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REVIEW

Immune Regulation by Sphingosine 1-Phosphate and Its Receptors

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Abstract It is well established that the lysophospholipid and signalling molecule sphingosine 1-phosphate (S1P) has many important functions in immune surveillance. S1P is produced from sphingosine by two distinct sphingosine kinases, SphK1 and SphK2, and acts as an intracellular messenger and as an extracellular ligand of five G protein-coupled cell surface receptors designated $S1P_1$–$S1P_5$. S1P not only regulates peripheral lymphocyte circulation, but also influences their differentiation, activation, infiltration, and local positioning. The therapeutic value of modulating S1P metabolism and S1P receptor function is currently tested in clinical trials and holds great promise for treatment of different autoimmune diseases. Despite its obvious contribution to immune regulation, the analysis of S1P is still challenging. A major obstacle is the difficulty to analyze S1P locally in tissues and within cells due to its high metabolic turnover and the limited resolution of current analytical techniques like liquid chromatography and mass spectrometry. This review focuses on recent advancements to our understanding how different sources of S1P contribute to immune function, and how changes in production, secretion, and degradation of S1P can influence immune responses.

Keywords Lymphocyte egress · Sphingosine kinase · S1P-lyase · Sphingolipid metabolism · Lymphopenia · FTY720

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Abbreviations

S1P	Sphingosine 1-phosphate
SGPL1	S1P-lyase
SphK	Sphingosine kinase
RBC	Red blood cells
SA	Serum albumin
HDL	High-density lipoproteins
LPP	Lipid phosphate phosphatase
PSA	Passive systemic anaphylaxis
MZB	Marginal zone B

Sphingosine 1-phosphate (S1P) is a versatile signalling molecule involved in many regulatory processes including immune surveillance (Gräler 2010; Rivera et al. 2008; Spiegel and Milstien 2011). The key step for the production of S1P is the phosphorylation of sphingosine catalyzed by sphingosine kinases SphK1 and SphK2 (Kohama et al. 1998; Liu et al. 2000) (Fig. 1). S1P can be reversibly dephosphorylated to sphingosine by different phosphatases like alkaline phosphatases (Andréani and Gräler 2006), lipid phosphate phosphatases (LPPs) (Roberts et al. 1998), and sphingosine phosphate phosphatases (Ogawa et al. 2003) (Fig. 1). The most important enzyme for its degradation is the retro-aldolase S1P-lyase (SGPL1), which irreversibly cleaves S1P into hexadecenal and phosphoethanolamine (Saba et al. 1997) (Fig. 1). Modulation of these enzymatic processes has profound influence on the immune system. S1P signals both intracellular and extracellular. Intracellularly it serves as a cofactor for tumor necrosis factor (TNF) receptor-associated factor-2, which is involved in nuclear factor κB activation via TNF and B cell receptor signalling (Alvarez et al. 2010), and it is potentially involved in epigenetic transcriptional regulation

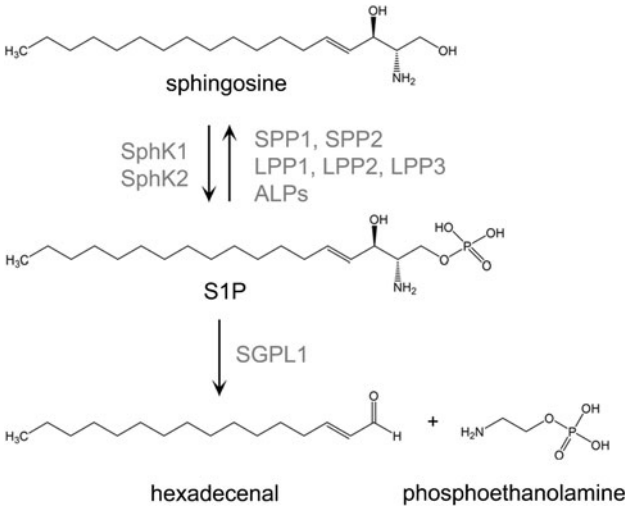

Fig. 1 Metabolism of S1P. Sphingosine is phosphorylated by sphingosine kinases type 1 (SphK1) and type 2 (SphK2), and sphingosine 1-phosphate (S1P) can be reversibly dephosphorylated by sphingosine phosphate phosphatases type 1 (SPP1) and type 2 (SPP2), lipid phosphate phosphatases type 1 (LPP1), type 2 (LPP2), and type 3 (LPP3), and by alkaline phosphatases (ALPs). Irreversible degradation of S1P is performed by the S1P-lyase (SGPL1)

