Targeting Sphingosine 1-phosphate (S1P) Levels and S1P Receptor Functions for Therapeutic Immune Interventions

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Key Words

Lymphocyte circulation • Thymocyte development • Dendritic cell • Antigen presentation • Marginal zone • Endothelial cell barrier • S1P-lyase • Sphingosine kinase

Abstract

Sphingosine 1-phosphate (S1P) is an important regulator of many different immune functions including lymphocyte circulation, antigen presentation, and T cell development. It stimulates five G protein-coupled receptors designated S1P₁₋₅, which are also expressed by immune cells. S1P receptors couple to different heterotrimeric G proteins including G α i, q, and 12/13, and elicit cellular signalling events by activating the small GTPases Rac and Rho and protein kinases Akt, ERK, and JNK, and by inducing cellular calcium flux and inhibiting cAMP accumulation, amongst others. S1P is the exit signal for lymphocytes leaving lymphoid organs and present in blood and lymph at high nanomolar concentrations due to the S1P-producing activity of sphingosine kinases (SK). The S1P-degrading enzyme S1P-lyase maintains low amounts of S1P in lymphoid organs. Disrupting this concentration difference by S1P receptor agonists

and antagonists like FTY720, SEW2871, and VPC23019, by an anti-S1P antibody, or by inhibiting the S1P-lyase has therapeutic potential for autoimmune diseases like multiple sclerosis (MS) and rheumatoid arthritis and for many other disorders like cancer, fibrosis, inflammation, macular degeneration, diabetic retinopathy, and glaucoma. This report aims to provide a brief overview of concepts, approaches, pharmaceutical compounds, and targets that are currently used to modulate S1P-driven immune functions.

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Regulation of lymphocyte circulation by S1P and immunomodulation by FTY720

One of the first observations leading to our current knowledge of S1P-driven lymphocyte circulation was a pronounced blood lymphopenia and defective recirculation of effector lymphocytes from peripheral lymph nodes after treatment of mice with FTY720, a novel immunosuppressive agent with a largely unknown molecular mechanism at that time [1]. Further studies revealed that this sphingosine analogue is phosphorylated *in vivo*, and FTY720-phosphate serves as an agonist for all S1P receptors ex-

cept S1P₂ [2, 3]. But it turned out that FTY720-phosphate not only activates S1P receptors, it also down-regulates them and consequently renders cells unresponsive to S1P [4]. S1P₁-receptor-deficient fetal liver chimera and T cell specific conditional knockout mice demonstrated that S1P₁ receptor expression in lymphocytes is required for their exit from thymus and secondary lymphoid organs [5, 6]. It was proposed that under normal conditions, endogenous S1P in blood and lymph serves as a stimulus for the S1P₁ receptor, expressed on lymphocytes, to initiate their egress from thymus into blood and from lymph nodes into lymph. This concept was supported with inducible gene deficient mice lacking SK1 and SK2 in all hematopoietic cells, vascular endothelial cells, and liver cells, amongst others [7]. SK1 and SK2 are the only known enzymes that phosphorylate sphingosine and produce S1P [8]. Blood-borne S1P is mainly generated by erythrocytes and vascular endothelial cells [9, 10]. Mice lacking both SK1 and SK2 in these cells do not produce S1P in blood and surprisingly also not in lymph [7]. While the adoptive transfer of bone marrow-derived wild type cells into lethally irradiated inducible sphingosine-kinase-deficient mice replenished S1P in blood, lymph-borne S1P was still lacking [7]. This result suggested a different radiation-resistant source for S1P in lymph, and it was speculated that lymphatic endothelial cells may be the main contributors [9]. These data strongly support the idea that S1P in blood and lymph serve as required exit signals for lymphocytes in thymus and lymph nodes by stimulating the S1P₁ receptor on their cell surface. FTY720-phosphate predominantly accumulates in lymphoid tissues and prematurely down-regulates the S1P₁ receptor on lymphocytes, which subsequently lose their responsiveness to S1P as the exit-mediating signal [11]. As a consequence lymphocytes are stuck in lymphoid organs, naïve T and B cells have lower chances to see their specific antigen, and effector cells do not reach the infected organs anymore. The result is a significant immunosuppression (Fig. 1).

