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The vascular S1P gradient—Cellular sources and biological significance

Timothy Hla*, **Krishnan Venkataraman**, and **Jason Michaud**
Center for Vascular Biology, Department of Cell Biology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06001, USA

Abstract

Sphingosine 1-phosphate (S1P), a product of sphingomyelin metabolism, is enriched in the circulatory system whereas it is estimated to be much lower in interstitial fluids of tissues. This concentration gradient, termed the vascular S1P gradient appears to form as a result of substrate availability and the action of metabolic enzymes. S1P levels in blood and lymph are estimated to be in the μM range. In the immune system, the S1P gradient is needed as a spatial cue for lymphocyte and hematopoietic cell trafficking. During inflammatory reactions in which enhanced vascular permeability occurs, a burst of S1P becomes available to its receptors in the extravascular compartment, which likely contributes to the tissue reactions. Thus, the presence of the vascular S1P gradient is thought to contribute to physiological and pathological conditions. From an evolutionary perspective, S1P receptors may have co-evolved with the advent of a closed vascular system and the trafficking paradigms for hematopoietic cells to navigate in and out of the vascular system.

Keywords

Endothelium; Sphingosine 1-phosphate; Vascular biology

1. Sphingosine 1-phosphate metabolism

Sphingomyelin (SM), a eukaryotic specific phospholipid, is essential for the formation of membrane rafts and caveolae [1]. SM is metabolized by the sphingomyelinase pathway to produce progressively polar molecules: ceramide, sphingosine and sphingosine 1-phosphate [2]. Ceramide is catalyzed by ceramidase into sphingosine. Sphingosine is phosphorylated by sphingosine kinase enzymes into sphingosine 1-phosphate (S1P) [3], which is degraded by the terminal enzyme S1P lyase into hexadecenal and phosphoethanolamine [4]. In addition, S1P-specific phosphatases [5] and lysolipid phosphatases [6] can dephosphorylate S1P into sphingosine.

The S1P metabolic pathway is coupled with the phospholipid biosynthetic pathway. Hexadecenal is metabolized into a fatty acyl-CoA derivative, an intermediate in glycerol-based phospholipid synthesis [7]. In addition, phosphoethanolamine is an essential intermediate in the remodeling of phospholipids. Sphingosine and ceramide also appear to play critical roles in metabolism, membrane homeostasis and endocytosis in lower eukaryotes such as the yeast *S. cerevesiae* [8].

*Corresponding author. Tel.: +1 860 679 4128; fax: +1 860 679 1201. *E-mail address*: hla@nso2.uchc.edu (T. Hla).

Metabolism of S1P occurs largely in the intracellular membranes at different subcellular locations. SM is enriched in the outer leaflet of plasma membrane as well as in the Golgi. Sphingomyelinases are localized in lysosomes, plasma membrane and the extracellular spaces [2,9]. Ceramidase is also localized in the extracellular space, outer leaflet of the plasma membrane, and the lysosome [10]. Sphingosine kinase is localized in the cytosol, the nucleus, and the membranes of the cells, as well as in the extracellular space [11]. In contrast, S1P lyase [12] and phosphatases [5,13] are exclusively located at the endoplasmic reticulum. In some situations, S1P formation likely occurs in the extracellular environment, as some of the biosynthetic enzymes such as sphingomyelinase [14,15] ceramidase [15] and sphingosine kinases [11,16] are secreted. Intracellular S1P is likely transported to the extracellular environment by specific transporters and some evidence exists that S1P export is mediated by ABC-type transporters [17,18]. However, mechanisms of export/secretion of S1P are poorly understood. It is likely that S1P formed in the inner surface of the plasma membrane would need to be flipped or transported to the outer leaflet of the plasma membrane in order to bind chaperones, such as HDL or albumin [19].

2. S1P receptors

Although originally considered a second messenger, our understanding of the actions of S1P improved significantly with the discovery of G protein-coupled receptors (GPCR) for S1P [20]. We now know that there are five GPCR for S1P ($S1P_{1–5}$), all of which bind to the ligand with nM affinity [21,22]. Although all S1PRs are G protein-coupled, each receptor subtype exhibits differential coupling efficacy to G protein alpha subunits. Most cells express one or more S1P receptor subtypes and therefore this bioactive lipid has effects on most organ systems.