as an inhibitor for the class I histone deacetylases HDAC1 and HDAC2 in the nucleus (Hait et al. 2009). S1P has long been recognized as a survival-promoting molecule that particularly counteracts the pro-apoptotic nature of its precursor ceramide, often referred to as the "S1P-ceramide rheostat" (Cuvillier et al. 1996). Although recent data indicate that the ratio of these two related molecules with antithetic functions is not always a predictive measure for cell fate (Weber et al. 2009), the basic idea of S1P promoting cell growth and survival is supported by numerous studies primarily in the cancer setting (Sabbadini 2006). Pro-survival signalling not only occurs intracellularly, but also extracellularly by stimulating five different cell surface S1P receptors (An et al. 1997; Im et al. 2000; Lee et al. 1998; Van Brocklyn et al. 2000). The combination of intra- and extracellular signalling via several different receptors and cellular target molecules with systemic and local metabolic regulation of S1P production and elimination establishes a powerful network for the interdependent regulation of different physiological networks including the immune system.

Regulation of Lymphocyte Circulation by S1P: The Basic Principle

The starting point of our current understanding how lymphocyte circulation is regulated was the functional characterization of a novel immunosuppressive agent called FTY720 (Suzuki et al. 1996). In contrast to classical immunosuppressive agents, FTY720 does not impair the interleukin-2 (IL-2) and IL-2 receptor-dependent proliferation of activated T and B cells, but alters their migration pattern (Pinschewer et al. 2000). FTY720 administration results in a severe depletion of T and B cells in peripheral blood, also known as lymphopenia. It does so by inhibiting their exit from lymphoid organs like thymus and lymph nodes. Further studies revealed that it is phosphorylated in vivo by sphingosine kinases, and that the phosphorylated compound is the active reagent that is able to activate four of the five known S1P receptors including S1P receptor type 1, 3, 4, and 5 (Mandala et al. 2002). The initial hypothesis that stimulation of S1P receptors by FTY720-phosphate actively blocks lymphocyte emigration from lymphoid organs was soon revised after the analysis of S1P receptor type 1 ($S1P_1$) knockout mouse chimeras without $S1P_1$ expression in T and B cells revealed a similar phenotype (Matloubian et al. 2004). Obviously, the loss of $S1P_1$ in lymphocytes has a similar effect as FTY720-phosphate administration. It turned out that FTY720 treatment leads to a major down-regulation of $S1P_1$ cell surface expression (Gräler and Goetzl 2004). Since then FTY720 is often denoted as a "functional antagonist", referring to its major inhibitory effect on $S1P_1$ signalling upon initial stimulation (Oo et al. 2007). In fact, it was shown that FTY720-phosphate and its chiral analog, AFD-R, not only induce short-term endosomal internalization of the $S1P_1$ receptor like S1P, but additionally evoke long-term desensitisation via polyubiquitination and lysosomal degradation (Gonzalez-Cabrera et al. 2007; Oo et al. 2007). Because of the capacity of AFD-R to target the $S1P_1$ receptor to lysosomal degradation instead of endosomal recycling to the cell membrane it was also denoted as a "supraphysiological agonist" (Gonzalez-Cabrera et al. 2007). The evolving mechanistic model, which is still valid, hypothesized that S1P in blood and lymph serves as an exit signal for lymphocytes that is transferred into the cells via activation of the $S1P_1$ receptor on their cell surface (Gräler and Goetzl 2004; Matloubian et al. 2004). Premature down-regulation of $S1P_1$ surface expression on lymphocytes by FTY720-phosphate, which accumulates significantly in lymphoid organs, desensitizes lymphocytes for the exit signal S1P, which is present at high concentrations in blood and lymph (Lo et al. 2005; Sensken et al. 2009). After publication of the $S1P_1$ knockout data a second hypothesis aroused that FTY720-phosphate blocks lymphocyte emigration from lymphoid organs by stimulating S1P receptors on sinus-lining endothelial cells and consequently establishes a firm endothelial cell barrier that cannot be overcome by exiting lymphocytes (Wei et al. 2005). Although these two models do not exclude each other, current data indicate that stimulation of $S1P_1$ on lymphocytes is more relevant for their emigration from