Efficacy of S1P₁ receptor agonists and antagonists - a different view of lymphocyte egress

In addition to the above-mentioned model with S1P serving as an active exit signal, another hypothesis evolved from studies using pharmacological S1P₁ receptor agonists and antagonists instead of genetically engineered mice. Based on the stimulatory activity of FTY720-phosphate

Fig. 1. Proposed mechanism of FTY720-induced immune modulation. FTY720 is taken up by cells and primarily phosphorylated by SK2. FTY720-phosphate is released by cells and activates the S1P₁ receptor on the cell surface. Activation-induced internalization of the S1P₁ receptor subsequently renders cells unresponsive towards the exit signal S1P. Extracellular FTY720-phosphate can be dephosphorylated again by lipid phosphate phosphatase 3 (LPP3).

for the S1P₁ receptor [2, 3] and the difficulty to detect S1P₁ receptor surface expression on lymphocytes [12], it was hypothesized that lymphatic endothelial cells instead of lymphocytes are targeted by FTY720-phosphate. Stimulation of the S1P₁ receptor on lymphatic sinus-lining endothelial cells would establish cell barriers that prevent lymphocytes from exiting [13]. Two-photon microscopy of explanted peripheral lymph nodes treated with the S1P₁ receptor agonist SEW2871 demonstrated emptied marginal sinusoids, while the S1P₁ receptor antagonist VPC23019 alone was inoperative, but reversed the egress blocking effect of the respective agonist [13]. Systemic application of *R* and *S* enantiomers of 3-amino-4-(3-hexylphenylamino)-4-oxobutylphosphonic acid, which are competitive antagonists of the S1P₁ receptor, additionally induced vascular leakage [14], and more recent studies with inducible SK1/2 deficient mice proved a supporting role of S1P in blood for the maintenance of vascular integrity [15]. These studies argue that stimulation of the S1P₁ receptor on lymphatic endothelial cells induces lymphopenia by enhancing endothelial cell barriers, and that S1P in blood and lymph have no active role in lymphocyte exit. While S1P₁ receptor agonists modulate lymphocyte circulation in both presented hypotheses, endogenous S1P and synthetic S1P₁ receptor antagonists would be inoperable for disrupting normal lymphocyte

egress according to the endothelial barrier theory [14]. In fact, all known S1P₁ receptor agonists induce lymphopenia, whereas no reported antagonist has this capability *in vivo* [13, 16]. But this lack of efficacy of S1P₁ receptor antagonists for inducing lymphopenia may just result from insufficient receptor affinity or compound distribution compared to the endogenous ligand S1P. Basically both theories are feasible and can coexist side by side. The contribution of these two mechanisms for lymphopenia induction remains to be determined. Until then the quest for better immunomodulators should also include S1P₁ receptor antagonists and blocking agents for S1P in blood and lymph as potential pharmaceutical candidates.

Anti-S1P antibodies as molecular sponges - a novel concept

In line with the potential usefulness of S1P-blocking reagents, an antibody against S1P was developed and is currently tested in clinical studies for treatment of cancer, fibrosis, inflammation, macular degeneration, diabetic retinopathy, glaucoma, and others. The wide variety of diseases that may be treated with the anti-S1P antibody in the future reflects the broad functional spectrum of S1P *in vivo*. This diversity is ensured primarily by several S1P receptors with different signalling purposes, expressed by many different cell types. The antibody is thought to bind and inactivate S1P, reducing the endogenous pool of bioactive S1P and inhibiting its stimulating activity [18]. In support of the egress-inducing function of endogenous S1P in blood and lymph, application of the anti-S1P antibody induces T and B cell lymphopenia [17]. Although the general function of this antibody as an S1P-blocking reagent is obvious, the contribution of local and systemic activities remains obscure. Erythrocytes serve as a major pool for S1P in blood and ensure a constant supply of S1P in plasma [10]. It is unclear whether or not the anti-S1P antibody can compete with continuous production of S1P by erythrocytes. Furthermore serum albumin and high density lipoproteins are major binding partners for S1P in plasma and likely compete for S1P-binding [19]. On the other hand, the local amount of S1P in tissues is low, and the antibody does not compete with endogenous soluble binding molecules in the interstitium. A thorough investigation of local and systemic contributions of the anti-S1P antibody for lymphopenia induction will potentially provide more insights regarding the exact role of S1P in blood, lymph, and lymphoid tissues for lymphocyte egress.

