The functional roles of S1P in immunity

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JB Review

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The lipid mediator sphingosine-1-phosphate (S1P) is generated within cells from sphingosine by two sphingosine kinases (SPHK1 and SPHK2). Intracellularly synthesized S1P is released into the extracellular fluid by S1P transporters, including SPNS2. Released S1P binds specifically to the G protein-coupled S1P receptors, including S1PR1/S1P1, activating a diverse range of downstream signalling pathways. Recent studies have proposed that one of the central physiological functions of intracellular S1P signalling is in lymphocyte trafficking in vivo because genetic disruption of SPHK1/2, SPNS2 or S1PR1/S1P1 in mice induces a lymphopenia phenotype. In this review, we discuss the current understanding of intercellular S1P signalling in the context of immunity.

Keywords: lipid mediator/sphingosine-1-phosphate/SPNS2/thymocyte egress/transporter.

Abbreviations: ABC, ATP-binding cassette; GPCRs, G protein-coupled receptors; LPPs, lipid phosphate phosphatases; NFκB, nuclear factor-κB; pI-pC, Polyninosinic-polycytidylic acid; PLC, phospholipase C; RIP1, receptor-interacting protein-1; S1P, sphingosine-1-phosphate; SPPs, S1P phosphatases; SPL, S1P lyase.

Sphingosine-1-phosphate (S1P), a bioactive lipid signalling molecule, plays an important role in multiple physiological phenomena, including angiogenesis, cardiovascular permeability, inflammation and immunity (1, 2). S1P is intracellularly produced from sphingosine by two sphingosine kinases (SPHK1 and SPHK2) and is dephosphorylated to regenerate sphingosine by S1P phosphatases (SPPs) and extracellular lipid phosphate phosphatases (LPPs) (Fig. 1) (3). S1P is also reversibly degraded by S1P lyase (SPL), resulting in the formation of ethanolamine phosphate and 2-hexadecenal (3).

Recent studies have shown that intracellularly produced S1P can function as a second messenger. SPHK1 is activated by tumour necrosis factor-α (TNF-α), resulting in S1P production. Intracellular S1P binds to and stimulates the E3 ubiquitin ligase activity of TNF receptor-associated factor 2 (TRAF2) (4), leading to the activation of receptor-interacting protein–1 (RIP1) and nuclear factor-κB (NFκB). In contrast, S1P in the nucleus specifically interacts with histone deacetylases such as HDAC1 and HDAC2 and inhibits their enzymatic activities (5), presumably influencing epigenetic gene regulation. However, the physiological functions of intracellular S1P have yet to be fully elucidated.

Accumulating evidence from mice defective in individual S1P receptors suggests that, in contrast, most physiological S1P activity is mediated by intracellular S1P signalling. S1P is released from cells through S1P transporters and specifically binds to S1P receptors on target cells, activating diverse downstream signalling pathways (Fig. 1). This review describes the molecular mechanisms that underlie intercellular S1P signalling and summarizes the physiological roles of S1P in immunity.

S1P Release from Cells by S1P Transporters

Recent findings regarding intercellular S1P signalling have revealed that ATP-binding cassette (ABC) transporters and SPNS2 (Spinster homologue 2) both function as S1P transporters. ABC transporters consist of two nucleotide-binding domains and two transmembrane domains and some of these transporters preferentially recognize lipophilic molecules as their substrates. Although S1P release from platelets and erythrocytes is dependent on ATP (6, 7), it is still unclear what type of molecule(s) regulates S1P transport in these cells. Several ABC transporters have been proposed to function as S1P transporters in various cell types. These transporters include ABCA1 in astrocytes (8), ABCC1 in mast cells and breast cancer cells (9–12) and ABCG2 in breast cancer cells (10). The knockdown of these ABC transporters by siRNA decreases S1P release (8–12), suggesting that these transporters are involved in S1P release. In addition, S1P release from erythrocytes and platelets can be inhibited by glyburide, an ABC transporter inhibitor (6, 7). However, the role of ABC transporters in S1P export remains controversial because S1P release is not detected when these ABC transporters are exogenously expressed in cultured cells (13), suggesting that they are not able to transport S1P by itself. Furthermore, the physiological functions of the ABC transporters remain obscure because the concentration of plasma...
SIP is unaltered in ABCA1-, ABCA7- or ABCC1-deficient mice (14).

