An update on the biology of sphingosine 1-phosphate receptors

Victoria A. Blaho and Timothy Hla*

Center for Vascular Biology, Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, 1300 York Ave., New York, NY 10065

* Corresponding author (email: tih2002@med.cornell.edu)
Abstract

Sphingosine 1-phosphate (S1P) is a membrane-derived lysophospholipid that acts primarily as an extracellular signaling molecule. Signals initiated by S1P are transduced by five G protein-coupled receptors (GPCR), named S1P$_{1-5}$ (1). Cellular and temporal expression of the S1P receptors (S1PR) determine their specific roles in various organ systems, but they are particularly critical for regulation of the cardiovascular, immune, and nervous systems, with the most well-known contributions of S1PR signaling being modulation of vascular barrier function, vascular tone, and regulation of lymphocyte trafficking. However, our knowledge of S1PR biology is rapidly increasing as they become attractive therapeutic targets in several diseases, such as chronic inflammatory pathologies, autoimmunity, and cancer. Understanding how the S1P receptors regulate interactions between biological systems will allow for greater efficacy in this novel therapeutic strategy as well as characterization of complex physiological networks. Because of the rapidly expanding body of research, this review will focus on the most recent advances in S1P receptors.

Introduction

Sphingosine 1-phosphate (2S-amino-1-(dihydrogen phosphate)-4E-octadecene-1,3R-diol) is a simple, membrane-derived lysophospholipid with regulatory roles in almost all facets of mammalian biology (2). Concentrations of S1P in blood and lymph plasmas are high, in the high nanomolar to low micromolar ranges, whereas S1P concentrations in tissues are kept low, creating an S1P gradient (3). S1P signals primarily through five highly-specific G protein-coupled receptors (GPCR), with nanomolar dissociation constants (4). Expression patterns of the five S1PRs vary in tissues and also during development and ageing. S1P$_1$, S1P$_2$, and S1P$_3$ are essentially ubiquitously expressed, whereas expression of S1P$_4$ and S1P$_5$ are highly restricted to distinct cell types (1).

Production of S1P can be initiated by external or internal signals, which leads to activation of the biosynthetic pathway beginning with metabolism of membrane
sphingomyelin (SM) to ceramide (Cer) by sphingomyelinases (SMase) (5, 6). Ceramide, an important signaling molecule itself, can be metabolized by ceramidase (CDase) to sphingosine (Sph) (7). Sph is then phosphorylated by one of two sphingosine kinases, Sphk1 or Sphk2, resulting in S1P genesis (8-10) (Figure 1).

Although there are proposed intracellular roles for S1P, it is often transported out of the cell where it can act in autocrine or paracrine manner on S1PR (11, 12). Transport out of the cell may occur via several transporters; however, the only bona fide transporter to date is Spns2, which is also capable of FTY720 export (13-22). Once outside of the cell, S1P can bind to two known carriers, albumin or Apolipoprotein M (ApoM) (6, 23, 24) (Figure 1). Approximately 35% of plasma S1P is bound to albumin and 65% to ApoM, which is found on a small percentage (~5%) of high-density lipoprotein (HDL) particles (24). This ApoM+HDL-bound S1P has been proposed as a primary contributor to the vasoprotective properties of HDL (25-27). How albumin or ApoM deliver S1P to specific S1PR has yet to be characterized.

**Agonists and antagonists**

There are several well-characterized agonists and antagonists of S1PR; however, most compounds have been directed toward modulating the activity of S1P₁. FTY720 (fingolimod/Gilenya; Novartis) is the prototypical S1PR agonist and was approved by the U.S. Food and Drug Administration as a first line oral therapy for relapsing-remitting multiple sclerosis (MS) (18, 28). Although FTY720 acts as an agonist at picomolar to nanomolar concentrations on S1P₁ and S1P₃-₅, it also acts as a functional antagonist for S1P₁ by inducing receptor endocytosis and degradation of this receptor (29-31). This promiscuity may be responsible for adverse affects, such as acute bradycardia (decreased heart rate) and hypertension, seen in fingolimod-treated patients (32, 33). Initial results from rodent studies indicated that FTY720-P activation of S1P₃ was responsible for both bradycardia and hypertension; however, treatment of humans with more selective agonists indicated that S1P₁ agonism was responsible for reduced heart rate, whereas S1P₃ signaling contributed to the development of hypertension (34-37). The divergent utilization of S1P₁ and S1P₃ in rodents versus primates for the regulation of these
coordinated physiological functions highlights the difficulties encountered upon extrapolation from rodent model-based characterization of S1PR function to human disease therapies.

