vascular system and inflammation

S1P and S1PRs have potent effects on the vascular system that further impact inflammatory responses. Indeed, temporally and spatially regulated vascular leak is a central feature of inflammation. The importance of S1P in the vascular system has long been known⁴⁵, and mice deficient in S1P₁ or in both SphK1 and SphK2 were embryonically lethal due to profound defects in vascular development². Nonetheless, the sources of S1P that regulates endothelial barrier function *in vivo* during inflammation were revealed only recently⁴⁶. Mutant mice engineered to selectively lack S1P in plasma displayed increased vascular leak and impaired survival after inflammatory challenges. Such increased leak can be reversed by

transfusion with wild-type erythrocytes (which restored plasma S1P levels) and by acute treatment with an agonist for S1P₁. Therefore, S1P supplied to plasma from erythrocytes activates S1P₁ signaling in endothelial cells to maintain vascular integrity and to prevent vascular leak during inflammatory responses⁴⁶.

In summary, S1P, either acting intracellularly or through surface GPCRs, generally elicits proinflammatory responses by modulating diverse receptor signaling. Animal models with diminished activities of SphKs are protected from or showed attenuated responses to various inflammatory insults such as bacterial sepsis³³, inflammatory arthritis^{47, 48}, colitis⁴⁹, allergic asthma⁵⁰, and anaphylaxis⁴³. Therefore, pharmacological blockade of the S1P–S1PR axis is a promising approach to treat these inflammatory and allergic diseases. However, it is important to note that S1P and S1PRs also mediate protective effects during inflammation such as maintaining vascular integrity and recovery from anaphylaxis^{44, 46}. Care should be taken in the design of therapeutic approaches to target S1P to control excessive inflammation.

Lymphocyte differentiation: the interface between immunity and tolerance

Compared with innate immune responses, adaptive immunity is slow to develop and mediates protection only several days or more post-infection. CD4 helper T cells are central regulators of adaptive immune responses and can either promote immune activation or induce tolerance or non-responsiveness, the balance of which is crucial to elicit immune defense, as well as to prevent autoimmune diseases such as type 1 diabetes and lupus⁵¹. CD4 T cells orchestrate the decision between immunity and tolerance through their ability to differentiate into diverse effector and regulatory populations (Figure 3A). Some of these populations are determined when they emerge from the thymus, such as Foxp3⁺ natural regulatory T (nT_{reg}) cells and natural killer T cells (NKT cells). The bulk of CD4 T cells develop from the thymus as conventional or naïve T cells, but can further differentiate into separate lineages in the peripheral lymphoid organs. In response to antigen stimulation, naïve CD4 T cells proliferate and differentiate into T helper type 1 (Th1) cells, Th2 cells, and Th17 cells to exert specific effector functions⁵¹. Naïve precursors can also develop into antigen-specific T_{reg} cells, known as induced T_{reg} cells (iT_{reg}), which act in synergy with nT_{reg} cells to establish immune tolerance and counter-balance effector T cell functions. S1P₁ has recently been implicated in the regulation of T cell differentiation and immune responses (Figure 3A).

Differentiation and function of nT_{reg} cells

Foxp3-expressing nT_{reg} cells play a central role in the maintenance of immune tolerance and prevention of autoimmunity⁵². However, the potent nT_{reg}-mediated suppression could abrogate adaptive immune responses and render the host susceptible to infection and cancer. How are nT_{reg} development and activity controlled to establish protective immunity toward pathogens and tumors without pathological anti-self reactivity? My colleagues and I have recently shown that S1P₁ signaling delivers an intrinsic negative signal to restrain thymic generation, peripheral maintenance and suppressive activity of nT_{reg} cells⁵³. S1P₁ was expressed in both nT_{reg} and naïve T cells, but upon activation, its expression was gradually reduced in nT_{reg} cells as compared with a more pronounced downregulation in conventional T cells. Mice deficient in S1P₁ contained an expanded nT_{reg} cell population, whereas S1P₁-transgenic mice showed diminished numbers of thymic nT_{reg} cells. Moreover, S1P₁ negatively affected the suppressive function of nT_{reg} cells, and S1P₁-transgenic T_{reg} cells exhibited defective suppressive activity *in vivo* that contributed to the development of systemic autoimmunity. S1P₁ impeded the differentiation and suppressive activity of nT_{reg} cells by inducing the selective activation of the Akt-mTOR pathway. Notably, FTY720 has been shown to enhance the suppressive activity and numbers of nT_{reg} cells^{54, 55}, similar to