We recently identified Spns2 as an S1P transporter based on genetic analysis of a cardiac-defective zebrafish mutant (15). Spns2, which has a structural similarity to the bacterial glycerol-3-phosphate transporter, is a member of the major facilitator superfamily (MFS), not the ABC transporter superfamily. When zebrafish spns2 was exogenously introduced in CHO (Chinese Hamster Ovary) cells expressing SPHK1, significant S1P release from the cells was detected (15). Furthermore, introduction of human SPNS2 in SPHK1-expressing CHO cells mediates S1P release in a similar manner to zebrafish Spns2, illustrating the conservation of S1P export by Spns2 between fish and mammals. The cardiac defect in zebrafish spns2 mutants is almost identical to the phenotype of the zebrafish s1pr2 mutant (16). This observation strongly suggests that Spns2-S1PR2 signalling is involved in zebrafish cardiac development. Notably, Spns2 was the first S1P transporter discovered to be physiologically functional in vivo.

**Intercellular S1P Signalling through S1P Receptors**

To date, five S1P receptors (S1PR1/S1P1–S1PR5/S1P3) expressed on various cell types have been identified as seven-membrane-spanning proteins, a characteristic feature of G protein-coupled receptors (GPCRs). S1pr1, S1pr2 and S1pr3 are widely expressed in a range of tissues including the brain, lung, spleen, heart and kidney, whereas S1pr4 and S1pr5 show a more restricted expression pattern. S1pr4 is expressed in the lymphoid tissues and lung, whereas S1pr5 is found in the brain and skin (2). S1P receptors associate with different heterotrimeric G protein α subunits. S1PR1 couples with Gi, whereas S1PR2 and S1PR3 associate with Gi, G12/13 and Gq. Both S1PR4 and S1PR5 couple with Gi and G12/13 (2).
Signalling through Gi reportedly associates with multiple signalling pathways, including the Ras/ERK pathway, promoting cell proliferation, the PI3K/Akt pathway, inhibiting apoptosis and the PI3K/Rac pathway, activating cell migration (17). SIP-mediated Gq activation predominantly associates with the phospholipase C (PLC) and calcium signalling pathways. Interestingly, signalling through G12/13 promotes Rho activation, preventing Rac-mediated cell migration (18) (Fig. 1). Therefore, combining SIP receptors with their distinct developmental expression patterns and G proteins coupled with the distinct signalling pathways that they are able to activate allows the diverse physiological functions of SIP in the context of development, homeostasis and diseases.

Plasma SIP
Plasma SIP exists at higher concentrations than in other compartments (from the nanomolar to the micromolar range) and is associated with albumin and high-density lipoprotein (HDL) (19, 20). In contrast, the SIP concentration in tissues, including lymphoid organs, is maintained at lower levels due to the abundance of SIP-degrading factors. An SIP analogue, C17-SIP, administered exogenously is rapidly degraded in plasma, with a half-life of ~15 min (21), indicating the presence of an active degradation pathway in plasma. Therefore, the high SIP levels found in plasma must be maintained by a continuous SIP supply from SIP-producing cells. Plasma SIP is thought to originate from erythrocytes, activated platelets and endothelial cells. Plasma SIP concentration is unaltered in NF-E2-deficient mice, which lack circulating platelets (22), suggesting that platelets only marginally contribute to plasma SIP level in the steady state. SIP released from activated platelets may function as a lipid mediator in inflammatory sites.