SEW2871 is an S1P1-specific agonist that activates ERK1/2, AKT, and Rac signals at nanomolar concentrations and induces receptor internalization and recycling; however, it has a relatively short half-life in vivo (38). AUY954 is another commonly used S1P1-selective agonist with an EC50 of approximately 1nM, inducing phosphorylation of ERK and AKT (39). At high concentrations, AUY954 also has some activity on S1P5 (39). Conversely, W146 antagonizes AKT and ERK phosphorylation and is the only widely utilized S1P1-specific antagonist (40). Administration of W146 enhances vascular leakage and induced pulmonary edema (40, 41). VPC23019 is a useful in vitro tool as a dual S1P1/3 antagonist; however, poor stability and in vivo efficacy limit its use (42-44). The only known compound with activity at S1P2 is JTE-013, an antagonist with an IC50 of approximately 20 nM, which blocks S1P2 signaling through ROCK and PTEN (45, 46). The S1P2 specificity of JTE-013 has been called into question by several studies that indicate it may have activity at S1P4 as well as non-S1PR-mediated effects (44, 47-49).

**Vascular and lymphatic systems**

Many effects of S1P on the vasculature are due to expression of S1P1 by the endothelium. S1P1, originally named EDG1 (endothelial differentiation gene) was discovered during a search for immediate early genes regulating endothelial cell differentiation (50). Although S1pr1−/− embryos developed a vascular network, they died in utero at E12.5- E14.5 due to defective coverage of large vessels by pericytes and vascular smooth muscle cell (VSMC) (51, 52). Specifically, the aorta exhibited severe morphological abnormalities, endothelial hypersprouting, and altered VSMC recruitment and localization (Figure 2) (53, 54). The generation of inducible, cell-specific S1P1 knockout mice has clarified the roles of EC or VSMC S1P1 in the regulation of post-natal vascular development, maturation, and function. In the developing retinal vasculature, S1P1 expression is restricted to the EC and increases with vessel maturity, as the lowest levels
of expression are found at the vascular leading front (55). Post-natal deletion of EC S1pr1 did not affect mural cell recruitment or vessel coverage in the retina; however, angiogenic hypersprouting occurred, characterized by dilated vessels and increases in the number of branch points and tip cells. Induced over-expression of EC S1P1 suppressed vascular sprouting (55). Changes in the vascular architecture of EC S1pr1−/− were accompanied by increased vascular permeability, resulting from altered VE cadherin localization at endothelial cell-cell junctions (54, 55). These data confirmed numerous earlier, in vitro studies describing the necessity of EC S1P1 for the maintenance of vascular barrier function through adherens junction formation induced by activation of Rac after Gαi coupling to S1P1 (Figure 2)(56, 57).

Maintenance and formation of adherens junctions was dependent on S1P1 signaling initiated not only by ligand, but also by fluid shear stress (Figure 2). Examination of murine aortae found that areas of turbulent flow (the lesser curvature) had poor endothelial cell alignment and S1P1 relocalized from the EC surface to endocytic vesicles, whereas the in the descending aorta, an area of laminar flow, S1P1 and VE cadherin co-localized to the cell surface (55). Additionally, maintenance of vascular homeostasis by the endothelial glycocalyx, which is also susceptible to changes in flow dynamics, was dependent upon S1P1-induced inhibition of matrix metalloproteinase (58).

Mice with endothelium-specific deletion of S1P1 developed severe pathology in a model of renal ischemia/reperfusion injury, both in the kidneys and the liver, characterized by elevated plasma creatinine, ALT, and tissue necrosis (59). Conversely, of the five S1PR, S1P2 mRNA in the kidney was most increased upon renal ischemia/reperfusion, and mice deficient in S1P2 developed significantly less pathology compared to wild-type controls (60). When S1pr2−/− mice were treated with the S1P1 antagonist, W146, before ischemia/reperfusion, they were no longer protected from renal injury, suggesting that S1P1 and S1P2 in the renal vasculature endothelium play protective and injurious roles, respectively, in kidney injury and disease (60).
The pro-inflammatory tendency of S1P₂ is supported by in vitro studies suggesting a paracrine feedback loop involving EC TNFα induction of S1P₂ expression leading to activation of NF-kB and increases in intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (61). In vivo studies utilizing S1pr2⁻/⁻ mice and a model of acute inflammation, endotoxemia, further support the conclusion that S1P₂ is an important regulator of vascular activation and therefore, permeability (62). Induction of endotoxemia in mice lacking S1pr2 in the stroma and not in the bone marrow compartment resulted in decreased vascular permeability and VCAM-1 and ICAM-1 expression, and more rapid resolution (62). Similarly, in vitro, S1P₂ actively suppressed angiogenic sprouting through leukemia associated RhoGEF (LARG) activation of RhoC (63). These recent studies reaffirm the conclusion that an antagonistic relationship exists between S1P₁ and S1P₂ in the vascular endothelium during tissue injury and disease.