The molecular details of how S1P activates its receptors are not well understood. An S1P docking site on the $S1P_1$ receptor was defined from modeling and mutagenesis studies, which places the phosphate group on the outer surface of the receptor and the hydrophobic chain of S1P in the inner pocket between the transmembrane helices [23]. It is not clear if extracellular S1P inserts itself into the outer leaflet of the plasma membrane followed by lateral entry into the receptor binding pocket, or whether it enters from the extracellular surface. The interaction of the chaperone-bound S1P with the cell, dissociation from the chaperone, and association with the receptor also need to be understood at the molecular level. Considering the S1P that is produced in response to agonist activation, it is likely that it is rapidly exported (or flipped) from the inner leaflet to the outer leaflet of the plasma membrane so that receptor activation can take place.

Once activated, S1P receptor signaling is likely transient. The $S1P_1$ receptor gets phosphorylated at the C terminal tail by G protein-coupled receptor kinase (GRK)-2 and/or protein kinase-C [24]. This allows the recruitment of the adapter protein β-arrestin, which induces receptor internalization via clathrin coated pits. Upon binding S1P, the $S1P_1$ receptor is efficiently internalized and recycles back to the plasma membrane within ~30 min [25]. Thus, under physiological conditions, S1P signaling is likely tonic. Interestingly, pharmacological agonists, such as FTY720P, induce rapid $S1P_1$ degradation and achieve functional antagonism [26,27]. In addition, some studies indicate that $S1P_3$ receptor is internalized less efficiently which may lead to more sustained signaling [28]. Differential internalization and recycling among S1P receptor subtypes may lead to complex signaling kinetics in various contexts of S1P stimulation.

3. Biological actions of S1P

S1P is a pleiotropic lipid mediator capable of influencing many cell types. However, in mammalian systems S1P is found mainly in the blood and lymph [29–31]. Furthermore, its

receptors are expressed highly in the vasculature and immune organs [32,33]. Thus, the functions of S1P in these two organ systems have been characterized most extensively.

In the vascular system, S1P regulates vessel maturation, angiogenesis, and vascular permeability both developmentally and in the adult [12]. In addition, S1P regulates basal vascular tone in the adult [34,35]. These effects are mediated by signaling through S1P receptors on endothelial and vascular smooth muscle cells. It is likely that S1P receptors present on vascular endothelial cells are constantly activated by plasma-borne S1P. Due to receptor desensitization and internalization, there is also likely to be continuous tonic signaling. However, during cell activation or stress, acute localized production of S1P occurs. For example, activation of endothelial cells by TNFa [36] or VEGF [37] activates sphingosine kinase enzyme and produces S1P. In such situations, localized receptor activation likely occurs via autocrine and paracrine mechanisms. Similarly, S1P receptor expression is under dynamic control. $S1P_1$ receptor mRNA was originally isolated as an immediate-early gene induced by phorbol myristic acetate in human endothelial cells [32]. Subsequent studies have shown that it is also induced by VEGF [38], FGF [39], laminar shear-stress [40] and statin treatment [41]. In the quiescent vasculature, $S1P_1$ is expressed at extremely low levels, is induced in angiogenic tumor vessels [42,43]. These data suggest that S1P signaling in endothelial cells is under dynamic regulation at the level of receptor expression and subcellular localization, as well as endogenous or exogenous S1P production.

In the immune system, S1P regulates the trafficking of T-, B - and dendritic cells [44]. Early work showed that S1P receptors are present on lymphocytes and that S1P modulated cell proliferation and cytokine expression. in both human and mouse cell systems [45,46]. Importantly S1P was also found to be chemotactic for lymphocytes. However, the work with FTY720, an experimental agent with its origins in Eastern traditional medicine, showed that $S1P_1$ signaling is a major mechanism for T-cell trafficking *in vivo* [47,48].