SHPK1- or SHPK2-deficient mice have no obvious phenotypes, in contrast to SHPK1/2 double knockout mice that are not viable due to defective brain and cardiovascular development, indicating that these kinases have redundant functions (23). Polynucleotidylic acid (pl-pc)-inducible SHPK1/2-deficient mice were generated to examine the physiological function of SHPK1/2 in immunity. Plasma and lymphatic SIP are undetectable in these mice (22), indicating that SHPK1 and SHPK2 are indispensable for SIP production in vivo. When SHPK1/2-deficient bone marrow cells are transplanted to lethally irradiated wild-type (WT) mice, plasma SIP levels decrease to 10% of controls, whereas lymphatic SIP remained unchanged. In contrast, transferring WT erythrocytes into pl-pc-inducible SHPK1/2-deficient mice restores plasma SIP levels (22). This suggests that radiation-resistant cells such as endothelial cells might contribute the remaining 10% of plasma SIP.

As mentioned previously, we recently identified SPNS2 as an SIP transporter. Expression of Spns2 can be detected in mouse endothelial cells. Surprisingly, vascular endothelial cells purified from the aorta of SPNS2-deficient mice are unable to release SIP (Fig. 2B) (24). On the other hand, SIP release from erythrocytes and platelets of SPNS2-deficient mice is comparable to those of WT mice, suggesting the contribution of some SIP transporters expect for SPNS2 in these cells. Thus, these results suggest that SPNS2 functions as the only SIP transporter in endothelial cells. In contrast, the plasma SIP level in SPNS2-deficient mice is 60% of that in WT mice, suggesting that endothelial cells contribute 40% of the total plasma SIP, a different value from that suggested by the transplantation experiment using the SHPK1/2-deficient mice-derived bone marrow cells. This discrepancy may indicate compensatory SIP release by other cell types and could be interpreted as illustrating the different roles of erythrocytes and endothelial cells in supplying the plasma with SIP.

Roles of SIP in lymphocyte trafficking
Recent studies have found that SIP signalling is indispensable for lymphocyte egress from the thymus in immune system (Fig. 3). When WT bone marrow cells were transplanted into pl-pc inducible SHPK1/2-deficient mice, lacking plasma and lymphatic SIP, thymocyte egress was abrogated (22), suggesting that non-bone marrow-derived cells are the source of the SIP necessary for thymocyte circulation. Furthermore, pericyte-specific SHPK1/2-deficient mice also showed an accumulation of mature thymocytes in the thymus (25). Therefore, pericyte is one of SIP source required for thymocyte egress.

When the SIP degradation activity of SPL in thymocytes is inhibited through shRNA treatment, SIP levels in the thymus increase. As a result, SIPR1 on mature thymocytes was internalized and subsequently unable to respond to SIP, resulting in thymocyte accumulation in the thymus (26). Because SPL is located on the endoplasmic reticulum membrane and its active catalytic domain is facing to the cytosol in thymocytes (27), inhibiting SPL activity causes an increase in intracellular SIP levels and a consequent increase in extracellular SIP in the thymus, which is necessary for thymocyte egress. In addition, inhibiting SPL activity might induce apoptosis by ceramide, a metabolite of SIP, as amounts both SIP and ceramide increase in the thymus of SPL-deficient mice, enhancing thymocyte apoptosis (28).

SIP is dephosphorylated by several lipid phosphatases (SPPs and LPPs) to yield sphingosine, and these SIP-dephosphorylating enzymes are expressed in the thymus (29, 30). Interestingly, mice lacking LPP3 in either epithelial cells or endothelial cells show inhibited thymocyte egress (30). The LPPs can contribute to the dephosphorylation of extracellular SIP because they have six transmembrane domains and their active catalytic domains are facing the outer surface of plasma membranes or luminal surface of internal membranes (31). LPP3 expression in thymic epithelial cells and endothelial cells is required for thymocyte egress, and LPP3-deficiency in either cell type causes SIPR1 internalization in thymocytes, suggesting that the extracellular SIP levels in the thymus are controlled by LPP3 derived from these cell types (30). In summary, the balance of SIP synthesizing and degrading
enzymes determines the concentration of extracellular S1P in the thymus and secondary lymphoid organs, which is essential for lymphocyte trafficking in vivo. One important question raised by these findings is which S1P receptor(s) is involved in lymphocyte circulations. Interestingly, S1PR1 expression is up-regulated during thymocyte maturation (25). T cell-specific S1PR1-deficient mice show disturbances in thymocyte egress, and S1PR1-deficient mature thymocytes lose their chemotactic response to S1P (32, 33). Furthermore, expression of S1PR1 by immature thymocytes increases in thymocyte-specific S1PR1-transgenic mice to levels comparable to those in mature thymocytes, and these immature thymocytes acquire the ability to exit to the peripheral blood (25). These results suggest that thymocyte egress is regulated by S1PR1 expression in thymocytes.