Lymphatic endothelium also expresses S1PR, although more interest has focused on the role it may play in S1P metabolism (21) (64). Examination of murine iliac collecting lymph vessels demonstrated that while S1P does not induce nitric oxide or prostaglandin release, signaling via S1P₂ regulates tonic contractility of lymph vessels, as shown using S1P₂ inhibition by JTE013 (65).

**Immune System**

S1P receptors regulate many aspects of immune cell biology. The best known is the regulation by S1P₁ of lymphocyte migration out of the secondary lymphoid organs into the blood and lymph (Figure 3) (66). Regulation of migration occurs by S1P₁ counteracting the retention signals provided by the chemokine receptor CCR7 (67). However, this is not the only role for S1P₁ in lymphocytes, and roles for the other four S1PR in the immune system have recently been revealed.
The contribution of S1PRs to regulation of the immune response has been studied extensively in the context of experimental autoimmune encephalomyelitis (EAE), the most commonly used animal model of multiple sclerosis (MS) (68). Although EAE/MS are considered to be primarily diseases of the immune system, the role of S1PR on neural cells is also gaining an appreciation and will be discussed later. FTY720 is a sphingosine analogue that is phosphorylated, acts on S1P1, 3-5, and was the first FDA approved oral therapy for MS (69). The presumed mechanism of action has been the trapping of autoreactive T and B cells in the lymphoid organs, away from the central nervous system (70) (71). However, T cell S1P1 may also regulate the activation and differentiation status of these immune cells. Deletion of T cell S1P1 significantly suppresses the ability of these cells to be polarized to Th17 in vitro (72). Conversely, when EAE was induced in mice expressing an internalization-defective S1P1 (S5A), this significantly increased polarization of T cells to the Th17 phenotype resulting in increased disease pathology and immune cell infiltration into the CNS (72).

S1P1 is also expressed on CD4 T cells isolated from human rheumatoid arthritis (RA) patients (73). S1P enhances TNFα-induced expression of receptor activator of nuclear factor kB (RANK) ligand by these cells, an effect replicated in a synovial cell-like cell line, MH7 (73). In collagen-induced models of RA, an S1P1-specific antagonist prevented or ameliorated disease by up-regulating lymphocyte CD69 expression, which down-regulates S1P1 surface expression, blocking thymic egress (73, 74) (75).

S1P1 also affect other populations of T cells, such as T regulatory cells (T_{reg}), which, as the name implies, play an important role in controlling immune responses, and T memory cells (76, 77). S1P1 suppresses T_{reg} development via the AKT/mTOR pathway and affects their migration from the thymus and out of the periphery by counteracting CCR7 retention signals, similar to the mechanism regulating the egress of effector T cells from lymph nodes (77) (78) (67). S1P1 signals may also modulate nuclear localization of the transcription factor FOXP3, which is necessary for T_{reg} generation (78). In human patients, FTY720 significantly increased the number of T regulatory cells while decreasing central memory T cells (79). In a specific subset of T memory cells, non-
lymphoid resident memory cells (T\textsubscript{RM}), cytokines that induce the T\textsubscript{RM} phenotype also down-regulate the transcription factor KLF2 and its target gene, \textit{S1pr1} (80). Subsequently, T\textsubscript{RM} are unable to sense S1P in circulation and are maintained in the periphery.

Although S1P\textsubscript{1} has been the focus of much research, not much is known of the roles of the other S1PR. In CD8 effector T cells, S1P\textsubscript{4} may influence their trafficking to LN, although it appears not to be a primary regulator (81). \textit{S1pr4}\textsuperscript{-/-} mice have decreased Th17 T cell polarization; however, reduced Th17 differentiation is likely T cell extrinsic and primarily due to functions of S1P\textsubscript{4} in dendritic cells (81).

