thematic review

Thematic Review Series: Lysophospholipids and their Receptors

An update on the biology of sphingosine 1-phosphate receptors

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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, named $S1P_{1–5}$. Cellular and temporal expression of the S1P receptors (S1PRs) 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 S1PRs 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 S1PRs.—Blaho, V. A., and T. Hla. **An update on the biology of sphingosine 1-phosphate receptors. *J. Lipid Res.* 2014.** 55: **1596–1608.**

Supplementary key words immunity • endothelium • vascular permeability • nervous system • migration • activation • immune cells • nervous system

Sphingosine 1-phosphate [2*S*-amino-1-(dihydrogen phosphate)-4E-octadecene-1,3*R*-diol] (S1P) is a simple membrane-derived lysophospholipid with regulatory roles in almost all facets of mammalian biology (1). 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 (2). S1P signals through five highly-specific G protein-coupled receptors with nanomolar dissociation constants (3, 4). Expression patterns of the five S1P receptors (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 (4).

Production of S1P can be initiated by external or internal signals, which lead to activation of the biosynthetic pathway beginning with metabolism of membrane SM to ceramide by SMases (5, 6). Ceramide, an important signaling molecule itself, can be metabolized by ceramidase to sphingosine (Sph) (7). Sph is then phosphorylated by one of two Sph kinases (Sphks), Sphk1 or Sphk2, resulting in S1P genesis (8–10) (**Fig. 1**).

Although there are proposed intracellular roles for S1P, it is often transported out of the cell where it can act in an autocrine or paracrine manner on S1PRs (11, 12). Transport out of the cell may occur via several transporters; however, the only bona fide transporter to date is spinster 2, which is also capable of FTY720 (fingolimod/Gilenya; Novartis) export (13–22). Once outside of the cell, S1P can bind to two known carriers, albumin or ApoM (6, 23, 24) (Fig. 1). Approximately 35% of plasma S1P is bound to albumin and 65% to ApoM, which is found on a small percentage (~5%) of HDL particles (24). This ApoM+HDL-bound S1P has been proposed as a primary contributor to the vasoprotective properties of HDLs (25–27). How albumin or ApoM deliver S1P to specific S1PRs has yet to be characterized.

AGONISTS AND ANTAGONISTS

There are several well-characterized agonists and antagonists of S1PRs; however, most compounds have been di-

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Abbreviations: BBB, blood brain barrier; BM, bone marrow; DC, dendritic cell; DLBCL, diffuse large B cell lymphoma; dNK, decidual natural killer; EAE, experimental autoimmune encephalomyelitis; EC, endothelial cell; ER, estrogen receptor; GC, germinal center; ICAM, intracellular adhesion molecule; LN, lymph node; MC, mast cell; MS, multiple sclerosis; MZ, marginal zone; NK, natural killer; ROCK, Rho-associated protein kinase; Sgpl, sphingosine 1-phosphate lyase; S1P, sphingosine 1-phosphate; Sph, sphingosine; Sphk, sphingosine kinase; Sph, sphingosine; S1PR, sphingosine 1-phosphate receptor; Th, T helper cell; T_{reg}, T regulatory cell; T_{RM}, T resident memory cell; VSMC, vascular smooth muscle cell.

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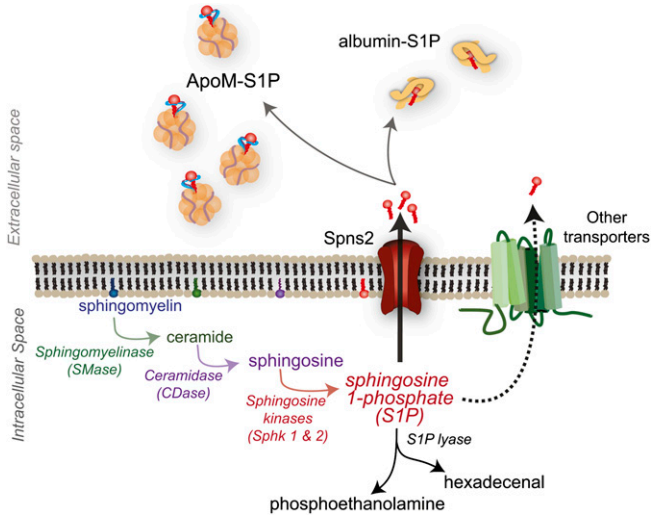