Fig. 2. Cyclical modulation of S1P₁ surface expression on marginal zone B cells (MZB). (1) MZB take up blood-borne antigens in the marginal zone. High S1P and low CXCL13 levels in the marginal zone result in low S1P₁ receptor surface expression and high CXCR5 signalling, and MZB consequently migrate to the B cell follicle with high CXCL13 expression. (2) Upon arrival in the B cell follicle, they deliver the loaded antigens to follicular dendritic cells (FDC). (3) Low S1P and high CXCL13 levels entail the up-regulation of S1P₁ receptor surface expression and down-regulation of CXCR5 signalling. (4) MZB consequently migrate back to the marginal zone with high S1P and low CXCL13 levels.

Marginal zone B cells enable blood antigen presentation by cyclical cell surface expression of the S1P₁ receptor

Besides its role for regulating systemic lymphocyte circulation, the S1P₁ receptor is also important for local lymphocyte positioning in lymphoid tissues. Particularly marginal zone B cells are dependent on a cyclical modulation of S1P₁ receptor cell surface expression to shuttle between the marginal zone of the spleen and B cell follicles [20]. B cells deficient for the S1P₁ receptor do not migrate into the marginal zone anymore. This defect is

compensated in S1P₁ and CXCR5 double-deficient B cells, demonstrating that S1P signalling via the S1P₁ receptor overrides chemokine signalling via the CXCR5 receptor [20, 21]. S1P₁ receptor cell surface expression however turned out to be variable, resulting in constant shuttling of marginal zone B cells from the marginal zone into the B cell follicle and vice versa. This process is thought to be driven by different S1P concentrations in the marginal zone and in the B cell follicle [20, 21]. High S1P concentrations in the marginal zone induce internalization of the S1P₁ receptor, thereby inducing responsiveness to the chemokine and CXCR5 ligand CXCL-13. Marginal zone B cells subsequently migrate to the CXCL-13 expressing B cell zone. S1P concentrations are thought to be low in B cell follicles, resulting in up-regulation of S1P₁ receptor cell surface expression again. The CXCL-13 chemokine signal is overruled by increasing S1P₁ receptor cell surface expression and signalling, and marginal zone B cells migrate back into the marginal zone (Fig. 2). By this mechanism, marginal zone B cells pick up antigens from blood in the marginal zone and transport them into the B cell follicle, where follicular dendritic cells incorporate, process, and present them to patrolling lymphocytes [20]. S1P₁ receptor agonists like FTY720-phosphate abolish this transport of blood-borne antigens by prolonged internalization of the S1P₁ receptor and consequently interfere with efficient presentation of blood-borne antigens, which likely enhances their immunosuppressive function.

Altered T cell development by S1P and the S1P₁ receptor

T cell development starts from early T cell progenitors (ETP) in the thymus. The thymic ETP pool is constantly replenished from the blood. ETP immigration is a periodic event and dependent on the expression of functional P-selectin ligands on ETP. Recent data suggest that expression of thymic P-selectin and the chemokine CCL-25 is regulated by the S1P concentration in blood [22]. High plasma S1P-levels correlated with high P-selectin expression in thymi and vice versa. Plasma S1P concentration and thymic P-selectin expression change periodically and may therefore be the basis for the observed periodicity of thymic ETP receptivity. The amount of S1P in plasma was dependent on the amount of peripheral lymphocytes in blood, with lymphopenia causing increased S1P-concentrations in plasma [22]. Accordingly, low numbers of circulating lymphocytes increased the receptivity

of ETP in thymus. S1P in plasma may therefore also be involved in the regulation of the T cell repertoire.

A later step in thymocyte development is the maturation of regulatory T cells. These cells are important for maintaining immunological tolerance by suppressing T cell-mediated immune responses. S1P₁ receptor signalling impaired the development of regulatory T cells and caused autoimmunity in T cell-specific S1P₁-receptor-transgenic mice, while T cell-specific conditional S1P₁ receptor knockout mice elicited increased numbers of regulatory T cells [23]. In addition to altered numbers of regulatory T cells in S1P₁ receptor transgenic and conditional knockout mice, S1P₁ receptor deficiency resulted in enhanced suppressive function, whereas overexpression of the S1P₁ receptor severely impaired the suppressive activity of these cells [23]. Constitutive high expression of S1P₁ in T cells may therefore result in a higher risk for autoimmune diseases due to reduced numbers of regulatory T cells, while low T cell expression of S1P₁ could enhance immunological tolerance.