FTY720, a sphingosine analog, is phosphorylated by sphingosine kinase-2 into FTY720P [49], which is a nM agonist for all S1P receptors except $S1P_2$ [47,48]. Although FTY720P is a strong agonist for S1P receptors, chronic treatment with this compound leads to sustained internalization of the receptor (particularly $S1P_1$) abrogating S1P-induced chemotaxis *in vitro* [26]. In endothelial cells and HEK293 cells, FTY720P binding and internalization of the receptor resulted in exaggerated ubiquitinylation of the $S1P_1$ receptor and proteosome-mediated degradation [27,50]. *In vivo* treatment with FTY720 inhibits leads to sustained lymphopenia by blocking lymphocyte egress from lymphoid organs and thymus into the S1P-rich blood and lymph. The deletion of the *S1pr1* gene in T-cells can have identical effects on lymphocyte trafficking [51,52]. A recent study proposed that S1P-dependent egress system acts in a competitive manner with the chemokine-based retention pathways and the net effect of such G protein-based signaling pathways leads to egress [53]. An alternative mechanism has been proposed whereby $S1P_1$ on lymphatic endothelium, at egress sites, is activated by FTY720P and “closes” the egress portals, presumably by the assembly of endothelial cell adherens junctions [54,55]. These studies strongly suggest that S1P in the lymph serves as a chemoattractant signal to allow lymphocyte egress from the thymus, secondary lymphoid organs and Peyer’s patches. This was further supported by a study which showed that inhibition of S1P lyase by a food colorant called 2-acetyl-4-tetrahydroxybutylimidazole (THI) raised S1P levels in the lymphoid organs and inhibited lymphocyte egress [56].

In addition to T-cell trafficking, B-cell recruitment from the bone marrow to the periphery is also regulated by S1P [57–59]. Furthermore, dendritic cell trafficking form periphery to lymph nodes, and hematopoietic progenitor cell recirculation are also regulated by the S1P signaling system [60–63], underscoring the generality of this signaling pathway immune cell trafficking.

The biological significance of S1P signaling pathways in immunity is not as clear. It seems that S1P-mediated egress is used by naive and memory T-cells. However, when T-cells become activated by antigen presenting cells, $S1P_1$ appears to be down-regulated by poorly understood mechanisms [53]. The effect of lack of $S1P_1$ is the retention of the activated T-cell in the lymphoid organ, which allows for clonal expansion. Thus, excessive egress would be anticipated to suppress the strength of the adaptive cell-mediated immune signal. Indeed, two transgenic models in which $S1P_1$ was expressed constitutively in T-cells led to attenuation of adaptive immunity in mouse models [64,65]. To date, the role of S1P signaling in innate immunity has not been described.

4. Vascular S1P gradient

Gradients of biologically active molecules are critical for development and physiological homeostasis in multicellular organisms. Therefore, significant mechanisms are devoted to establishing and maintaining such gradients. Developmental pattern formation is critically dependent on accurate spatial expression of morphogenic factors, such as growth factors and cytokines [66]. Polypeptide growth factors are structurally designed to achieve differential affinity to extracellular matrix and thus maintain a stable gradient. For example, vascular endothelial cell growth factor (VEGF) is spliced into heparin binding and soluble isoforms, which are critical for accurate blood vessel patterning [66]. In contrast, bioactive lipid gradients are not well understood. Gradients of S1P are likely influenced by the unique physiochemical properties of this lipid. For example, the affinity for selected chaperones such as albumin and HDL [19], and its affinity for hydrophobic membranes would likely contribute to the diffusion and movement of the lipid. In addition, S1P gradients would clearly be influenced by the activities and distribution of degradative and biosynthetic enzymes.

In mammals, S1P is enriched in blood. Plasma contains μM concentration of S1P [67], bound mostly to HDL and albumin. Similarly, S1P in lymph is ~1/4 of plasma levels [30,68]. In contrast, estimates of S1P levels in tissue interstitial fluids are in the nM concentration [44, 56]. Thus, S1P gradients will be encountered by cells that have to traverse in and out of the blood and lymphatic circulatory system, S1P gradients will be encountered during their navigation.

The presence of the S1P gradient *in vivo* prompts several questions. For example, the cellular sources, kinetics of synthesis and degradation, activity and expression of metabolic enzymes, and the substrate availability are key issues that need to be understood.

5. S1P secretion/export and enrichment in plasma

Even though blood plasma contains high levels of S1P, its half-life *in vivo* is quite short. Studies using C17-S1P indicated that blood S1P has a $t_{1/2}$ of ~15 min [31]. Interestingly, incubation of C17-S1P with whole blood *ex vivo* at 37 °C did not degrade it appreciably within 1–4 h. These data suggest that S1P is not degraded by blood components, but possibly specific organs or the vascular wall. Indeed, work from the groups of Yatomi, Natarajan and Gräler showed that extracellular S1P is dephosphorylated and the resultant sphingosine is taken up rapidly by many cultured cells, including endothelial cells [6,69,70]. Furthermore, these data imply that significant biosynthetic capacity for S1P exists *in vivo* to replenish S1P that is turned over rapidly in plasma.