Fig. 1. Synthesis and export of S1P. S1P synthesis primarily begins with metabolism of membrane SM. Once synthesized, S1P can be irreversibly degraded to phosphoethanolamine and hexadecenal by S1P lyase, or actively transported out of the cell. Once outside of the cell, S1P is found bound to ApoM or albumin. Spns2, spinster 2.

rected toward modulating the activity of $S1P_1$. FTY720 is the prototypical S1PR agonist and was approved by the US 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 $S1P_1$ and $S1P_{3–5}$, it also acts as a functional antagonist for $S1P_1$ 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 phosphate activation of $S1P_3$ was responsible for both bradycardia and hypertension; however, treatment of humans with more selective agonists indicated that $S1P_1$ agonism was responsible for reduced heart rate, whereas $S1P_3$ signaling contributed to the development of hypertension (34–37). The divergent utilization of $S1P_1$ and $S1P_3$ 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 $S1P_1$-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 $S1P_1$-selective agonist with an EC_{50} of approximately 1 nM, which induces phosphorylation of ERK and AKT (39). At high concentrations, AUY954 also has some activity on $S1P_5$ (39). Conversely, W146 antagonizes AKT and ERK phosphorylation and is the only widely utilized $S1P_1$-specific antagonist (40). Administration of W146 enhances vascular leakage and induces pulmonary edema (40, 41). VPC23019 is a useful in vitro tool as a dual $S1P_{1/3}$ antagonist; however, poor stability and in vivo efficacy limit its use (42–44). The only known compound with activity at $S1P_2$ is JTE-013, an antagonist with an IC_{50} of approximately 20 nM, which blocks $S1P_2$ signaling through Rho-associated protein kinase (ROCK) and phosphatase and tensin homologue (45, 46). The $S1P_2$ specificity of JTE-013 has been called into question by several studies that indicate it may have activity at $S1P_4$ 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 $S1P_1$ by the endothelium. $S1P_1$, 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 cells (VSMCs) (51, 52). Specifically, the aorta exhibited severe morphological abnormalities, endothelial hypersprouting, and altered VSMC recruitment and localization (**Fig. 2**) (53, 54). The generation of inducible cell-specific $S1P_1$ knockout mice has clarified the roles of endothelial cells (ECs) or VSMC $S1P_1$ in the regulation of postnatal vascular development, maturation, and function. In the developing retinal vasculature,

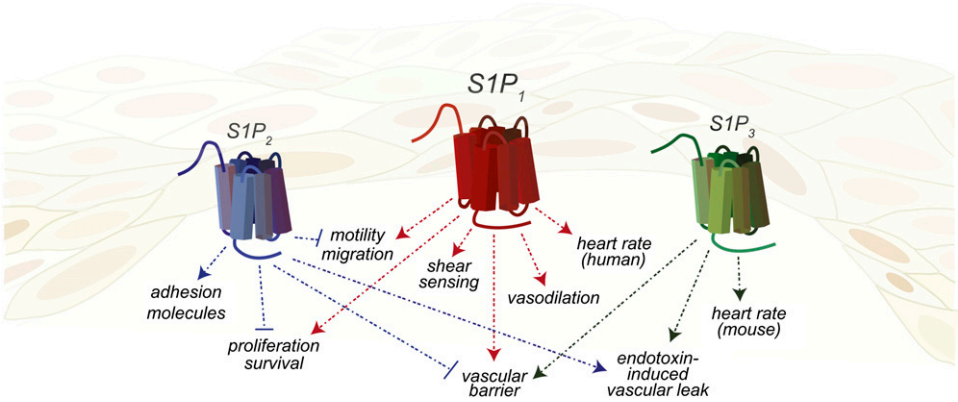

Fig. 2. Expression of S1PRs and responses by endothelial cells. Endothelial cells express $S1P_1$, $S1P_2$, and $S1P_3$ protein. Endothelial cells may express different S1PRs depending on activation status.