Treatment of autoimmune diseases by S1P-lyase inhibition

A different approach for treatment of autoimmune diseases emerged from studies demonstrating that inhibition of the S1P-lyase causes lymphopenia similar to treatment with S1P₁ receptor agonists [24]. S1P-lyase catalyzes the irreversible degradation of intracellular S1P to hexadecenal and phosphoethanolamine. It is the major S1P-degrading enzyme in tissue cells and constantly active. As a result, S1P concentrations in tissues are maintained at very low levels. Inhibition of the S1P-lyase entails the accumulation of S1P in tissues, including lymphoid tissues [24]. Similar to treatment with FTY720, accumulation of S1P in lymphoid tissues induces premature internalization of the exit-signal-sensing S1P₁ receptor on lymphocytes, rendering them unresponsive to S1P and preventing their egress from thymus and lymph nodes [25]. Furthermore the gradient between high S1P-levels in blood and lymph and low S1P-levels in lymphoid tissues is neutralized, and activation of the S1P₁ receptor on lymphatic endothelial cells establishes cell barriers that additionally prevent lymphocytes from exiting [13]. The result is a severe T and B cell lymphopenia. A S1P-lyase inhibitor is currently tested in clinical trials for treatment of rheumatoid arthritis [26]. While partial inhibition of the S1P-lyase predominantly induced lymphopenia, S1P-lyase deficiency in mice additionally causes severe thymus at-

rophy and early death [27]. Thymus atrophy is caused by enhanced apoptosis of all developing thymocytes due to an increase of the pro-apoptotic factor ceramide [27]. The reason for the observed early lethality is not known. S1P-lyase inhibition should therefore be carefully monitored for the accumulation of ceramides and the occurrence of severe side effects.

Treatment of autoimmune diseases by FTY720

FTY720 passed phase II clinical trials for treatment of the autoimmune disorder MS [28]. The initial concept was to reduce the amount of circulating lymphocytes, as outlined above, in order to prevent their infiltration and disastrous effector functions against self antigens in peripheral tissues like brain [29]. However, it turned out that FTY720 is most effective for MS treatment at dosages that are suboptimal for peripheral blood lymphocyte depletion. Since many S1P receptors including S1P₁ are also expressed in neuronal cells, FTY720 may exert additional direct effects on different S1P receptors expressed in the brain [30]. S1P receptors are present in oligodendrocytes, neurons, astrocytes, and microglia [31–34]. It was recently suggested that FTY720 could also reduce astrogliosis by down-regulating the S1P₁ receptor on astrocytes [30]. The therapeutic effect of FTY720 on MS may therefore derive from both, its systemic immunomodulatory activity and its local activity on neuronal cells in the brain. This is, of course, only possible because of the ubiquitous presence of S1P receptors in many different systems including lymphocytes and neuronal cells. And the low specificity of FTY720 across different S1P receptors, which was proposed as the reason for unwanted side effects like bradycardia [35], may as well be beneficial for the overall therapeutic effect in MS treatment.

Modulating dendritic and natural killer cell function

Lymphocytes are not the only immune cells that respond to S1P. Dendritic cells upregulate S1P₁ and S1P₃ during maturation [36], and NK cells express the S1P₅ receptor at high levels [37]. The S1P receptor agonist FTY720-phosphate impairs dendritic cell mobilization from peripheral tissues into secondary lymphoid organs, their local positioning within spleen, and their potency to

activate naive and effector T cells [38, 39]. This was shown for skin- and lung-derived dendritic cells. Local targeting strategies for dendritic cells in the lung turned out to be very effective in experimental asthma [39]. Inhalation of FTY720 suppressed eosinophilic airway inflammation and bronchial hyperresponsiveness without causing systemic lymphopenia [39]. S1P and FTY720 were similarly potent in inhibiting the capacity of dendritic cells to activate and polarize antigen-specific T cells *in vitro* [39]. It is therefore possible that activation of S1P receptors, presumably S1P₁, accounts for the described inhibition of allergic airway inflammation. However, it cannot be completely ruled out that activation-induced down-regulation of S1P receptors is the underlying mechanism by which both FTY720 and S1P suppress asthma. Nonetheless, inhibition of dendritic cell migration and lymphocyte activation by *local* application of S1P receptor agonists could help to gain relatively high specificity for treatment of certain diseases like asthma by restricting the site of action and consequently avoiding systemic effects. Whether or not potent S1P receptor antagonists are also active in this scenario remains to be investigated.