the effects observed in S1P₁-deficient nT_{reg} cells⁵³. Thus, FTY720 probably acts as a functional antagonist to down-modulate S1P₁ in nT_{reg} cells. Notably, the effects exerted by S1P₁ are intrinsic to the development and function of nT_{reg} cells, highlighting a key negative role of S1P₁ signaling in this T cell subset⁵³.

Reciprocal differentiation of iT_{reg} and Th1 cells

iT_{reg} cells are a second subset of T_{reg} cells that are preferentially generated in the mucosal environment in a process dependent upon transforming growth factor (TGF)- β stimulation⁵². Also, iT_{reg} cells generated *in vitro* represent a potent population for cellular immunotherapy of autoimmune diseases and transplant rejection. Consistent with a suppressive role for the development of nT_{reg} cells, S1P₁ also dampened the differentiation of iT_{reg} cells⁵⁶. In addition, treatment of T cells with FTY720 upregulated iT_{reg} cell generation *in vitro* and *in vivo*^{56, 57}. Moreover, S1P₁ signaling drove Th1 cell generation in a reciprocal manner. Mechanistically, S1P₁ signaled through mTOR (mammalian target of rapamycin) and antagonized TGF- β function by attenuating sustained Smad3 activity. Interestingly, TGF- β treatment upregulated the expression of SphKs in differentiating T cells, and experiments using SphK inhibitors indicated that S1P₁ function is dependent upon endogenous sphingosine kinase activity, suggesting for the first time the involvement of S1P inside-out signaling in T cell differentiation. These studies establish an S1P₁-mTOR axis that controls T cell lineage specification and immune homeostasis (Figure 3B)⁵⁶. Consistent with this observation, increased S1P₁ expression is associated with autoimmune diseases⁵⁸, and S1P enhances IFN- γ production by human T cells from patients with the autoimmune disease primary Sjögren's syndrome⁵⁸.

Differentiation of other T cell lineages

S1P₁ signaling has also been implicated in the regulation of Th2 and Th17 responses. T cells from transgenic mice expressing the human S1P₁ cDNA showed higher levels of IL-4 and IL-17^{59, 60}. The reason for the distinct phenotypes observed in the transgenic mice expressing the mouse and human S1P₁ genes is unclear⁵⁶, and whether this reflects species-specific functions of S1P₁ or a different context of immune responses remains to be established. Also, unlike the role of S1P₁ in T_{reg} and Th1 responses, the role of S1P₁ in Th2 and Th17 responses requires additional testing with the use of loss-of-function approaches. Interestingly, unlike S1P₁, S1P₄ mediates immunosuppressive effects of S1P by inhibiting proliferation and secretion of effector cytokines, while enhancing secretion of the suppressive cytokine IL-10⁶¹.

Therapeutic targeting of S1P and S1PRs

FTY720

FTY720 (fingolimod), 2-amino-2-propane-1,3-diol hydrochloride, is the first-in-class S1PR modulator (Table 1). As a pro-drug, FTY720 is phosphorylated *in vivo* by SphK2 to form the active moiety FTY720-phosphate, a structural analog of S1P that binds to four of the five S1PR subtypes. FTY720 had initially been considered as a promising immunosuppressant for transplant rejection, but recent clinical trials failed to demonstrate a significant advantage of FTY720 over standard care³⁹. Serious adverse events included bradycardia that was likely caused by the activity of FTY720 on cardiac S1P₃ receptors, because FTY720 failed to induce bradycardia in S1P₃-deficient mice⁶².