$S1P_1$ expression is restricted to the ECs and increases with vessel maturity, as the lowest levels of expression are found at the vascular leading front (55). Postnatal 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 overexpression of EC $S1P_1$ suppressed vascular sprouting (55). Changes in the vascular architecture of EC $S1pr1^{-/-}$ mice were accompanied by increased vascular permeability, resulting from altered vascular endothelial cadherin localization at endothelial cell-cell junctions (54, 55). These data confirmed numerous earlier in vitro studies describing the necessity of EC $S1P_1$ for the maintenance of vascular barrier function through adherens junction formation induced by activation of Rac after $G_{\alpha i}$ coupling to $S1P_1$ (Fig. 2) (56, 57).

Maintenance and formation of adherens junctions was dependent on $S1P_1$ signaling initiated not only by ligand, but also by fluid shear stress (Fig. 2). Examination of murine aortae found that areas of turbulent flow (the lesser curvature) had poor endothelial cell alignment and $S1P_1$ relocalized from the EC surface to endocytic vesicles, whereas in the descending aorta, an area of laminar flow, $S1P_1$ and vascular endothelial cadherin colocalized 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 $S1P_1$-induced inhibition of matrix metalloproteinase (58).

Mice with endothelium-specific deletion of $S1P_1$ developed severe pathology in a model of renal ischemia/reperfusion injury, both in the kidneys and the liver, characterized by elevated plasma creatinine, alanine transferase, and tissue necrosis (59). Conversely, of the five S1PRs, $S1P_2$ mRNA in the kidney was most increased upon renal ischemia/reperfusion, and mice deficient in $S1P_2$ developed significantly less pathology compared with WT controls (60). When $S1pr2^{-/-}$ mice were treated with the $S1P_1$ antagonist, W146, before ischemia/reperfusion, they were no longer protected from renal injury, suggesting that $S1P_1$ and $S1P_2$ in the renal vasculare endothelium play protective and injurious roles, respectively, in kidney injury and disease (60).

The pro-inflammatory tendency of $S1P_2$ is supported by in vitro studies suggesting a paracrine feedback loop involving EC TNFα induction of $S1P_2$ expression leading to activation of nuclear factor (NF)-κB 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 $S1P_2$ 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 (BM) compartment resulted in decreased vascular permeability, VCAM-1 and ICAM-1 expression, and more rapid resolution (62). Similarly, in vitro, $S1P_2$ 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 $S1P_1$ and $S1P_2$ in the vascular endothelium during tissue injury and disease.

Lymphatic endothelium also expresses S1PRs, 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 $S1P_2$ regulates tonic contractility of lymph vessels, as shown using $S1P_2$ inhibition by JTE013 (65).

IMMUNE SYSTEM

S1PRs regulate many aspects of immune cell biology. The best known is the regulation by $S1P_1$ of lymphocyte migration out of the secondary lymphoid organs into the blood and lymph (**Fig. 3**) (66). Regulation of migration occurs by $S1P_1$ counteracting the retention signals provided by the chemokine receptor CCR7 (67). However, this is not the only role for $S1P_1$ in lymphocytes, and roles for the other four S1PRs 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 MS (68). Although EAE and MS are considered to be primarily diseases of the immune system, the role of S1PRs on neural cells is also gaining an appreciation and will be discussed later. FTY720 is a Sph analog that is phosphorylated, acts on $S1P_{1,3-5}$, and was the first US Food and Drug Administration 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 $S1P_1$ may also regulate the activation and differentiation status of these immune cells. Deletion of T cell $S1P_1$ significantly suppresses the ability of these cells to be polarized to T-helper (Th)17 in vitro (72). Conversely, when EAE was induced in mice expressing an internalization-defective $S1P_1$ (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).