While T and B cells typically emigrate from lymphoid tissues after stimulation of the S1P₁ receptor, natural killer cells exit bone marrow and lymph nodes by activating the S1P₅ receptor on their cell surface [40]. Natural killer cells are innate-like lymphocytes and active in the early phases of infection. The use of S1P₅ instead of S1P₁ as exit-mediating receptor enables them to emigrate from lymph nodes even after activation and CD69 up-regulation, which typically results in down-regulation of S1P₁ receptor cell surface expression [41]. S1P₅ receptor antagonists could therefore be valuable pharmaceutical compounds for treatment of specific infectious diseases that are dependent on natural killer cell function like herpes virus or *Leishmania* parasite infections.

S1P receptor function and signalling

S1P₁ was the first identified receptor of the *endothelial differentiation gene (edg)* family [42]. It is the only S1P receptor that solely couples to the G α i (G_i) class of trimeric G proteins [43]. It was reported to induce cellular calcium flux and to activate extracellular signal-regulated kinases (ERK) [44], the small GTPase Rac, the protein kinase Akt [45], and STAT1, a member of the Signal Transducers and Activators of Transcription family of transcription factors [46]. Its main function

on immune cells is chemotaxis [47]. S1P₁ is also involved in the maintenance of the vascular integrity [15]. S1P₂ couples to G_i, G_q, and G_{12/13} and was described as a counteracting receptor for S1P-mediated chemotaxis [43, 48]. Deficiency of S1P₂ in mice causes deafness and diffuse large B cell lymphoma formation [49-51]. The main signalling pathways elicited by S1P₂ include activation of ERK, c-Jun, c-Fos, Rho, and Rac, and formation of cAMP and cellular calcium flux [49]. S1P₃ also mediates chemotaxis and regulates the splenic marginal sinus organization [52, 53]. It couples to G_i, G_q, and G_{12/13}, and elicits cellular signalling via calcium flux, endothelial nitric oxide synthase (eNOS), Akt, ERK, and Rho [43, 54]. S1P₄ is predominantly expressed in immune cells [55]. The exact function of this receptor is still not known. S1P₄ couples to G_i and G_{12/13} and activates phospholipase C (PLC), ERK and Rho [56]. Cellular events mediated by S1P₄ include increased peripheral stress fiber formation, cell rounding, and cell motility [56]. S1P₅ also couples to G_i and G_{12/13} [57]. It influences natural killer (NK) cell trafficking, inhibits cAMP accumulation and ERK phosphorylation, and activates c-Jun N-terminal kinases (JNK) [37, 40, 57].

Summary

S1P and its receptors are powerful regulators of various critical immune functions. Starting with the original observation that S1P and its receptor S1P₁ are required for unobstructed lymphocyte circulation, many more cell types like dendritic cells, natural killer cells, and marginal zone B cells were identified to respond to S1P via different receptors like S1P₁, S1P₃, and S1P₅. Targeting these cells with specific S1P receptor agonists or antagonists can modulate specific immune responses locally and systemically by altering migration, differentiation, antigen presentation, and lymphocyte activation. Manipulation of endogenous local and systemic amounts of bioactive S1P by inhibiting metabolic enzymes like the S1P-lyase or by applying S1P-blocking antibodies is a promising approach for treatment of autoimmune and inflammatory diseases.