S1P formation and enrichment in plasma could be due to the abundance of precursors and/or the activity of biosynthetic enzymes. The precursors, sphingomyelin and ceramide are enriched in lipoproteins. Sphingomyelinases are secreted, ceramidase can be either secreted or found as an ectoenzyme, and sphingosine can be generated in the extracellular space. Due to its hydrophobicity, sphingosine is avidly taken up by cells. Therefore, it is thought that majority

of S1P is synthesized in the cytosol of cells in either the vasculature or hematopoietic cells and secreted/exported into the plasma. Alternatively, small quantities of sphingosine kinase activity exists as a soluble enzyme in plasma (5.3 nmol/g of plasma), so secreted sphingosine kinase could phosphorylate residual sphingosine in the plasma into S1P [11].

S1P export form the cytosol to extracellular environment is not fully understood. It is thought that broad-spectrum lipid transporters of the ABC family are involved. In mast cells ABCC1 (MRP1) [17], in astrocytes ABCA1 [18], and in platelets ABCA7 [71], were proposed to be involved in the export/secretion of S1P. We analyzed the requirement for ABC transporters in the export of plasma S1P in various knock out mice including ABCA1, ABCA7 and ABCC1 null mice [68]. None of these mice showed reduced S1P levels in plasma, suggesting that each of the ABC transporters is not rate-limiting in the release of S1P into plasma (unpublished results). Alternatively, compensatory mechanisms where several ABC transporters export S1P *in vivo* may mask the effects in mice lacking a single ABC subtype.

After S1P is flipped or exported by the transporter, it is likely to bind to chaperones such as HDL and/or albumin near the cell surface. However, molecular details of this process, including the existence of docking proteins for the chaperones are not understood.

6. Cellular sources of S1P in blood

S1P levels are 3–4 times higher in serum than plasma. In addition, platelet activation causes the release of S1P, which led to the notion that platelets are a major source of S1P. However, whether platelets contribute to plasma S1P *in vivo* was not examined critically, until recently. *Nfe1*$^{-/-}$ mice lack platelets altogether but had normal plasma S1P levels [30]. In addition, platelet depletion, by infusion of an anti-GPIb antibody, also did not decrease plasma S1P levels [31]. These data suggest that platelets are unlikely to be the significant source of plasma S1P under physiological conditions.

Significant progress towards identifying the cellular source(s) of S1P were made when Pappu et. al. used a novel knockout mouse system to examine the sources of S1P in plasma. They showed that when sphingosine kinase-1 and -2 genes were deleted by an inducible Cre system (poly-IC-induced Mx-Cre), plasma S1P levels dropped to undetectable levels. In this system, sphingosine kinase-1 and -2 genes are likely to be broadly deleted, including in hematopoietic cells, endothelial cells and other stromal elements [30]. By conducting adoptive transfer experiments, they demonstrated that wild-type red blood cells have the capacity to restore plasma S1P to normal levels in mice lacking *Sphk1* and *-2*, suggesting that red cells are a major source of S1P in plasma. Surprisingly, lymphatic S1P levels were not altered by red cell transfusion, suggesting the existence of an alternative source of S1P for lymphatic plasma. These conclusions were supported by findings from groups of Igarashi and Gräler who also reported that red cells phosphorylate sphingosine into S1P and release it *in vitro* or *ex vivo* [72,73].

However, our own findings suggest that red cells may not be the only source of S1P in plasma. Two lines of evidence suggest that RBC and other hematopoietic cells are not the only source of S1P. When mice were subjected to phenylhydrazine-induced anemia, plasma S1P levels were not reduced. In addition, lethal irradiation, which induced pancytopenia between days 1–14, did not reduce plasma S1P; indeed, there was an early rise in S1P that was not sustained. To confirm these findings, we conducted reciprocal bone marrow transplants between wild-type and *Sphk1*$^{-/-}$*Sphk2*$^{+/-}$ (*Sphk* triple allele null mice), which have ~60% reduction in plasma S1P. When *Sphk* triple allele mice were transplanted with wild-type bone marrow, plasma S1P levels were restored to wild-type levels, suggesting that hematopoietic cells secrete enough S1P to bring plasma S1P back to normal concentrations. However, in the reciprocal

experiment, when wild-type recipients were given *Sphk* triple allele null bone marrow, S1P levels in plasma remained at wild-type levels, suggesting that a nonhematopoietic source exists to supply S1P in plasma.