Recently, we found that the S1P transporter SPNS2 contributes regulating thymocyte egress (Figs 2 and 3) (24). In SPNS2-deficient mice, thymocytes are able to differentiate into mature cells (CD4+CD62Lhi or CD8+CD62Lhi) and migrate towards S1P, while there are decreased numbers of lymphocytes in peripheral blood and mature thymocytes accumulate in the thymus (24). In fact, Spsn2 mRNA can be detected in the endothelial cells of thymic peripheral blood vessels. Spsn2 mRNA transcription predominantly occurs in endothelial cells and is stronger in peripheral and venous endothelial cells than in arterial ones. These results indicate that SPNS2, an endothelial S1P transporter, supplies S1P from peripheral vascular endothelial cells in the thymus, which is essential for thymocyte entry into the circulation. Other groups have also reported this lymphopenia phenotype observed in SPNS2-deficient mice, reinforcing the importance of SPNS2 in S1P signalling (34, 35).

SPNS2-mediated S1P release would activate S1P receptor(s) in the process of thymocyte egress. S1PR1 and S1PR2 are expressed in both mature thymocytes and endothelial cells (32, 36). The activation of S1PR1 on thymocytes enables thymocytes transit into peripheral blood stream as describe above (25, 32, 33). Furthermore, the balance between S1PR1 and S1PR2 signalling in endothelial cells determines vascular permeability that is inhibited through S1PR1 and increased through S1PR2 (37). These results raise the possibility that thymocyte egress might be regulated by SPNS2-S1PR1/2 signalling in thymocytes and endothelial cells.

The Immunosuppressant FTY720

One of the most remarkable roles of intercellular S1P signalling is its regulation of lymphocyte circulation. Indeed, S1P-mediated lymphocyte trafficking is inhibited by the immunosuppressive pro-drug FTY720...
the proteins that act as FTY720-P transporters have not been determined. The cells expressing both erythrocytes (be synthesized and released from platelets but not from cytes or platelets (SPNS2 does not affect the S1P release from erythrocytes as those from WT mice, showing that a disruption of SPNS2-deficient mice show similar S1P release activity effect. Both erythrocytes and platelets isolated from the plasma S1P level and enables thymocyte egress.

![Fig. 3 Schematic illustration of thymocyte egress into the blood via S1P signalling.](image)

Concluding Remarks
There are still many questions that need to be answered regarding intercellular S1P signalling: (i) What is the S1P exporting molecules in erythrocytes and platelets?, (ii) How is the S1P gradient between the plasma and the lymph maintained?, and (iii) What is the molecular mechanism for transferring S1P through the lipid bilayer? Because intercellular S1P signalling is involved in human cancer and autoimmune diseases, the S1P transporter SPNS2 and S1P receptors are attractive drug targets for treating these diseases.

Furthermore, in addition to S1P, several other types of lipids have been revealed to function as intercellular signalling molecules, including lysophosphatidic acid (LPA) and steroid hormones. However, their lipophility makes them difficult to analyse, particularly their movement through the lipid bilayer. As mentioned in this review, SPNS2, which plays a physiological role in S1P transport in vivo, was the first lipid mediator transporter to be identified. With this as a model of lipid transporters, we expect that our understanding of lipid mediator signalling mechanisms will make rapid progress.

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Conflict of interest
None declared.

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