$S1P_1$ is also expressed on CD4 T cells isolated from human rheumatoid arthritis patients (73). S1P enhances TNFα-induced expression of the 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 rheumatoid arthritis, a $S1P_1$-specific antagonist prevented or ameliorated disease by upregulating lymphocyte CD69 expression, which downregulates $S1P_1$ surface expression, blocking thymic egress (73–75).

$S1P_1$ also affects 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). $S1P_1$ suppresses T_{reg} development via the AKT/mammalian target of rapamyacin pathway and affects their migration from the thymus and out of the periphery by counteracting CCR7 retention signals, similar to

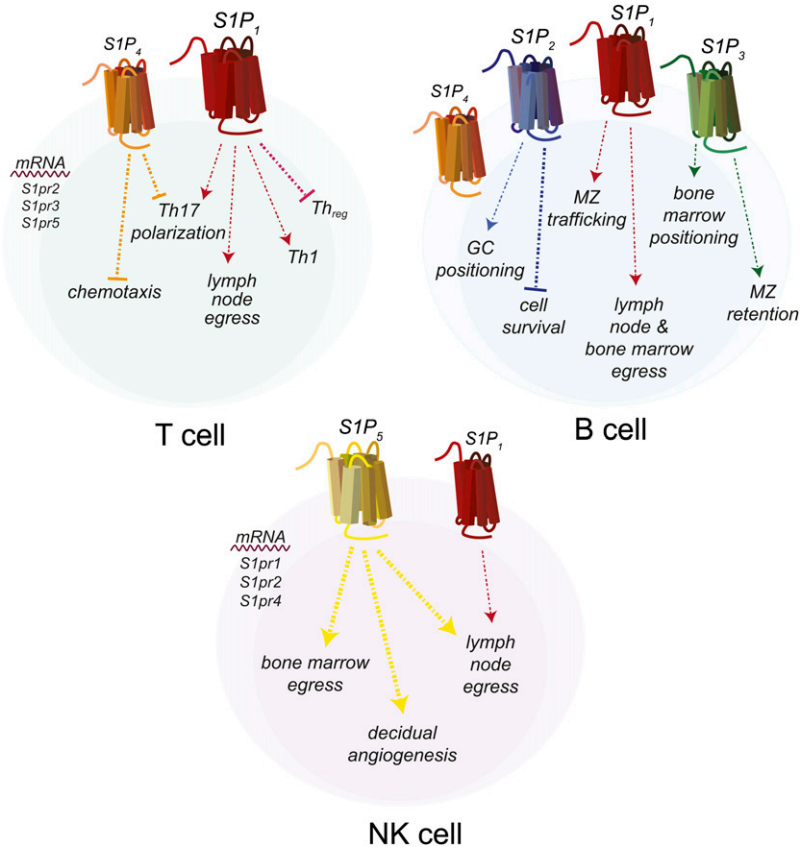

Fig. 3. Expression of S1PRs and responses by cells of the acquired immune system. T cells express $S1P_1$ and $S1P_4$, B cells express $S1P_1$, $S1P_2$, $S1P_3$, and $S1P_4$, and NK cells express $S1P_1$ and $S1P_5$. Cells do not necessarily express all of the illustrated S1PRs at one time, but may have differential expression during different stages of maturation or activation.

the mechanism regulating the egress of effector T cells from lymph nodes (67, 77, 78). $S1P_1$ signals may also modulate nuclear localization of the transcription factor forkhead box P3, which is necessary for T_{reg} generation (78). In human patients, FTY720 significantly increased the number of T_{reg} while decreasing central memory T cells (79). In a specific subset of T memory cells, nonlymphoid resident memory cells (T_{RM}), cytokines that induce the T_{RM} phenotype also downregulate the transcription factor Krüppel-like factor2 and its target gene, *S1pr1* (80). Subsequently, T_{RM} are unable to sense S1P in circulation and are maintained in the periphery.