lymphoid organs than gatekeeping of sinus-lining endothelial cells. Firstly, S1P in blood and lymph is required for unobstructed lymphocyte circulation. Depletion of S1P in blood inhibits thymocyte exit, and depletion of S1P from lymph blocks lymphocyte egress from lymph nodes (Pappu et al. 2007). Secondly, lymphocytes expressing a $S1P_1$ mutant with defective receptor internalization also show impaired efficacy of FTY720 for lymphopenia induction (Thangada et al. 2010). $S1P_1$ internalization upon treatment with FTY720 is therefore a crucial event for the onset of lymphopenia. These results point to $S1P_1$ receptor internalization and recycling as a third level of egress control, which is influenced by different S1P concentrations in the microenvironment. It is therefore not only crucial to have high amounts of S1P in blood and lymph as an exit-mediating signal. S1P concentrations in lymphoid organs need to be kept low in order to secure sufficient surface expression of $S1P_1$ on exiting lymphocytes. High S1P concentrations in blood and lymph together with low S1P concentrations in lymphoid organs maintain a constant flow of lymphocytes between lymphoid organs, with blood and lymph as connecting routes (Lo et al. 2005). $S1P_1$ serves as the major exit signal-sensing receptor, which guides lymphocytes out of lymphoid organs upon stimulation, followed by its internalization to prevent constitutive signalling in an environment with high S1P levels. After chemokine-guided re-entrance into a different lymphoid organ, low S1P-levels in the local tissue promote re-expression of $S1P_1$ on the surface of lymphocytes and render them sensitive for the exit signal S1P in blood and lymph, preparing them to leave the organ again into circulation (Fig. 2).

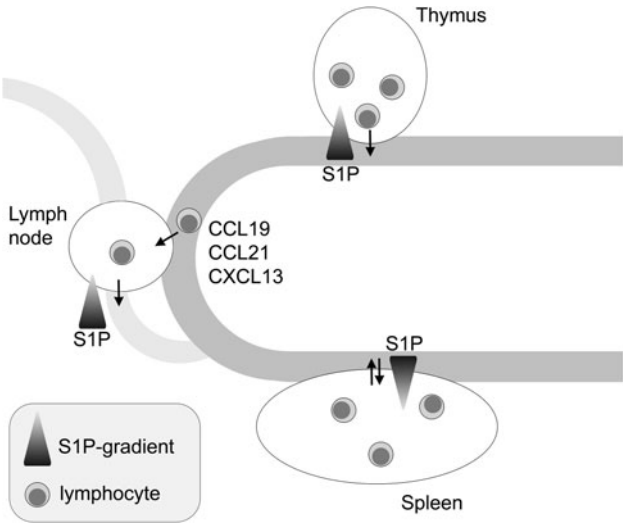

Fig. 2 Basic model of lymphocyte circulation. S1P concentrations are low in lymphoid organs and high in lymph and blood. These different S1P concentrations trigger lymphocytes to leave lymphoid organs and enter blood circulation. They re-enter T and B cell zones in lymphoid organs via chemokine signals like CCL19, CCL21, and CXCL13

Sources of S1P and Their Importance for Lymphocyte Emigration

The above-mentioned basic model proposes a step gradient of S1P between lymphoid organs with low S1P levels and blood and lymph with high S1P levels as a crucial determinant for lymphocyte egress. Nevertheless, what are the sources for S1P in blood and lymph? The analysis of human blood samples revealed red blood cells (RBC) as the predominant source for S1P in plasma (Hänel et al. 2007). While RBC-associated S1P is protected from degradation, S1P in plasma is mostly associated with either serum albumin (SA) or high-density lipoproteins (HDL) and is accessible to S1P receptors and metabolizing enzymes. S1P release from RBC is predominantly initiated by S1P binding molecules (Bode et al. 2010). Recently the HDL constituent ApoM was identified as a specific S1P binding molecule (Christoffersen et al. 2011). The stoichiometry of one S1P molecule associated with 500 SA molecules in a saturated solution indicates that SA may not directly bind S1P, but shape an environment that supports S1P dissolving (Bode et al. 2010). SA and HDL are the major endogenous molecules that extract S1P from the cell membrane of RBC in order to maintain high plasma levels of S1P, which is supported by scramblase-dependent transbilayer movement of S1P in RBC membranes (Bode et al. 2010). The reported modulation of S1P release from RBC by ABC transporters could not be confirmed in two different studies and therefore remains controversial (Bode et al. 2010; Kobayashi et al. 2009; Lee et al. 2007). Efficient depletion of S1P in blood and lymph was found in complete SphK2 and inducible SphK1 double-deficient mice with resultant depletion of both sphingosine kinases predominantly in hematopoietic cells, vascular endothelium, and liver (Pappu et al. 2007). RBC were identified as the main producers of blood-borne S1P in this mouse model as well. Subsequent studies also described vascular endothelial cells as potential contributors (Venkataraman et al. 2008). While transfusion of wild-type bone marrow in the above-mentioned complete SphK2 and inducible SphK1 double-deficient mice after lethal irradiation was sufficient to regain high S1P levels in plasma, S1P in lymph was not reconstituted (Pappu et al. 2007). Further studies identified lymphatic endothelial cells positive for the lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) as the major source for S1P in lymph (Pham et al. 2010). Complete SphK2 and LYVE-1 specific SphK1 ablation revealed specific depletion of S1P in lymph. Lymphocyte egress from peripheral and mesenteric lymph