S1PR expression choreographs many aspects of B cell subset localization within lymphoid organs, thereby affecting their functionality; however, there are some direct effects of S1P signaling on B cell survival (Figure 3) (82, 83). While S1P\textsubscript{1} has some regulatory functions in B cells, it appears that S1P\textsubscript{2} has a greater impact on these cells. Aged \textit{S1pr2}\textsuperscript{-/-} mice develop diffuse large B cell lymphoma (DLBCL), characterized by increased germinal center (GC) B cells and spontaneous GC formation, which correlates with an approximate 26% mutation incidence for \textit{S1PR2} in human DLBCL (84). Under homeostatic conditions, S1P\textsubscript{2} signals via G\textsubscript{12/13} to activate Rho/ROCK, antagonizing activation of AKT and pro-survival signals (82). B cell S1P\textsubscript{2} also regulates follicular positioning of B cells by directing their clustering to GC in response to follicular dendritic cell (DC)-derived S1P (82, 85). The ability of follicular B cells to exit the follicle was, however, dependent upon S1P\textsubscript{1} expression (86). Additionally, trafficking of marginal zone (MZ) B cells between the MZ and the follicle was regulated by S1P\textsubscript{1}, which maintains these cells in the MZ in order for them to capture blood-borne antigens (86-88).

Studies of non-obese diabetic (NOD) mice have shown that up-regulation of S1P\textsubscript{3} by MZ B cells and their T2 MZ precursors may also play a role in enhancing MZ retention in these mice (89, 90). S1P\textsubscript{3} has already been shown to regulate B cell migration \textit{in vitro} but not \textit{in vivo} in wild type mice (83, 87). However, it may be important for positioning of
immature B cells and their progenitors within the bone marrow, whereas S1P$_1$ participates in directing their migration from the bone marrow parenchyma into sinusoids and subsequently into circulation (83).

Natural killer cells (NK) are considered innate lymphoid cells that develop from lymphoid progenitors in the BM but do not undergo genomic changes that occur in the B or T cell receptor genes (91, 92). They are important for anti-tumor immunity and are prolific producers of interferon γ (IFNγ)(92). Mouse NK cells have low levels of transcript for S1pr1, S1pr2, and S1pr4 and high S1pr5 mRNA levels (Figure 3) (93, 94). S1P$_5$ normally antagonizes NK CXCR4 bone marrow retention signals, and S1pr5$^{-/-}$ mice have decreased numbers of NK cells in the periphery and increased numbers in lymph nodes and BM due to defective migration (93, 95). This phenotype is also observed in the mouse model of Niemann Pick disease, type C (NPC), a lysosomal storage disorder presenting as an accumulation of cholesterol and sphingolipids in the lysosome and decreased concentrations of circulating S1P in human patients (96, 97). Studies utilizing FTY720 indicated that S1P$_1$ also contributes to NK migration from LN to lymph, but the contribution is relatively minor compared to that of S1P$_5$, which is not subject to CD69 regulation (75, 94). Decidual NK cells (dNK) are a specialized NK subset that regulates trophoblast invasion during early pregnancy by secreting pro-angiogenic and growth factors, including vascular endothelial growth factor (VEGF) (98). S1P$_1$ and S1P$_5$ are increased in human dNK compared to circulating NK, and S1P$_5$ expression decreases after the first trimester (99). FTY720 treatment decreased dNK S1P$_5$ expression, VEGF production, and trophoblast invasion in vitro (99).

Macrophages are important sentinel cells that develop from monocytes to fight infection and repair damaged tissue (100). S1PR expressed by monocytes and macrophages regulate their migration and activation, and the receptors responsible are cell subtype- and situation-specific (Figure 4). In general, S1P$_1$ and S1P$_3$ appear to induce migration toward S1P, whereas S1P$_2$ expression repulses macrophages from S1P (101) (102). S1pr2$^{-/-}$ mice on a pro-atherogenic genetic background (Apoe$^{-/-}$) developed significantly less atherosclerosis, accompanied by decreased macrophage and monocyte retention in
atherosclerotic plaques, indicating effects on migration, tissue retention, and activation (103). In comparison, S1pr3\textsuperscript{-/-} mice on the same Apoe\textsuperscript{-/-} background do not have altered development of atherosclerosis, but do have decreased monocytes and macrophages with atherosclerotic lesions (101). In wild-type mice, treatment with FTY720 results in decreased circulating monocytes; however, use of the S1P\textsubscript{1/4/5} agonist, BAF312 yielded similar results, both at homeostasis and during EAE, indicating that S1P\textsubscript{3} is not the sole regulator of monocyte circulation (104). This could be a cell subtype specific effect, or dependent on environment, as local administration of FTY720 appeared to enhance recruitment of anti-inflammatory, pro-angiogenic monocytes (105). This supports an earlier report that macrophage S1P\textsubscript{3} induces a pro-regenerative phenotype in a model of renal ischemia/reperfusion (106).