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References

- 1 Pinschewer DD, Ochsenbein AF, Odermatt B, Brinkmann V, Hengartner H, Zinkernagel RM: Fty720 immunosuppression impairs effector t cell peripheral homing without affecting induction, expansion, and memory. *J Immunol* 2000;164:5761-5770.
- 2 Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thornton R, Shei GJ, Card D, Keohane C, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H: Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* 2002;296:346-349.
- 3 Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumruker T, Hiestand P, Foster CA, Zollinger M, Lynch KR: The immune modulator fty720 targets sphingosine 1-phosphate receptors. *J Biol Chem* 2002;277:21453-21457.
- 4 Gräler MH, Goetzl EJ: The immunosuppressant fty720 down-regulates sphingosine 1-phosphate g-protein-coupled receptors. *Faseb J* 2004;18:551-553.
- 5 Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, Allende ML, Proia RL, Cyster JG: Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on slp receptor 1. *Nature* 2004;427:355-360.
- 6 Allende ML, Dreier JL, Mandala S, Proia RL: Expression of the sphingosine 1-phosphate receptor, slp1, on t-cells controls thymic emigration. *J Biol Chem* 2004;279:15396-15401.
- 7 Pappu R, Schwab SR, Cornelissen I, Pereira JP, Regard JB, Xu Y, Camerer E, Zheng YW, Huang Y, Cyster JG, Coughlin SR: Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science* 2007;316:295-298.
- 8 Mizugishi K, Yamashita T, Olivera A, Miller GF, Spiegel S, Proia RL: Essential role for sphingosine kinases in neural and vascular development. *Mol Cell Biol* 2005;25:11113-11121.
- 9 Venkataraman K, Lee YM, Michaud J, Thangada S, Ai Y, Bonkovsky HL, Parikh NS, Habrukowich C, Hla T: Vascular endothelium as a contributor of plasma sphingosine 1-phosphate. *Circ Res* 2008;102:669-676.
- 10 Hänel P, Andréani P, Gräler MH: Erythrocytes store and release sphingosine 1-phosphate in blood. *Faseb J* 2007;21:1202-1209.
- 11 Sensken SC, Bode C, Gräler MH: Accumulation of fingolimod (fty720) in lymphoid tissues contributes to prolonged efficacy. *J Pharmacol Exp Ther* 2009;328:963-969.
- 12 Singer II, Tian M, Wickham LA, Lin J, Matheravidathu SS, Forrest MJ, Mandala S, Quackenbush EJ: Sphingosine-1-phosphate agonists increase macrophage homing, lymphocyte contacts, and endothelial junctional complex formation in murine lymph nodes. *J Immunol* 2005;175:7151-7161.