Since 2002, a large number of studies have reported that FTY720 is effective in models of autoimmune diseases, particularly experimental autoimmune encephalomyelitis (EAE), a model of human multiple sclerosis (MS)^{11, 63}. In 2010, the results of two phase III clinical trials for MS (each enrolling more than 1000 patients with relapsing-remitting MS) were

reported ^{64, 65}. In one study (FREEDOMS – “FTY720 Research Evaluating Effects of Daily Oral Therapy in Multiple Sclerosis”), MS patients received oral FTY720 at a dose of 0.5 mg or 1.25 mg daily or placebo. As compared with placebo, both doses of oral FTY720 ameliorated the relapse rate, the risk of disability progression, and CNS lesions measured on magnetic resonance imaging (MRI) ⁶⁴. In a second study (TRANSFORMS – “Trial Assessing Injectable Interferon versus FTY720 Oral in Relapsing–Remitting Multiple Sclerosis”), MS patients were randomized to receive either oral FTY720 or intramuscular interferon beta-1a, an established therapy for MS. This trial showed the superior efficacy of FTY720 with respect to relapse rates and MRI outcomes ⁶⁵. Adverse events associated with FTY720 included bradycardia and atrioventricular block, certain infections, increased liver enzyme levels, hypertension and macular edema. Because of these encouraging clinical results, FTY720 was approved by FDA in September 2010 to become the first oral therapy for relapsing MS.

Mechanism of action for FTY720 include effects on lymphocyte trafficking, in which the initial activation and eventual downregulation and degradation of S1P₁ prevent lymphocyte egress from lymphoid tissues, thereby reducing autoaggressive lymphocyte infiltration into sites of inflammation ⁶³. Within this context, it is interesting to note that FTY720 differentially affects the recirculation of lymphocyte subsets; in MS patients, FTY720 primarily reduced the numbers of CCR7⁺CD45RA⁺ naive T cells and of CCR7⁺CD45RA⁻ central memory T cells in blood, whereas CCR7⁻CD45RA⁻ and CCR7⁻CD45RA⁺ effector memory T cell subsets remained largely unaffected ⁶³. In addition, FTY720 exerts direct effects on T cell differentiation and function by enhancing the generation and function of T_{reg} cells and inhibiting the differentiation of proinflammatory Th1 cells ⁵⁴⁻⁵⁷. Moreover, because of its lipophilic nature, FTY720 crosses the blood-brain barrier, and down-modulates S1P₁ in neural cells/astrocytes to reduce astrogliosis, a phenomenon associated with neurodegeneration in MS. Additional effects might result from a modulation of S1P₃ in astrocytes and of S1P₁ and S1P₅ in oligodendrocytes ⁶³. Therefore, FTY720 has effects on lymphocyte trafficking, development and function of T cell subsets, and CNS cells, all of which may contribute to its immunosuppressive mechanisms.

S1P receptor selective modulators

Because FTY720-mediated immune regulation is mainly dependent upon its effects on S1P₁, there is a distinct advantage to develop next-generation S1P receptor modulators by targeting S1P₁ selectively. The S1P₁-selective agonist SEW2871 is structurally unrelated to S1P but is capable of activating multiple signals that are triggered by S1P. Both SEW2871 and S1P activate Erk, Akt, and Rac signaling pathways and induced S1P₁ internalization and recycling ⁶⁶. SEW2871 stimulates lymphocyte trafficking *in vitro* and induces lymphopenia in mice via a S1P₁-dependent mechanism ^{22, 62}. In animal models, SEW2871 ameliorates renal ischemia/reperfusion injury by inhibiting lymphocyte egress and reducing pro-inflammatory molecules ^{9, 39}.

Another S1P₁ receptor-selective agonist, KRP-203, has structural similarity to FTY720. In animal models of organ transplantation, KRP-203 prolonged skin and heart allograft survival and attenuated chronic rejection ⁶⁷. More recent preclinical studies using animal models for inflammatory and autoimmune diseases have revealed that KRP-203 treatment ameliorated the injury in concanavalin A-induced hepatitis, experimental autoimmune myocarditis, chronic colitis, and lupus pathogenesis ^{39, 68}. KRP-203 sequestered circulating lymphocytes into lymphoid tissues and inhibited Th1 proinflammatory cytokine release. Notably, because KRP-203 selectively targets S1P₁ (but not S1P₃), the use of KRP-203 can potentially avoid the adverse effects associated with use of FTY720 ³⁹.