A report utilizing the zymosan peritonitis model proposed that the resulting apoptotic neutrophils induced S1P\textsubscript{1} expression on recruited macrophages and that S1P\textsubscript{1} is necessary for emigration from the inflamed peritoneum, but has no role in efferocytosis or activation (107). S1P\textsubscript{2} on alveolar macrophages (AM) may regulate their phagocytic capacity, as S1pr2\textsuperscript{-/-} AM displayed decreased phagocytosis of the fungus Cryptococcus neoformans due to decreased expression of Fc receptors necessary for phagocytosis of antibody-opsonized fungus (108).

Neutrophils are the first immune cell line of defense and can shape the immune response (109). Neutrophils express mRNA for all S1PR; however, the level of expression and the ability of S1P to affect changes in their responses depends upon their activation status (Figure 4) (110). More recently, it was reported that S1P lyase (Sgpl) \textsuperscript{-/-} mice are unable to degrade S1P and have neutrophilia (111). Although S1P\textsubscript{4} deficiency in Sgpl knockouts resulted in circulating neutrophil numbers that were close to WT, S1P\textsubscript{4} was not specifically deleted in neutrophils, raising the possibility that multiple cell types were responsible for the effect. Specific deletion of neutrophil S1P\textsubscript{1} did not normalize neutrophil numbers in Sgpl\textsuperscript{-/-} mice. However, in rat models of hyperalgesia dependent upon neutrophil infiltration, S1P\textsubscript{1} was necessary for neutrophil recruitment (112).
Specific S1P₁ antagonism blocked neutrophil infiltration, whereas agonism increased sensitivity.

Eosinophils (Eos) and mast cells (MC) are both involved in anti-parasite immune responses and allergic immunity (113). Eos from mice over-expressing IL-5, an eosinophil growth factor, express high levels of S1P₃ and demonstrate increased chemotactic responses to S1P in vitro (Figure 4) (114). In a model of allergic rhinitis, FTY720 treatment significantly decreased the numbers of infiltrating MC and Eos, resulting in resolution (115). In vitro, FTY720 induced MC apoptosis in a dose-dependent manner (115). Similar to lymphocytes, S1P₁ regulates MC migration toward antigen, whereas S1P₂ regulates their activation status upon FcεRI ligation, inducing degranulation and CCL2 secretion (116).

Dendritic cells (DC) are professional antigen presenting cells and as such, are required for proper induction and direction of the acquired immune response (117). Both human and mouse DC express mRNA for S1P₁-5 and exhibit varied responses to S1P stimulation in vitro and in vivo (Figure 4) (118, 119) (120). Langerhans cell, skin resident DC, require S1P₁ for migration to LN, whereas kidney resident DC require S1P₃ for maturation in ischemia/reperfusion (121, 122). This is also the case in models of sepsis, where DC S1P₃ is required for IL-1β production (123). In EAE, although S1P₁ agonism decreased disease pathology, it did not affect entry into the CNS of a subset of DC (plasmacytoid DC (pDC)). However, pDC in the CNS were necessary for the efficacy of S1P₁ agonist treatment (124).

S1P₄ was cloned from mature human DC, yet not much is known about the role this receptor plays in these cells (125). In models of autoimmune disease, Th2-type immune responses, such as allergic airway inflammation and cutaneous hypersensitivity, S1pr4⁻/⁻ mice had increased pathology and up to 50% increase in DC in draining LN after topical antigen application (81). This implies that S1P₄ may antagonize S1P₁ in DC, regulating their ability to migrate from the periphery after antigen uptake.
Nervous System

Neural progenitors express mRNA for S1P$_{1-5}$ and respond to S1P stimulation with induction of Ca$^{2+}$ mobilization (Figure 5) (126). S1P regulates embryonic nervous system development, as the neuroepithelial layers of the developing telencephalon in S1pr1$^{-/-}$ embryos have significantly increased apoptosis and decreased mitosis (127). S1P$_2$ may also play a role in regulating neural progenitors, as post-ischemic administration of the S1P$_2$ antagonist JTE-013 or short hairpin RNA against S1P$_2$ significantly increased progenitor migration to the ischemic region (128). This indicates that S1P$_2$ may repel neural progenitors from areas of high S1P concentration in the same manner as it regulates macrophage migration (102). Indirectly, S1P signaling on astrocytes affects neural progenitor by increasing lamin production, thereby encouraging maturation and neurite outgrowth by progenitors (129). Interestingly, neural stem cells were protected from radiation-induced apoptosis by nanomolar FTY720 treatment in vitro, although it is unknown which receptor is involved in this protection (130).