Further experiments using adenoviral expression of sphingosine kinase-1 in *Sphk1*$^{-/-}$ mice, suggest that expression of the enzyme in the liver sinusoidal endothelial cells and hepatocytes is sufficient to restore S1P levels in plasma. Indeed, endothelial cells, but not hepatocytes, released S1P avidly *in vitro*. Moreover, laminar shear-stress treatment of vascular endothelial cells in culture down-regulated S1P lyase gene expression and stimulated S1P accumulation and release. Together, these experiments suggest that endothelial cells produce S1P and release it in response to physiological shear-stress during normal blood flow [11].

Since multiple cell types are thought to contribute to plasma S1P, it will be interesting to determine the relative contribution of each cell type. This could be addressed experimentally with tissue specific knockout or knockdown of *Sphk* genes.

7. Biological significance of S1P gradient

As stated above, S1P gradient plays a major role in the trafficking of T-cells from lymphoid organs to lymph. However, various hematopoietic cells may use this paradigm. For example, von Andrian's group showed recently that patrolling of hematopoietic progenitor/stem cells (HSC) into peripheral tissues also utilize $S1P_1$ system [63]. In their study, signaling of chemokines and integrins controls the influx of bone marrow-derived cells to tissues. However, in the absence of an inflammatory or differentiation signal, HSC use $S1P_1$ to sense the S1P gradient between the tissue and the lymph and egress into the lymphatic system, from which they gain access back to the bone marrow niche via the circulation. However, when an inflammatory signal is present, for example toll-like receptor-4 activation by LPS, $S1P_1$ is degraded and cells are retained, resulting in expansion and multi-lineage differentiation. Given that S1P gradients are also involved in trafficking of B-cells from bone marrow, intestinal immune cells, dendritic cells and CXCR4+ progenitor cells [74], it might be used as a general mechanism to traffic hematopoietic cells between vascular and tissue compartments.

The role of S1P gradients in vascular system is less clear. Endothelial cells are bathed in high levels of plasma-borne S1P, and are likely to be activated by S1P receptors, when they are expressed. As stated above, it appears that S1P receptors are dynamically regulated in vascular cells at the level of gene expression. *In vivo* $S1P_1$ expression is induced in microvessels within or juxtaposed to growing tumors compared to quiescent vessels [42]. In addition, $S1P_1$ expression in vascular and inflammatory cells is induced in inflamed lesions of rheumatoid arthritis [75]. Thus, S1P receptor levels are likely to change dramatically under various pathophysiological conditions.

Once expressed, S1P receptors in vascular endothelial cells are likely to be tonically activated by extracellular S1P in the plasma. Although total plasma S1P levels are in μM concentrations, the bioactive pool of S1P is likely lower. Cyster and colleagues estimated that bioactive S1P in plasma is ~10 nM [44,56], which is near the K_d of the ligand for the receptor. Alternatively, S1P receptors could be stimulated by acutely activated sphingosine kinase in an autocrine manner.

$S1P_1$ undergoes ligand-induced desensitization and down-regulation, where phosphorylation by GRK-2 and PKC appears to be involved in these responses. The receptor then recycles back to the plasma membrane, within ~30 min [25]. Treatment with the pharmacological agonist FTY720P down-regulates $S1P_1$ in a sustained manner and induces ubiquitination-dependent proteosomal degradation [27]. In lymphocytes, $S1P_1$ was shown to be associated with the lymphocyte activation molecule, CD69, which appears to down-regulate the $S1P_1$ [76–78].

Physiological regulation of S1P receptor protein levels are poorly understood and need to be further defined.

S1P is a potent regulator of vascular permeability, and as such, the S1P gradient may also be important in regulating this component of inflammation. Our studies showed that S1P signaling regulates the Rho- and Rac-dependent assembly of VE-cadherin-based adherens junctions in endothelial cells [79]. Garcia et al. showed that transmonolayer permeability of macromolecules was suppressed by S1P signaling, in particular $S1P_1$ [80]. Acute agonism of S1P receptors with FTY720 suppressed dermal microvessel permeability induced by VEGF treatment *in vivo* [49]. Treatment with $S1P_1$ antagonists induced spontaneous permeability in the mouse lung, suggesting that tonic signaling of $S1P_1$ under physiological conditions is important in maintaining basal lung vascular permeability [81]. Indeed, $S1P_1$ is transactivated by activated protein C, which was previously shown to be effective in suppressing sepsis-induced death in humans [82]. Thus, $S1P_1$ signaling may be a key event in the regulation of microvascular permeability under pathological conditions.