Several antagonists that interfere with S1P receptor activation by the physiological agonist S1P have recently been described, including 3-amino-4-(3-hexylphenylamino)-4-oxobutylphosphonic acid (W146), VPC23019, and JTE-013⁶⁹⁻⁷¹. Some of these compounds are useful for basic research but might not have potential use in humans (Table 1)^{9, 39}.

Sphingosine kinase inhibitors and silencing

Among the first compounds to be discovered as a competitive inhibitor of sphingosine kinase activity were DL-*threo*-dihydrosphingosine (DHS, Saphingol) and *N,N*-dimethylsphingosine (DMS)³⁹. Later, five non-lipid selective inhibitors that block the ATP binding site of sphingosine kinases were identified (compounds SKI I–V). Among them, SKI-II, 2-(*p*-Hydroxyanilino)-4-(*p*-chlorophenyl) thiazole, showed the highest selectivity against SphKs and promoted tumor cell apoptosis and *in vivo* antitumor activity^{39, 72}. One limitation for these early-stage SphK inhibitors is the lack of selectivity toward SphK1 and SphK2. Despite their sequence and functional similarity, these two isoenzymes can exhibit distinct immunomodulatory roles: for example, *in vivo* knockdown of SphK1 and SphK2 ameliorated and exacerbated inflammatory arthritis, respectively⁷³. As a result, more selective inhibitors are being developed. The first isoenzyme-specific inhibitor, (2R,3S,4E)-*N*-methyl-5-(4'-pentylphenyl)-2-aminopent-4-ene-1,3-diol, designated SK1-I (BML-258), acts on SphK1 but not SphK2⁷⁴. SK1-I induced apoptosis in tumor cells and inhibited growth of various xenograft tumors^{74, 75}. Another SphK1-selective compound, 5c, was efficacious in bacterial sepsis^{33, 76}. A SphK2-selective inhibitor, 3-(4-chlorophenyl)-adamantane-1-carboxylic acid (pyridin-4-ylmethyl)amide (ABC294640), promoted tumor cell autophagy, leading to nonapoptotic cell death and delay of tumor growth *in vivo*^{77, 78}. Several natural products with inhibitory effects on SphK activity have also been described, although the specificity of these compounds remains unclear⁷².

Inhibition of SphKs has been shown to affect immune cell functions *in vitro* and animal models of immune-mediated diseases. DMS modulated functions of murine DCs³⁹. Both DMS and SKI had the effects to promote iT_{reg} generation and inhibit Th1 cell differentiation *in vitro*⁵⁶. More importantly, *in vivo* application of SphK inhibitors ameliorated murine disease models of allergic asthma, inflammatory arthritis and bacterial sepsis^{33, 48, 50, 73}. Some of these therapeutic effects were recapitulated by siRNA-mediated silencing of SphK1 *in vivo*, suggesting an important role for SphK1 to promote inflammation. Therefore, SphK1 inhibition represents a promising tool to treat inflammatory and immune-mediated diseases.

Other strategies to target S1P and S1PRs

Sabbadini and colleagues developed a specific monoclonal antibody to S1P, which reduced tumor progression and eliminated measurable tumors in xenograft and allograft models⁷⁹. The anti-S1P antibody also neutralized S1P-induced proliferation, the release of cytokines, and the ability of S1P to protect tumor cells from apoptosis in several tumor cell lines⁷⁹. A humanized form of the monoclonal S1P-specific antibody, LT1009, has entered Phase I clinical trials for cancer and age-related macular degeneration⁸⁰. The effects of anti-S1P antibody on inflammatory diseases have not been reported.

S1P lyase can be inhibited by treatment with the food colorant 2-acetyl-4-tetrahydroxybutylimidazole (THI), deoxypyridoxine, or the compound LX2931 of Lexicon Pharmaceuticals. This resulted in lymphopenia and ablation of the S1P gradient. The described effect of S1P lyase inhibition is similar to that observed after treatment with the FTY720, suggesting a shared mechanism at targeting the S1P–S1P₁ axis^{7, 81}.