Although analyses of entire mouse dorsal root ganglion found that S1P$_3$ was the most highly expressed S1PR, single cell mRNA analysis of individual neurons found that S1P$_1$ was most highly expressed, regardless of neuronal subtype, indicating that high expression of S1P$_3$ occurs in ganglion cell types other than neurons (Figure 5) (131, 132). One group found that pain responses induced by intradermal S1P injection or models of post-operative pain were significantly decreased in S1pr3$^{-/-}$ mice, whereas minimal differences were seen in S1pr1$^{-/-}$ mice (131); however, another group found that mice lacking S1P$_1$ specifically in nociceptor neurons were protected from S1P-induced pain (133). Finally, in the murine model of the neurodevelopmental disease Rett syndrome, FTY720 or S1P$_1$-specific agonist SEW2871 in vivo treatment increased neuron production of brain-derived neurotrophic factor (BDNF) and decreased neurological symptoms (134).

Oligodendrocytes are the myelinating cells of the CNS and the primary cell type affected in MS and in the mouse EAE model (135). Process retraction, Rho/ROCK-mediated inhibition of immature oligodentrocyte precursor migration, and Gi/AKT-mediated
survival in mature oligodendrocytes occurs via S1P5 (Figure 5) (136, 137). Ex vivo studies using cerebellar slice cultures indicated that S1PR agonism, particularly S1P1, could prevent or reverse demyelination, explaining the ability of FTY720 to induce remyelination and process extension in the same system (138, 139). Data from a different *in vitro* system, myelinated neurospheres, indicated that FTY720 decreased microglial activation and oligodenrocyte apoptosis, and induced remyelination primarily by S1P5 agonism (140). An *in vivo* study provides conflicting evidence to these *in vitro* studies, reporting no effects on myelin repair with FTY720 treatment; however, the models of demyelination utilized in both the *in vitro* and *in vivo* studies were induced chemically and are meant to exclude possible effects of immune or vascular cells (141). As such, they cannot model complex neuroinflammatory disease and care must therefore be taken when attempting to extrapolate results to *in vivo* disease, such as EAE or MS.

The resident immune cells of CNS, microglia, express all S1PR (142). *In vitro* studies indicated that FTY720 down-regulated production of pro-inflammatory molecules by microglia while increasing neurotrophic factor production, resulting in an overall neuroprotective phenotype (142). FTY720 also inhibited secretory vesicle mobility and exocytic release by astroglia, thus inhibiting the release of proinflammatory mediators by this cell type, as well (143). Astrocytic gliosis also occurs in EAE and MS (Figure 5) (71). *In vitro* treatment of a human astrocyte cell line with FTY720 suppressed S1P-induced production of pro-inflammatory cytokines (144). *In vivo*, specific deletion of astrocyte S1P1 resulted in decreased EAE pathology and a loss of FTY720 efficacy, indicating that the primary target of FTY720 during EAE was S1P1 specifically on astrocytes (145). Additionally, in a model of spinal cord injury, FTY720 affected the later stages of vascular permeability and astrogliosis, partially through agonism of S1P1 (146). Another target of FTY720, S1P3, was also found on reactive astrocytes in human MS lesions and up-regulated by LPS stimulation of astrocytes *in vitro*, although it is unknown if expression of S1P3 is protective or pathogenic in the context of MS/EAE (147). Mice deficient in the one S1PR not targeted by FTY720, S1P2, are prone to seizures resulting in 40% mortality and have enhanced hippocampal gliosis accompanied by behavioral defects (148). Importantly, MS patients treated with fingolimod show reduced brain
volume loss and lesional activity, suggesting the importance of S1PR pathways in neuroprotection (149-151).