Although $S1P_1$ has been the focus of much research, not much is known of the roles of the other S1PRs. In CD8 effector T cells, $S1P_4$ may influence their trafficking to lymph nodes (LNs), although it appears not to be a primary regulator (81). *S1pr4*$^{-/-}$ mice have decreased Th17 T cell polarization; however, reduced Th17 differentiation is likely T cell extrinsic and primarily due to functions of $S1P_4$ in dendritic cells (DCs) (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 (Fig. 3) (82, 83). While $S1P_1$ has some regulatory functions in B cells, it appears that $S1P_2$ has a greater impact on these cells. Aged *S1pr2*$^{-/-}$ 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 *S1PR2* in human DLBCL (84). Under homeostatic conditions, $S1P_2$ signals via $G_{12/13}$ to activate Rho/ROCK, antagonizing activation of AKT and pro-survival signals (82). B cell $S1P_2$ also regulates follicular positioning of B cells by directing their clustering to GC in response to follicular DC-derived S1P (82, 85). The ability of follicular B cells to exit the follicle is, however, dependent upon $S1P_1$ expression (86). Additionally, trafficking of marginal zone (MZ) B cells between the MZ and the follicle is regulated by $S1P_1$, which maintains these cells in the MZ in order for them to capture blood-borne antigens (86–88).

Studies of nonobese diabetic mice have shown that upregulation of $S1P_3$ by MZ B cells and their T2 MZ precursors may also play a role in enhancing MZ retention in these mice (89, 90). $S1P_3$ has already been shown to regulate B cell migration in vitro, but not in vivo, in WT mice (83, 87). However, it may be important for positioning of immature B cells and their progenitors within the BM, whereas $S1P_1$ participates in directing their migration from the BM parenchyma into sinusoids and subsequently into circulation (83).

Natural killer (NK) cells 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 IFNγ (92). Mouse NK cells have low levels of transcript for *S1pr1*, *S1pr2*, and *S1pr4* and high *S1pr5* mRNA levels (Fig. 3) (93, 94). $S1P_5$ normally antagonizes NK CXCR4 BM 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, 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 cell migration from LN to lymph, but the contribution is relatively minor compared with that of $S1P_5$, which is not subject to CD69 regulation (75, 94). Decidual NK (dNK) cells are a specialized NK cell 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 cells compared with circulating NK cells, 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). S1PRs expressed by monocytes and macrophages regulate their migration and activation, and the receptors responsible are cell subtype- and situation-specific (**Fig. 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*$^{-/-}$ mice on the same *Apoe*$^{-/-}$ background do not have altered development of atherosclerosis, but do have decreased monocytes and macrophages with atherosclerotic lesions (101). In WT mice, treatment with FTY720 results in decreased circulating monocytes; however, use of the $S1P_{1/4/5}$ agonist, BAF312, yielded similar results, both at homeostasis and during EAE, indicating that $S1P_3$ 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 $S1P_3$ 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 $S1P_1$ expression on recruited macrophages and that $S1P_1$ is necessary for emigration from the inflamed peritoneum, but has no role in efferocytosis or activation (107). $S1P_2$ on alveolar macrophages may regulate their phagocytic capacity, as *S1pr2*$^{-/-}$ alveolar macrophages displayed decreased phagocytosis of the fungus *Cryptococcus neoformans* due to decreased expression of Fc receptors necessary for phagocytosis of antibody-opsonized fungus (108).