nodes and from Peyer's patches was efficiently blocked. Thymocyte egress, however, was not dependent on lymph S1P, but on blood-borne S1P, supporting the hypothesis that mature T cells exit the thymus directly into blood circulation (Pappu et al. 2007). A similar observation was made with splenocytes. The amount of splenocytes did not change when S1P was depleted in blood and lymph, but it decreased significantly when S1P was only absent in lymph and not in blood (Pappu et al. 2007). Lymphocytes therefore leave the spleen via S1P into blood, enter lymph nodes, and re-enter blood circulation via S1P in lymph. Depletion of S1P in lymph causes a block of lymphocyte egress from lymph nodes while splenocytes still enter circulation, which decreases the pool of resident cells in the spleen (Fig. 2).

Besides S1P in blood and lymph it was noted that local S1P sources in the microenvironment of lymphoid organs also contribute to lymphocyte egress. Neural crest-derived perivascular cells for example produce S1P locally within the thymus and contribute to thymocyte exit (Zachariah and Cyster 2010). Deletion of SphK1 and SphK2 in these cells resulted in incomplete mature thymocyte egress. Additional studies investigating the chemotactic response of mouse lymphocytes isolated from different lymphoid organs also suggest that locally produced S1P in the microenvironment of peripheral lymph nodes contributes to lymphocyte egress (Sensken et al. 2011). Although current techniques do not allow to directly analyze differences of low S1P concentrations in the microenvironment of lymphoid tissues, these functional data support a contributing role of local S1P concentrations in lymphoid tissues for lymphocyte egress in blood and lymph.

The Role of Sphingosine Kinases for S1P Production and Immune Function

Genetic mouse mutants demonstrate that both SphK1 and SphK2 are contributing to normal S1P production, and both kinases have to be deleted to eliminate S1P locally and/or systemically (Mizugishi et al. 2005). Recent studies however, indicate that both kinases also have unique characteristics that cannot be fully replaced by the other one. Extracellular S1P is mainly produced by SphK1. This result is predominantly supported by cancer cells that upregulate SphK1 expression to produce S1P and consequently activate S1P receptors (Pyne and Pyne 2010). Complete deficiency of SphK1 translates into significantly reduced plasma levels of S1P in mice (Allende et al. 2004). SphK1 is translocated from the cytoplasm to the cell membrane upon different stimuli (Jarman et al. 2010; Johnson et al. 2002). It was proposed that the cell membrane localization of SphK1 supports the production and export of S1P. In contrast, SphK2 is provided with nuclear localization and export signals that allow its expression in the cytosol and in the nucleus (Hait et al. 2009; Igarashi et al. 2003). While SphK1 can be regarded as an enzyme important for S1P exportation, recent data suggest that SphK2 is important for cellular S1P importation. SphK2 deficient mice are largely protected from S1P and sphingosine accumulation in lymphoid organs induced by SGPL1 inhibition and respond with only a mild lymphopenia (Sensken et al. 2010). Surprisingly they have elevated amounts of S1P in blood, and transfusion of RBC loaded with traceable C17-S1P demonstrated a major block of S1P distribution from blood into peripheral tissues as the predominant defect in these mice. The failure of SphK2 deficient mice to develop a lymphopenia in response to FTY720 treatment may as well be explained by the failure of lymphocytes to import FTY720-phosphate from blood (Kharel et al. 2005; Sensken et al. 2009; Zemann et al. 2006), in addition to the better efficiency of SphK2 in comparison to SphK1 to phosphorylate FTY720 (Billich et al. 2003; Paugh et al. 2003). SphK1 can therefore be regarded as the predominant producer of receptor-available extracellular S1P while SphK2 is involved in cellular uptake of S1P and generates SGPL1-accessible cellular and also nuclear S1P. Thus both kinases are relevant for the maintenance of local and systemic cellular and extracellular S1P levels.