The blood brain barrier (BBB) forms through unique interactions between brain endothelial cells, astrocyte foot-processes, and pericytes, and regulates interactions between the immune and nervous systems (152). Alterations in the BBB are implicated or present in numerous neurological diseases, including MS, stroke, and dementias (153). S1P5 was highly expressed by human brain capillary endothelial cells, and antagonism of S1P5 in an in vitro model of BBB decreased vascular permeability and monocytic transmigration (154). Studies of FTY720 treatment in the context of transient cerebral ischemia and reperfusion have demonstrated neuroprotection in mouse and rat models; however, these effects may be due to effects on interactions between the neurovasculature and immune cells (155, 156). FTY720 treatment reduced brain edema as well as expression of the vascular adhesion molecule ICAM-1, resulting in decreased neutrophil infiltration (155). Additionally, when transient cerebral ischemia was induced in lymphocyte-deficient Rag1−/− mice, the protective effect of FTY720 was lost, further implying that FTY720-mediated protection is due to effects on the neurovasculature and its interactions with immune cells (156). Conversely, a study utilizing a model of permanent cerebral ischemia demonstrated no effect on pathology with FTY720 treatment, whereas another group demonstrated efficacy after delaying FTY720 treatment for 3 days after photothrombosis induction, with increased functional capacity and decreased astrogliosis (157, 158). Thus, protection by FTY720 may be dependent on method of ischemia induction and temporal regulation of cell activation and recruitment.

**Involvement of S1PRs in cancer and oncogenesis**

S1P receptors have also been implicated in cancer pathogenesis, playing roles in tumor maintenance similar to their roles in maintenance of homeostasis, such as modulation of survival and proliferation (159-161). Wild-type hamster lung fibroblasts were protected from nutrient deprivation-induced apoptosis by expression of S1P1, which induced the
anti-apoptotic protein McI1 via the P13K and PKC pathways (162). Lung adenocarcinoma cell lines respond to S1P with increased proliferation and invasion through S1P₃-mediated expression of epidermal growth factor receptor (EGFR) (163).

ER⁺ breast cancer cells also responded to S1P via S1P₃ to coordinately regulate EGFR localization and signaling (164). High expression of S1P₁ or S1P₃ by ER⁺ breast cancer cells correlated with poor prognosis and high S1P₁ expression induced decreased expression of pro-apoptotic markers (165, 166). In estrogen receptor negative (ER⁻) breast cancer cells, S1P₄ expression activated the ERK1/2 pathway and correlated with poor prognosis (167). In vitro, several breast cancer cell lines respond to S1P or S1P₁ agonist SEW2871 with increased proliferation (168).

Another malignancy that S1P signaling may play a prominent role in is colonic inflammation and the resultant cancer (169). In a model of ulcerative colitis, considered a possible precursor for colon cancer, increased colonic bleeding and mortality resulted from S1P₁ deletion (170). In a model of colitis-associated cancer, S1P₁ signaling was necessary for persistent activation of NF-kB and STAT3 transcription factors needed for maintaining the chronic inflammatory state and could be blocked by FTY720 treatment (171). In human colon cancer cells, expression of the chemotherapeutic resistance and cancer stem cell marker CD44 was regulated by S1P₂-induced ERK phosphorylation (172). Interestingly, FTY720 treatment impaired the mucosal immune response to the extracellular bacterium, Citrobacter rodentium, including decreased DC numbers, as well as macrophages and T cells in the colon, while increasing bacterial burden (173). These data suggest that FTY720 or other S1PR modulators could be beneficial or detrimental, depending upon how they influence the immune response.

In prostate adenocarcinoma, Sphk1-derived S1P activated AKT pro-survival pathways through activation of S1P₂ (174). AKT and BAD pro-survival pathways were also reduced by FTY720 administration to neuroblastoma cells in vitro and an in vivo xenograft model, resulting in decreased cancer cell viability (175).
S1PR expression in several hematological malignancies has also been described, including S1P₁ expression by classical Hodgkin’s lymphoma (CHL) cells, B cell chronic lymphocytic leukemia (B-CLL), activated B cell-like diffuse large B cell lymphoma (ABC-DLBCL) (176-178). Chronic myeloid leukemia (CML) cells expressed S1P₂, which resulted in increased stability of the BCR-Abl1 fusion protein and subsequently, increased proliferation (179). Expression of S1PR by blood cancer cells may directly regulate their survival or by controlling the localization of cells within permissive environments such as the lymph nodes.

**Concluding Remarks**

S1PR are gaining appreciation as powerful modulators of homeostasis and pathogenesis. In all biological systems, S1PR play some role in regulating cell survival, migration, phenotype, activation status, and proliferation. In the current review, we have attempted to summarize the most recent advances in the field of S1PR biology and to provide novel insights into the biological responses regulated. As more cell-specific animal models of gene deletion or over-expression are created, and agonists and antagonists with greater S1PR subtype specificity are developed, further studies with such tools will clarify the contributions of specific S1PR in each physiological or pathological context. This is especially true of the less explored members of the S1PR family, S1P₄ and S1P₅. Additionally, we anticipate that the development of more compounds for clinical use will expand our understanding of the complex signaling networks regulated by S1PR and their role in human homeostasis and disease.