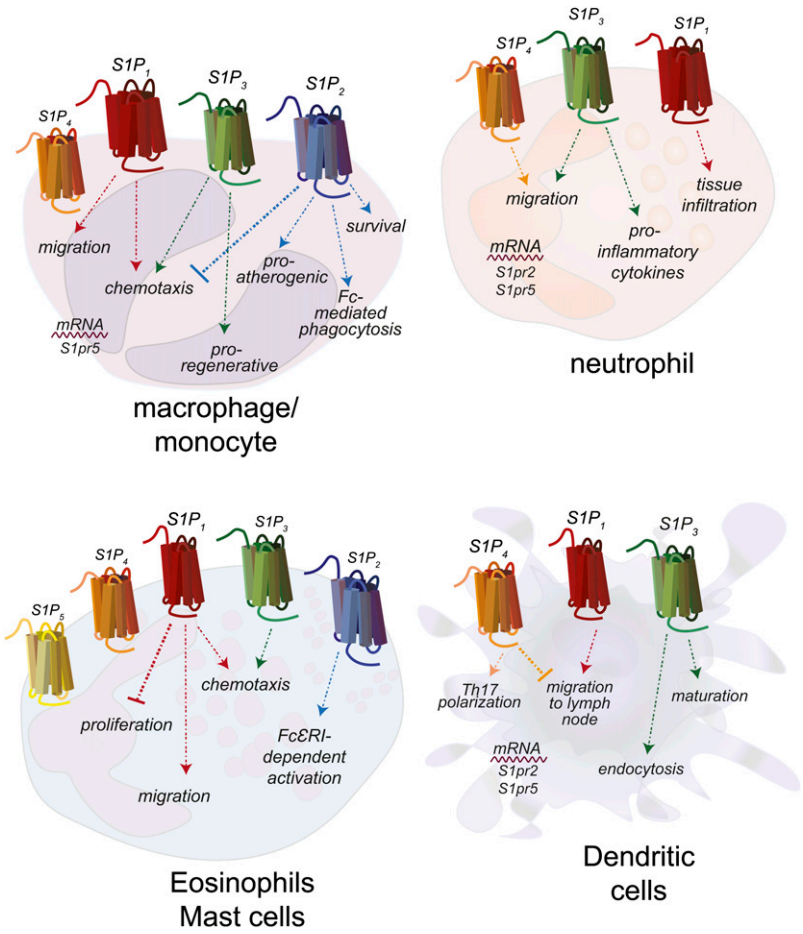

Fig. 4. Expression of S1PRs and responses by cells of the innate immune system. Monocytes and/or macrophages express $S1P_{1–4}$, neutrophils express $S1P_1$, $S1P_3$, and $S1P_4$, eosinophils and MCs express all S1PRs, and DCs express $S1P_1$, $S1P_3$, and $S1P_4$. Cells do not necessarily express all of the illustrated S1PRs at one time, but may have differential expression during different stages of maturation or activation.

Neutrophils are the first immune cell line of defense and can shape the immune response (109). Neutrophils express mRNA for all S1PRs; however, the level of expression and the ability of S1P to affect changes in their responses depend upon their activation status (Fig. 4) (110). More recently, it was reported that S1P lyase ($Sgpl$)$^{-/-}$ mice are unable to degrade S1P and have neutrophilia (111). Although $S1P_4$ deficiency in *Sgpl* knockouts resulted in circulating neutrophil numbers that were close to WT, $S1P_4$ was not specifically deleted in neutrophils, raising the possibility that multiple cell types were responsible for the effect. Specific deletion of neutrophil $S1P_1$ did not normalize neutrophil numbers in $Sgpl^{-/-}$ mice. However, in rat models of hyperalgesia dependent upon neutrophil infiltration, $S1P_1$ was necessary for neutrophil recruitment (112). Specific $S1P_1$ antagonism blocked neutrophil infiltration, whereas agonism increased sensitivity.

Eosinophils and mast cells (MCs) are both involved in anti-parasite immune responses and allergic immunity (113). Eosinophils from mice over-expressing interleukin-5, an eosinophil growth factor, express high levels of $S1P_3$ and demonstrate increased chemotactic responses to S1P in vitro (Fig. 4) (114). In a model of allergic rhinitis, FTY720 treatment significantly decreased the numbers of infiltrating MCs and eosinophils, resulting in resolution (115). In vitro, FTY720 induced MC apoptosis in a dose-dependent manner (115). Similar to lymphocytes, $S1P_1$ regulates MC migration toward the antigen, whereas $S1P_2$ regulates their activation status upon FcεRI ligation, inducing degranulation and CCL2 secretion (116).