Maintenance of S1P Gradients by Degrading Enzymes

Besides sphingosine kinases as the main producers of S1P, phosphatases and SGPL1 are important enzymes to disarm and eliminate S1P. While SGPL1 is the major intracellular enzyme that irreversibly degrades S1P (Allende et al. 2011; Bektas et al. 2010; Vogel et al. 2009; Weber et al. 2009), LPP3 seems to be important for the elimination of extracellular S1P in thymic interstitium (Breart et al. 2011). Inducible LPP3 deficient mice demonstrated impaired T cell egress from thymus, and conditional deletion of LPP3 in endothelial or epithelial cells was sufficient for compromised thymocyte exit. Although no changes in local tissue S1P concentrations were found, the proposed explanation for this phenotype is the lacking dephosphorylation and consequent elimination of S1P in thymic interstitium (Breart et al. 2011). High extracellular S1P concentrations in the microenvironment of the thymus are thought to down-regulate $S1P_1$ surface expression in mature thymocytes, which consequently prevents their exit into circulation. An even more pronounced immunological phenotype was seen in SGPL1 deficient mice, which accumulate up to 1000-fold more S1P in lymphoid organs than wild-type control mice and develop a severe B and T

cell lymphopenia due to the premature internalization of the $S1P_1$ receptor in T and B cells of those organs (Weber et al. 2009). They additionally develop a severe thymus atrophy 2 weeks after birth caused by the accumulation of the pro-apoptotic sphingolipid ceramide up to 10-fold above the amount measured in control thymus tissue (Weber et al. 2009). These data point to a persistently active role of S1P degrading enzymes like SGPL1 and LPP3 for the maintenance of low S1P levels in lymphoid tissues. Intriguingly the incomplete inhibition of SGPL1 with the vitamin B_6-antagonist 4-deoxypyridoxine (DOP) results in S1P accumulation mainly in lymphoid organs, and although more than 100 metabolic enzymes are dependent on the coenzyme pyridoxalphosphate, mice treated with its antagonist DOP are predominantly characterized by increased S1P and sphingosine levels in organs of the lymphatic system (Schwab et al. 2005). Expression of functional SGPL1 is therefore particularly important for the lymphatic system and may be a good target molecule for treatment of immune and autoimmune diseases and lymphomas.

Vascular Permeability and Leukocyte Infiltration

Regulation of vascular permeability is an important characteristic in inflammation. While locally and temporally limited permeation of the vascular bed enables the extravasation of plasma during local infections and supports the clearance of pathogens, systemic responses like bacterial sepsis, viral haemorrhagic fever, and allergic anaphylactic shock can be life threatening. S1P is an important constituent of blood that regulates the integrity of the vascular system. Depletion of S1P in blood of complete SphK2 and inducible SphK1 deficient mice exhibited basal vascular leak and demonstrated an increased local response to leak-inducing agents like serotonin and histamine (Camerer et al. 2009). Modulation of vascular integrity was dependent on $S1P_1$ receptor signalling most likely in vascular endothelial cells. Investigations regarding the role of S1P during anaphylactic shock revealed an important function of the $S1P_2$ receptor particularly on mast cells, but presumably also on vascular endothelial cells mediating different effects at early and late stages of immunoglobulin E induced passive systemic anaphylaxis (PSA) (Olivera et al. 2007, 2010; Oskeritzian et al. 2010). S1P enhances PSA probably via activation of $S1P_2$ on mast cells (Olivera et al. 2007; Oskeritzian et al. 2010), but it also supports the recovery from PSA after its induction (Olivera et al. 2010). Notably SphK1 deficient mice have lower and SphK2 deficient mice higher S1P levels in plasma than wild-type control mice (Allende et al. 2004; Sensken et al. 2010), and the amount of histamine measured in plasma directly after PSA induction paralleled plasma S1P levels in these mice (Olivera et al. 2010). This suggests that plasma S1P levels influence the severity of the anaphylactic shock. A possible explanation could be that the $S1P_2$ receptor of tissue residing mast cells is activated by S1P during early extravasation of plasma (Oskeritzian et al. 2010). At later stages high plasma S1P levels help to recover from the anaphylactic shock by counteracting hypotension and promoting the elimination of histamine from plasma. This effect was attributed to enhanced stimulation of $S1P_2$ in the vasculature (Olivera et al. 2010).