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**Figure Legends**

**Figure 1.** Synthesis and export of sphingosine 1-phosphate (S1P). S1P synthesis primarily begins with metabolism of membrane sphingomyelin. Once synthesized, S1P
can be irreversibly degraded to phosphoethanolamine and hexadecenal by S1P lyase, or actively transported out of the cell. Once outside of the cell, S1P is found bound to ApoM or albumin. Abbreviations: Spns2: spinster 2; ABC: ATP-binding cassette; ApoM: apolipoprotein M.

Figure 2. Expression of S1PR and responses by endothelial cells Endothelial cells express S1P1, S1P2, and S1P3 protein. Endothelial cells may express different S1PR depending on activation status. The subcellular localization of heterotrimeric G proteins are not meant to be on the plasma membrane.

Figure 3. Expression of S1PR and responses by cells of the acquired immune system. T cells express S1P1 and S1P4, B cells express S1P1, S1P2, S1P3, and S1P4, and natural killer (NK) cells express S1P1 and S1P3. Cells do not necessarily express all of the illustrated S1PR at one time, but may have differential expression during different stages of maturation or activation. The subcellular localization of heterotrimeric G proteins are not meant to be on the plasma membrane.

Figure 4. Expression of S1PR and responses by cells of the innate immune system. Monocytes and/or macrophages express S1P1-4, neutrophils express S1P1, S1P3, and S1P4, eosinophils and mast cells express all S1PR, and dendritic cells express S1P1, S1P3, and S1P4. Cells do not necessarily express all of the illustrated S1PR at one time, but may have differential expression during different stages of maturation or activation. The subcellular localization of heterotrimeric G proteins are not meant to be on the plasma membrane.

Figure 5. Expression of S1PR and responses by neural cells. Neural progenitors express S1P1 and S1P2, neurons express S1P1 and S1P3, oligodendrocytes express S1P1 and S1P5, and astrocytes express S1P1 and S1P2. S1P1 couples exclusively to Gαi. S1P2 and S1P3 can couple to Gαi, Gα12/13, or Gαq, and S1P5 can couple to Gαi or Gα12/13. Cells do not necessarily express all of the illustrated S1PR at one time, but may have differential expression during different stages of maturation or activation. The subcellular localization of heterotrimeric G proteins are not meant to be on the plasma membrane.
motility
migration
vascular barrier
vasodilation
proliferation
survival
S1P1
S1P2
S1P3
adhesion molecules
proliferation survival
motility migration
shear sensing
vasodilation
vascular barrier
endotoxin-induced vascular leak
heart rate (human)
heart rate (mouse)

Figure 2
Figure 3

- **T cell**
  - Chemotaxis
  - Lymph node egress
  - Th17 polarization
  - Th1

- **NK cell**
  - Decidual angiogenesis
  - Bone marrow egress

- **B cell**
  - S1P1
  - MZ retention
  - Bone marrow positioning
  - Lymph node & bone marrow egress

- **S1pr2**
- **S1pr3**
- **S1pr5**

- **S1P1**
- **S1P2**
- **S1P3**
- **S1P4**

- mRNA expression:
  - S1pr1
  - S1pr2
  - S1pr3
  - S1pr4
Figure 4

- **S1P1**
  - Migration
  - Chemotaxis
  - Pro-atherogenic mRNA
  - Macrophage/Monocyte

- **S1P2**
  - Migration
  - Chemotaxis
  - Pro-regenerative mRNA

- **S1P3**
  - Migration
  - Pro-atherogenic mRNA

- **S1P4**
  - Migration

- **S1P5**
  - Migration

**Neutrophil**

**Eosinophils**

**Mast cells**

**Dendritic cells**

- **Th17 polarization**
  - Migration to lymph node

- **Migration**
  - Endocytosis

**FcɛRI-dependent activation**

**mRNA S1pr2 S1pr5**

**mRNA S1pr5**

**Pro-inflammatory cytokines**

**Tissue infiltration**

**Migration**

**Survival**

**Pro-inflammatory cytokines**

**Pro-regenerative survival**

**Pro-infiltration**

**Pro-inflammatory**

**Pro-inflammatory cytokines**

**Pro-infiltration**

**Migration**

**Survival**

**Pro-inflammatory cytokines**

**Pro-infiltration**

**Migration**

**Survival**

**Pro-inflammatory cytokines**

**Pro-infiltration**