DCs 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 DCs express mRNA for $S1P_{1–5}$ and exhibit varied responses to S1P stimulation in vitro and in vivo (Fig. 4) (118, 119, 120). Langerhans cells, skin resident DCs, require $S1P_1$ for migration to LN, whereas kidney resident DCs require $S1P_3$ for maturation in ischemia/reperfusion (121, 122). This is also the case in models of sepsis, where DC $S1P_3$ is required for interleukin-1β production (123). In EAE, although $S1P_1$ agonism decreased disease pathology, it did not affect entry into the CNS of a subset of DCs (plasmacytoid DCs). However, plasmacytoid DCs in the CNS were necessary for the efficacy of $S1P_1$ agonist treatment (124).

$S1P_4$ was cloned from mature human DCs, 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 DCs in draining LN after topical antigen application (81). This implies that $S1P_4$ may antagonize $S1P_1$ in DCs, 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

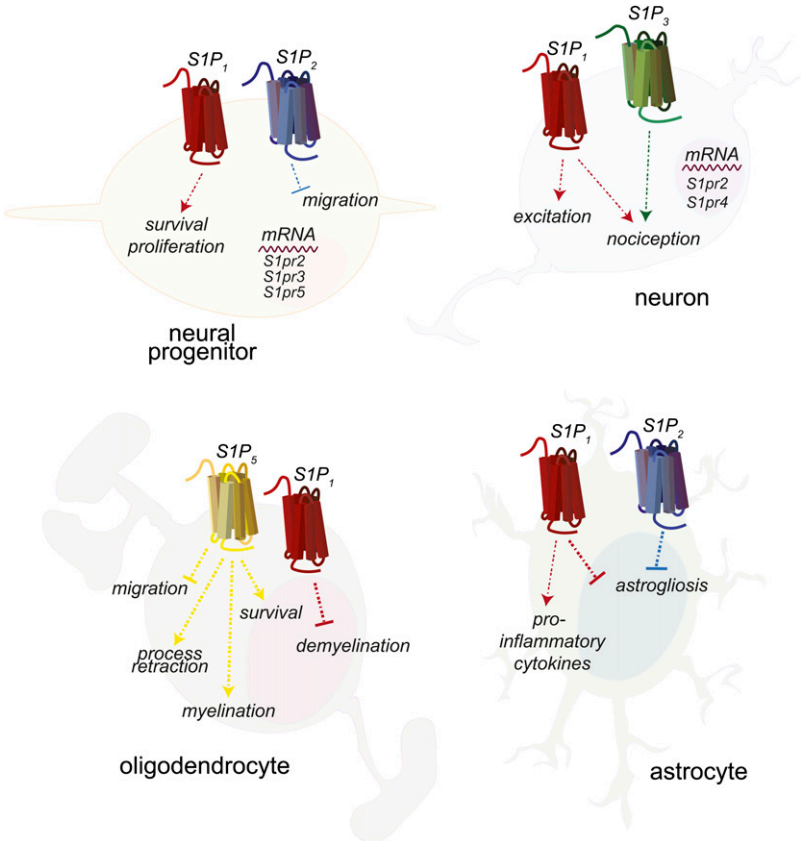

Fig. 5. Expression of S1PRs and responses by neural cells. Neural progenitors express $S1P_1$ and $S1P_2$, neurons express $S1P_1$ and $S1P_3$, oligodendrocytes express $S1P_1$ and $S1P_5$, and astrocytes express $S1P_1$ and $S1P_2$. $S1P_1$ couples exclusively to $G_{\alpha i}$. $S1P_2$ and $S1P_3$ can couple to $G_{\alpha i}$, $G_{\alpha 12/13}$, or $G_{\alpha q}$, and $S1P_5$ can couple to $G_{\alpha i}$ or $G_{\alpha 12/13}$. Cells do not necessarily express all of the illustrated S1PRs at one time, but may have differential expression during different stages of maturation or activation.