Activation of $S1P_3$ on dendritic cells (DCs) occurred in a sepsis model using a severe, but not completely lethal lipopolysaccharide (LPS) challenge (Niessen et al. 2008). Similar to PSA induction, SphK1 deficiency reduced the severity of LPS-induced sepsis. $S1P_3$ deficient mice were also largely protected from LPS-induced lethality, suggesting the SphK1-S1P-$S1P_3$ axis as one critical pathway that is activated during LPS-induced sepsis (Niessen et al. 2008). Although the source of S1P was not determined in this study, it could be that plasma S1P plays a critical role in this sepsis model as well since SphK1 deficient mice are characterized by reduced plasma S1P levels (Allende et al. 2004). The authors additionally discovered a crosstalk of $S1P_3$ with the protease-activated receptor 1 (PAR1) in DCs (Niessen et al. 2008). Restoration of PAR1 and $S1P_3$ deficient mice with wild-type DCs completely restored their responsiveness to systemic inflammation. A major role of SphK1 in sepsis was also postulated in a different study using an inhibitor and siRNA against SphK1, although the authors proposed a very different mechanism with LPS-induced upregulation of SphK1 in macrophages and neutrophils (Puneet et al. 2010). Although source and local function of S1P in these studies are not completely resolved, it seems possible that changes in blood-borne S1P may alter local and systemic immune responses through inflammation-induced extravasation of plasma in local tissues. Plasma itself carries by far more S1P than is required for S1P receptor stimulation, which renders it unlikely that rather small changes in plasma S1P levels have a direct effect on exposed S1P receptors. The timely and locally restricted extravasation of plasma into tissue microenvironment, however, could amplify even slight changes of plasma S1P into significant changes of S1P distributed to tissue interstitium. This may also explain the observation that rather small relative changes in plasma S1P have a significant impact on the peripheral lymphocyte pool and thymic P-selectin expression which regulate thymic progenitor homing and lymphocyte homeostasis (Gossens et al. 2009). Besides local production of S1P via upregulation and activation of SphK1 or SphK2,

extravasation of plasma into tissue may serve as an additional regulated pathway to stimulate S1P receptors.

Leukocyte Recruitment and Positioning by S1P Receptors

Among the five known S1P receptors $S1P_1$ is the predominant receptor that induces cell migration in many different immune cells including mature T and B lymphocytes and their haematopoietic progenitors, DCs, osteoclasts, and mast cells. Emigration of B cells from the bone marrow and immigration of thymic progenitor cells into thymic niches are partially dependent on $S1P_1$ (Allende et al. 2010; Gossens et al. 2009; Pereira et al. 2010). Trafficking of osteoclast precursors to the bone surface is also mediated by $S1P_1$ (Ishii et al. 2009). Recent data suggest that chemorepulsion away from high S1P levels in blood mediated by $S1P_2$ supports the movement of osteoclast precursors to the bone surface by preventing their migration to high S1P levels in blood, allowing them to migrate towards low S1P levels on the bone surface (Ishii et al. 2010). Migration to and positioning within spleen and lymph nodes of DCs in response to S1P is mediated predominantly by $S1P_1$, while $S1P_3$ signalling can contribute to $S1P_1$ mediated chemotaxis depending on the developmental stage and origin of DCs (König et al. 2010; Rathinasamy et al. 2010). Mast cells migrate in response to S1P via stimulation of the $S1P_1$ receptor, but degranulation is mediated by $S1P_2$ activation (Oskeritzian et al. 2010). Some other cells of the innate immune system use different S1P receptors for migration. Natural killer (NK) cells for example use $S1P_5$ to emigrate from lymph nodes and bone marrow (Jenne et al. 2009; Walzer et al. 2007). While surface expression of the $S1P_1$ receptor is counterregulated by CD69 expression which leads to the temporal retention of activated lymphocytes in lymphoid organs (Bankovich et al. 2010; Shiow et al. 2006), $S1P_5$ expression is not influenced by CD69 and allows NK cells to reach inflamed sites via circulation right after their activation. Trafficking of neutrophils does not seem to be dependent on $S1P_1$ receptor signalling either, but in SGPL1 and $S1P_4$ double-deficient mice the amount of circulating neutrophils in peripheral blood is significantly decreased compared to SGPL1 single knockout mice (Allende et al. 2011). This phenotype however, is likely an indirect effect due to lower amounts of pro-inflammatory cytokines in the double-deficient mice. The exact role of S1P receptors for macrophage trafficking is also not clear. Recent studies suggest that $S1P_2$ and $S1P_3$ are involved in the pro-inflammatory activities of macrophages in ApoE deficient mice as a model for atherosclerosis (Keul et al. 2011; Skoura et al. 2011; Wang et al. 2010). Whether or not S1P and $S1P_{2/3}$ play a direct role in the recruitment of macrophages to inflamed sites remains to be determined. An anti-mycobacterial activity of S1P in macrophages was reported after overexpression of SphK1, which rendered them more resistant to *Mycobacterium smegmatis* infection due to enhanced generation of nitric oxide and enhanced expression of the antimicrobacterial proteins inducible nitric oxide synthase, phosphoprotein pp38, and lysosomal-associated membrane protein-2 (Prakash et al. 2010).