Given the specific signaling pathways activated by S1P receptors, targeting the downstream pathways can show therapeutic effects. Recently, my colleagues and I found that S1P₁ signals through mTOR to control Th1 and iT_{reg} cell differentiation⁵⁶. Treatment of S1P₁-transgenic mice with the mTOR-selective inhibitor rapamycin restored the normal development of T cell subsets in these mice, similar as the treatment with FTY720. Notably, although both drugs are effective in a number of preclinical models of autoimmune diseases and graft rejection, FTY720 and rapamycin were thought to affect distinct molecular and cellular pathways in T cells, by acting on S1P₁ to induce lymph node sequestration and on mTOR to block cell cycle entry, respectively. Therefore, our results suggest that these two immunosuppressants target the same S1P₁-mTOR axis that likely contribute to their immunosuppressive functions *in vivo*.

Concluding remarks

The SphK-S1P-S1PR axis has been implicated to regulate immune responses by affecting lymphocyte trafficking, activating innate immune cells and inflammation, and directing T cell differentiation. S1P can be produced by the actions of SphKs to directly act on intracellular targets, independent of surface receptors; alternatively, S1P can be secreted out of the cells to activate surface receptors in an autocrine/paracrine manner, or activate upon other cells to regulate immune cell trafficking. Currently, drugs that target the ligand production and receptor function and signaling have been developed and are at various stages of preclinical and clinical development. The remarkable advancement of this area is facilitated by both genetic systems and pharmacological approaches, and further integration of these complementary tools will likely lead to even greater discoveries in this future.

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Figure 1. S1P synthesis and degradation

S1P is synthesized by the phosphorylation of the precursor sphingosine (Sph) mediated by sphingosine kinases (SphKs). S1P is irreversibly degraded by S1P lyase or dephosphorylated by S1P phosphatases. S1P regulates immune responses by signaling through plasma membrane G protein-coupled receptors (S1P₁–S1P₅) or acting on intracellular targets such as TRAF2. Cer, ceramide.

Figure 2. Function of SphK1 and S1P in innate immune receptor signaling

Engagement of TLR, TNFR and PAR1 results in the activation of SphK1, which catalyzes the formation of S1P from the precursor sphingosine (Sph). Once formed, S1P can bind and activate intracellular targets such as TRAF2. Alternatively, S1P can be secreted outside of the cells via transporter proteins and activate cell surface S1PRs in an autocrine or paracrine manner. The binding partners that link SphK1 to TLR and PAR1 signaling remain unknown (indicated by the blue-colored proteins).

Figure 3. S1P₁ functions in lymphocyte differentiation

(A). Naïve and natural T_{reg} (nT_{reg}) cells are generated in the thymus and migrate to peripheral tissues in a S1P₁-dependent manner. Thymic differentiation of nT_{reg} cells was inhibited by S1P₁. In response to antigen stimulation, naïve T cells can differentiate into iT_{reg} cells or Th1 that is reciprocally regulated by S1P₁ signaling. (B). Model for S1P-S1P₁-dependent regulation of reciprocal Th1 and iT_{reg} differentiation. TGF-β treatment induces the expression of SphKs and the initiation of S1P-S1P₁-mTOR axis, which feedback inhibits the TGF-β-Smad3 signaling, thereby modulating T cell lineage differentiation.

Table 1
Major compounds that target S1P and S1P receptors

Compound	Target specificity	Status of clinical and preclinical development
FTY720	Agonist/functional antagonist for S1P ₁ , S1P ₃ , S1P ₄ , S1P ₅	Approved by FDA for multiple sclerosis treatment (2010)
KRP-203	Agonist for S1P ₁	Phase I clinical trials for multiple sclerosis
SEW2871	Agonist for S1P ₁	Animal models of renal ischemia-reperfusion injury
W146	Antagonist for S1P ₁	N/A
VPC23019	Antagonist for S1P ₁ , S1P ₃	N/A
JTE-013	Antagonist for S1P ₂	N/A
DMS	Inhibitor for SphKs	Animal inflammatory models such as asthma and arthritis
SKI-II	Inhibitor for SphKs	Animal tumor models
SK1-I	Inhibitor for SphK1	Animal tumor models
5c	Inhibitor for SphK1	Animal models of bacterial sepsis
ABC294640	Inhibitor for SphK2	Animal tumor models
LT1009	Humanized antibody for S1P	Phase I clinical trials for cancer and macular degeneration
LX2931	Inhibitor for S1P lyase	Phase II clinical trials for rheumatoid arthritis