The presence of the S1P gradient also ensures that extravascular cells are exposed to low levels of S1P. Thus stromal fibroblasts, mast cells, macrophages, dendritic cells, etc are unlikely to be constantly activated by S1P, unless stimulated by appropriate local activating stimuli or when vascular leak occurs. We showed recently that in the hypoxia-induced model of retinopathy in newborn mice, $S1P_2$ mediates enhanced vascular permeability, which is likely important in driving the local inflammatory response. Thus, $S1P_2$ is important in the formation of abnormal vascular structures called “vascular tufts” in the vitreous chamber [83]. Similarly, H_2O_2-induced lung injury also induces vascular permeability that is dependent on $S1P_2$ signaling [84]. S1P that is released into the interstitial fluid due to enhanced vascular permeability would likely induce fibroblast proliferation, immune cell activation and chemotaxis and as such contribute to the tissue responses in inflammation. In many cases, S1P signaling acts as a modulator of immune and inflammatory responses such as mediator secretion of mast cells, macrophage survival, fibroblast and vascular smooth muscle cell proliferation and migration [85,86], and gene expression [87]. Such events are likely mediated by S1P receptor subtypes. Future challenges will be to define such events in physiologically relevant models so that better understanding of pathological processes is achieved which will allow rational use of S1P receptor-based therapeutics.

8. Evolutionary significance of the S1P gradient

S1P and related lipids (for example phytosphingosine in fungi) are widely present in the eukaryotic phylum [2]. However, known S1P receptors of the so-called “EDG family” are only found in the genomic databases of vertebrates [88]. Indeed, S1PR isoforms are present in all vertebrate genomes, including the recently determined cartilaginous fish (elephant shark) genome (http://esharkgenome.imcb.a-star.edu.sg/). The urochordate genomic databases (*Ciona*) contain a distant family member, which may be an ancestral lipid mediator receptor [88]. This suggests that S1P receptors evolved during the development of vertebrates.

The vascular and immune systems underwent dramatic changes as the vertebrate species emerged. The vascular system became closed, whereby continuous endothelial cells developed tight junctions and became ensheathed with vascular smooth muscle. Along with the closed vascular system, new regulatory systems emerged to regulate blood pressure, vascular permeability and blood flow [89]. The immune system also underwent dramatic changes; from a primarily innate-based immunity of invertebrates, adaptive immune system was developed [90,91]. More critically, in invertebrates, an open vascular system allowed easy traverse of phagocytic innate immune cells, whereas in the vertebrate immune system specialized

mechanisms using chemokines, integrins, and high endothelial cell venules are needed for efficient and coordinated function of both systems.

We speculate that enrichment of S1P in the closed vascular system occurred with the emergence of vertebrates. This enrichment within the vasculature would allow the formation of the vascular S1P gradient, as interstitial S1P levels are low. Thus, from a teleological perspective, that S1P gradients are well positioned to be used in the trafficking of hematopoietic and immune cells as they traverse in and out of the vascular system. Furthermore, S1P is well suited as a signaling lipid in the vascular system in various physiological and pathological contexts.

9. Conclusions

Recent studies have highlighted the presence of the S1P gradient between the vascular and extravascular compartments. Factors such as substrate availability, metabolic enzyme activity and the presence of regulatory factors contribute to the establishment and maintenance of the S1P gradient. Both hematopoietic as well as vascular cells likely contribute to the high levels of S1P in plasma. Future studies are needed to further define mechanisms involved in the formation, release and regulation of plasma S1P levels. The S1P gradient was shown to be functionally relevant in the trafficking of immune cells *in vivo* as they navigate in and out of the vascular and lymphatic compartments. The vascular cells are also very much affected by S1P signaling and actively participate to maintain the gradient. Future studies need to better define the effect of S1P gradients on vascular and immune physiology and pathology. Such efforts will enable a deeper understanding of this lipid mediator and allow the rational design and use of S1P-based therapeutics.

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