The Interplay of Chemokines and S1P in Spleen and Lymph Nodes

As discussed so far, S1P and S1P receptors can influence the migration pattern of many adaptive and innate immune cells by providing a direct chemotactic stimulus or by indirectly altering receptor expression profiles and adhesion molecules by autocrine and paracrine cell activation and modulation of cytokine profiles. A third way of S1P and its receptors to alter immune cell positioning is a vivid crosstalk with the chemokine system. Chemokines guide lymphocytes to enter tissues, which can be sites of inflammation, peripheral tissues, or lymphoid organs. Homing chemokines like CCL19 and CCL21 for T cells and CXCL13 for B cells enable lymphocytes to enter T and B cell zones in lymphoid organs via the specific chemokine receptors CXCR5 on B cells and CCR7 on T cells (Förster et al. 1996, 1999). Chemokines and chemokine receptors provide the major admittance signals for B and T cells to enter lymph nodes. On the other hand, S1P in blood and lymph and the $S1P_1$ receptor on T and B cells serve as the major exit-mediating molecules (Matloubian et al. 2004; Pappu et al. 2007). However, chemokines not only guide circulating T and B cells in lymphoid organs, they also delay their exit via S1P and the $S1P_1$ receptor. The chemokine receptor CCR7 for example provides a retardation signal for T cells in the T cell zone of lymph nodes and delays their exit (Pham et al. 2008). Even more sophisticated is the proposed crosstalk of $S1P_1$ and CXCR5 in marginal zone B (MZB) cells in the spleen: MZB cells in the marginal zone can pick up antigens from blood and migrate into the B cell follicle via CXCL13, where they pass antigens over to follicular DCs. Since S1P levels are low in the B cell follicle, MZB cells start to express the $S1P_1$ receptor on their cell surface, which overrides the chemokine signal and guides them back into the marginal zone along an increasing S1P gradient. High S1P levels in the marginal zone eventually lead to internalization of the $S1P_1$ receptor, and MZB cells start again to migrate to the B cell follicle via CXCR5 stimulation after antigen capture (Cinamon et al. 2004,

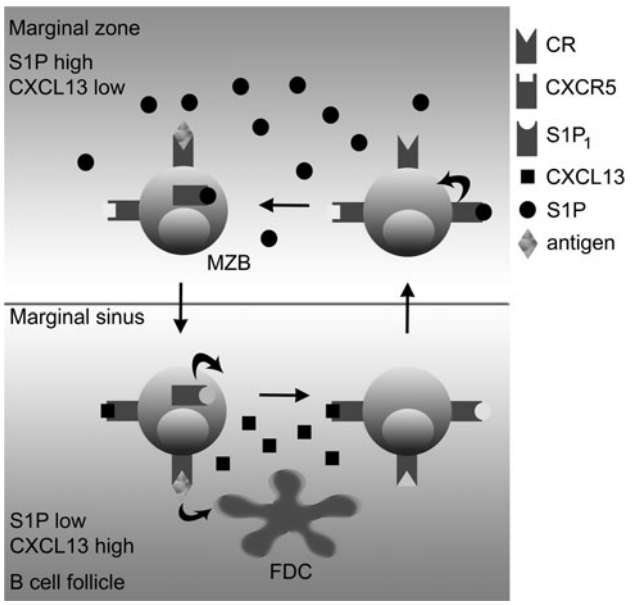

Fig. 3 Model of marginal zone B (MZB) cell shuttling. MZB cells capture complement-opsonized antigens via complement receptors (CR) from blood (*upper left*) and migrate to the B cell follicle along a CXCL13 concentration gradient where they present antigens to follicular dendritic cells (FDC, *lower left*). Due to low S1P concentrations in the B cell follicle, MZB cells increase surface expression of $S1P_1$, which overrules the chemokine signal and prompt MZB cells to migrate back into the marginal zone along increasing S1P concentrations (*lower right*). High S1P concentrations induce internalization of $S1P_1$ and re-sensitize MZB cells for the CXCL13 signal, initiating MZB cells to migrate back into the B cell follicle after antigen capture from blood (*upper right*)

2008) (Fig. 3). This model once again draws upon different S1P concentrations in the microenvironment of B cell follicles and the marginal zone which still needs to be confirmed. Unfortunately, current analytical techniques like liquid chromatography and mass spectrometry do not allow such high-resolution analysis. Further improvements of the chip-based robotic nanoelectrospray ion source for tissue analysis by mass spectrometry may provide further insights in the local tissue distribution of S1P in the future (Kertesz and Van Berkel 2010).