(**Fig. 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 postischemic 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 progenitors 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 (Fig. 5) (131, 132). One group found that pain responses induced by intradermal S1P injection or models of postoperative 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 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 G_i/AKT-mediated survival in mature oligodendrocytes occurs via $S1P_5$ (Fig. 5) (136, 137). Ex vivo studies using cerebellar slice cultures indicated that S1PR agonism, particularly $S1P_1$, 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 $S1P_5$ 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 were 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 the CNS, microglia, express all S1PRs (142). In vitro studies indicated that FTY720 downregulated 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 pro-inflammatory mediators by this cell type, as well (143). Astrocytic gliosis also occurs in EAE and MS (Fig. 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 $S1P_1$ resulted in decreased EAE pathology and a loss of FTY720 efficacy, indicating that the primary target of FTY720 during EAE was $S1P_1$ 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 $S1P_1$ (146). Another target of FTY720, $S1P_3$, was also found on reactive astrocytes in human MS lesions and upregulated by lipopolysaccharide stimulation of astrocytes in vitro, although it is unknown if expression of $S1P_3$ is protective or pathogenic in the context of MS/EAE (147). Mice deficient in the one S1PR not targeted by FTY720, $S1P_2$, 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). $S1P_5$ was highly expressed by human brain capillary endothelial cells, and antagonism of $S1P_5$ 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 the method of ischemia induction and temporal regulation of cell activation and recruitment.

INVOLVEMENT OF S1PRs IN CANCER AND ONCOGENESIS

S1PRs 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). WT hamster lung fibroblasts were protected from nutrient deprivation-induced apoptosis by expression of $S1P_1$, which induced the anti-apoptotic protein Mcl1 via the phosphoinositide 3-kinase and PKC pathways (162). Lung adenocarcinoma cell lines respond to S1P with increased proliferation and invasion through $S1P_3$-mediated expression of epidermal growth factor receptor (EGFR) (163).

Estrogen receptor positive (ER^+) breast cancer cells also responded to S1P via $S1P_3$ to coordinately regulate EGFR localization and signaling (164). High expression of $S1P_1$ or $S1P_3$ by ER^+ breast cancer cells correlated with poor prognosis and high $S1P_1$ expression induced decreased expression of pro-apoptotic markers (165, 166). In ER^- breast cancer cells, $S1P_4$ expression activated the ERK1/2 pathway and correlated with poor prognosis (167). In vitro, several breast cancer cell lines respond to S1P or $S1P_1$ 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 $S1P_1$ deletion (170). In a model of colitis-associated cancer, $S1P_1$ signaling was necessary for persistent activation of nuclear factor-kB and signal transducer and activator of transcription3 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 $S1P_2$-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 $S1P_2$ (174). AKT and Bcl-associated death promoter pro-survival pathways were also reduced by FTY720 administration to neuroblastoma cells in an 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 $S1P_1$ expression by classical Hodgkin's lymphoma (CHL) cells, B cell chronic lymphocytic leukemia (B-CLL) cells, and activated B cell-like DLBCL cells (176–178). Chronic myeloid leukemia (CML) cells expressed $S1P_2$, which resulted in increased stability of the B cell receptor-Abl1 fusion protein and subsequently, increased proliferation (179). Expression of S1PRs 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

S1PRs are gaining appreciation as powerful modulators of homeostasis and pathogenesis. In all biological systems, S1PRs 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 overexpression are created, and agonists and antagonists with greater S1PR subtype specificity are developed, further studies with such tools will clarify the contributions of specific S1PRs in each physiological or pathological context. This is especially true of the less explored members of the S1PR family, $S1P_4$ and $S1P_5$. Additionally, we anticipate that the development of more compounds for clinical use will expand our understanding of the complex signaling networks regulated by S1PRs and their role in human homeostasis and disease.

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