Keeping Lymphocytes in Check: Pharmacological Interventions

Probably the best known compound in the field of S1P receptor modulation is FTY720, which was approved by the American Food and Drug Administration in September 2010 under the brand name Gilenya as the first oral medication for treatment of the autoimmune disease multiple sclerosis (Brinkmann et al. 2010). As outlined in the first chapter of this review, FTY720 is phosphorylated to FTY720-phosphate and serves as an agonist for four of the five known S1P receptors (Brinkmann et al. 2002; Mandala et al. 2002). Its biological activity however, is mostly attributed to its "functional antagonism" on the $S1P_1$ receptor, which induces peripheral blood lymphopenia and immunosuppression (Oo et al. 2007). Recent data suggest that its efficacy in multiple sclerosis is predominantly based on $S1P_1$ receptor modulation in astrocytes and not in lymphocytes where it was first noticed (Choi et al. 2011). Other S1P receptor ligands are currently tested, and it remains tantalizing whether or not $S1P_1$ receptor agonists and antagonists will both be efficacious immunosuppressants, as the current theory would predict.

Besides S1P receptor agonists and antagonists that are also developed for S1P receptors other than $S1P_1$, the development of SGPL1 inhibitors appears to be promising for treatment of rheumatoid arthritis and potentially other autoimmune diseases. Lexicon Pharmaceuticals compound LX2931 is currently tested in phase II clinical trials (Bagdanoff et al. 2010). The fact that lymphocyte counts in the periphery are diminished by a quite selective increase of S1P and sphingosine levels in organs of the lymphatic system renders the occurrence of unwanted side effects in other areas like the cardiovascular or the neuronal system unlikely. But current SGPL1 inhibitors can be neutralized by additional application of vitamin B_6, indicating that they most likely antagonize the coenzyme pyridoxalphosphate rather than SGPL1 itself (Schwab et al. 2005). This strategy implies potential unspecific inhibition of other vitamin B_6-dependent enzymes as well, more than 100 of which are known. A pyridoxine independent SGPL1 specific inhibitor could therefore be a valuable improvement of current drug candidates.

An antibody against S1P was developed by LPath for treatment of age-related macular degeneration (Isonep) and cancer (Asonep) (Sabbadini 2011). It binds and inactivates S1P and therefore prevents S1P receptor stimulation (Wojciak et al. 2009). Application of the anti-S1P antibody in mice induced a mild but prolonged lymphopenia (O'Brien et al. 2009). Although the antibody is very effective in binding and neutralizing S1P, its ability to extract S1P also from RBC as the main repository of S1P in blood leads to substantially increased S1P concentrations in plasma, suggesting that its neutralizing effect in plasma is compromised by the additional supply of S1P from RBC (Sensken et al. 2011). Recent data however, indicate that the anti-S1P antibody may be efficacious in blocking S1P predominantly in the local environment of tissues, although current analytical techniques again impede a direct proof (Sensken et al. 2011). Isonep is currently tested in a phase 2a study and Asonep is planned to follow.

Beyond Migration: S1P in Leukocyte Activation and Differentiation

Although S1P plays a major role in systemic lymphocyte migration and local leukocyte positioning, the functional diversity of this lipid-signalling molecule is much broader. For example, we demonstrated that constitutive T cell specific transgenic expression of $S1P_1$ in mice resulted in depressed T cell functions (Gräler et al. 2005). More recent studies revealed impaired numbers and function of regulatory T cells and reciprocally enhanced development of T helper type 1 (T_H1) cells in T cell specific $S1P_1$ transgenic mice (Liu et al. 2009, 2010). Studies with non-obese diabetic mice indicated that S1P negatively regulates T cell activation via $S1P_1$ stimulation and consequently enhanced expression of hypoxia-inducible factor-1α short isoform I.1 (Srinivasan et al. 2008). Furthermore, it was shown that the S1P-$S1P_1$ axis augments IL-17 production and enhances the number and activity of T_H17 cells (Huang et al. 2007; Liao et al. 2007). Expression of the $S1P_4$ receptor was linked to megakaryocyte differentiation and proplatelet development (Golfier et al. 2010). S1P exerts additional cellular functions in DCs, mast cells, and macrophages, which were extensively reviewed elsewhere (Olivera and Rivera 2011; Rivera et al. 2008; Spiegel and Milstien 2011; Weigert et al. 2009).

Summary

S1P metabolism and the S1P receptor network are important constituents of our immune system. Processes like lymphocyte circulation, leukocyte recruitment and positioning, antigen presentation, and inflammation are influenced by local and systemic S1P levels and by S1P receptors expressed in immune cells. S1P metabolizing enzymes like SphK1 and SphK2, SGPL1, and phosphatases like LPP3 ensure the site-specific supply and elimination of S1P and profoundly influence S1P receptor signalling. Pharmacological modulation of receptors and enzymes involved in S1P signalling therefore emerges as a promising approach for the treatment of immune and autoimmune diseases.

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