- 13 Wei SH, Rosen H, Matheu MP, Sanna MG, Wang SK, Jo E, Wong CH, Parker I, Cahalan MD: Sphingosine 1-phosphate type 1 receptor agonism inhibits transendothelial migration of medullary t cells to lymphatic sinuses. *Nat Immunol* 2005;6:1228-1235.
- 14 Sanna MG, Wang SK, Gonzalez-Cabrera PJ, Don A, Marsolais D, Matheu MP, Wei SH, Parker I, Jo E, Cheng WC, Cahalan MD, Wong CH, Rosen H: Enhancement of capillary leakage and restoration of lymphocyte egress by a chiral s1p1 antagonist in vivo. *Nat Chem Biol* 2006;2:434-441.
- 15 Camerer E, Regard JB, Cornelissen I, Srinivasan Y, Duong DN, Palmer D, Pham TH, Wong JS, Pappu R, Coughlin SR: Sphingosine-1-phosphate in the plasma compartment regulates basal and inflammation-induced vascular leak in mice. *J Clin Invest* 2009;119:1871-1879.
- 16 Foss FW, Jr., Snyder AH, Davis MD, Rouse M, Okusa MD, Lynch KR, Macdonald TL: Synthesis and biological evaluation of gamma-aminophosphonates as potent, subtype-selective sphingosine 1-phosphate receptor agonists and antagonists. *Bioorg Med Chem* 2007;15:663-677.
- 17 O'Brien N, Jones ST, Williams DG, Cunningham HB, Moreno K, Visentin B, Gentile A, Vekich J, Shestowsky W, Hiraawa M, Matteo R, Cavalli A, Grotjahn D, Grant M, Hansen G, Campbell MA, Sabbadini R: Production and characterization of monoclonal anti-sphingosine-1-phosphate antibodies. *J Lipid Res* 2009;50:2245-2257.
- 18 Sabbadini RA: Targeting sphingosine-1-phosphate for cancer therapy. *Br J Cancer* 2006;95:1131-1135.
- 19 Yatomi Y: Plasma sphingosine 1-phosphate metabolism and analysis. *Biochim Biophys Acta* 2008;1780:606-611.
- 20 Cinamon G, Zachariah MA, Lam OM, Foss FW, Jr., Cyster JG: Follicular shuttling of marginal zone b cells facilitates antigen transport. *Nat Immunol* 2008;9:54-62.
- 21 Cinamon G, Matloubian M, Lesneski MJ, Xu Y, Low C, Lu T, Proia RL, Cyster JG: Sphingosine 1-phosphate receptor 1 promotes b cell localization in the splenic marginal zone. *Nat Immunol* 2004;5:713-720.
- 22 Gossens K, Naus S, Corbel SY, Lin S, Rossi FM, Kast J, Ziltener HJ: Thymic progenitor homing and lymphocyte homeostasis are linked via s1p-controlled expression of thymic p-selectin/ccl25. *J Exp Med* 2009;206:761-778.
- 23 Liu G, Burns S, Huang G, Boyd K, Proia RL, Flavell RA, Chi H: The receptor s1p1 overrides regulatory t cell-mediated immune suppression through akt-mtor. *Nat Immunol* 2009;10:769-777.
- 24 Schwab SR, Pereira JP, Matloubian M, Xu Y, Huang Y, Cyster JG: Lymphocyte sequestration through s1p lyase inhibition and disruption of s1p gradients. *Science* 2005;309:1735-1739.
- 25 Lo CG, Xu Y, Proia RL, Cyster JG: Cyclical modulation of sphingosine-1-phosphate receptor 1 surface expression during lymphocyte recirculation and relationship to lymphoid organ transit. *J Exp Med* 2005;201:291-301.
- 26 Vogel P, Donoviel MS, Read R, Hansen GM, Hazlewood J, Anderson SJ, Sun W, Swaffield J, Oravec T: Incomplete inhibition of sphingosine 1-phosphate lyase modulates immune system function yet prevents early lethality and non-lymphoid lesions. *PLoS One* 2009;4:e4112.
- 27 Weber C, Krueger A, Münk A, Bode C, Van Veldhoven PP, Gräler MH: Discontinued postnatal thymocyte development in sphingosine 1-phosphate-lyase-deficient mice. *J Immunol* 2009;183:4292-4301.
- 28 Comi G, O'Connor P, Montalban X, Antel J, Radue EW, Karlsson G, Pohlmann H, Aradhye S, Kappos L: Phase ii study of oral fingolimod (ft720) in multiple sclerosis: 3-year results. *Mult Scler* 2010;16:197-207.
- 29 Mehling M, Brinkmann V, Antel J, Bar-Or A, Goebels N, Vedrine C, Kristofic C, Kuhle J, Lindberg RL, Kappos L: Fty720 therapy exerts differential effects on t cell subsets in multiple sclerosis. *Neurology* 2008;71:1261-1267.
- 30 Brinkmann V: Fty720 (fingolimod) in multiple sclerosis: Therapeutic effects in the immune and the central nervous system. *Br J Pharmacol* 2009;158:1173-1182.
- 31 Kimura A, Ohmori T, Ohkawa R, Madoiwa S, Mimuro J, Murakami T, Kobayashi E, Hoshino Y, Yatomi Y, Sakata Y: Essential roles of sphingosine 1-phosphate/s1p1 receptor axis in the migration of neural stem cells toward a site of spinal cord injury. *Stem Cells* 2007;25:115-124.
- 32 Jaillard C, Harrison S, Stankoff B, Aigrot MS, Calver AR, Duddy G, Walsh FS, Pangalos MN, Arimura N, Kaibuchi K, Zalc B, Lubetzki C: Edg8/s1p5: An oligodendroglial receptor with dual function on process retraction and cell survival. *J Neurosci* 2005;25:1459-1469.
- 33 Sorensen SD, Nicole O, Peavy RD, Montoya LM, Lee CJ, Murphy TJ, Traynelis SF, Hepler JR: Common signaling pathways link activation of murine par-1, lpa, and s1p receptors to proliferation of astrocytes. *Mol Pharmacol* 2003;64:1199-1209.
- 34 Wu YP, Mizugishi K, Bektas M, Sandhoff R, Proia RL: Sphingosine kinase 1/s1p receptor signaling axis controls glial proliferation in mice with sandhoff disease. *Hum Mol Genet* 2008;17:2257-2264.
- 35 Sanna MG, Liao J, Jo E, Alfonso C, Ahn MY, Peterson MS, Webb B, Lefebvre S, Chun J, Gray N, Rosen H: Sphingosine 1-phosphate (s1p) receptor subtypes s1p1 and s1p3, respectively, regulate lymphocyte recirculation and heart rate. *J Biol Chem* 2004;279:13839-13848.
- 36 Czeloth N, Bernhardt G, Hofmann F, Genth H, Forster R: Sphingosine-1-phosphate mediates migration of mature dendritic cells. *J Immunol* 2005;175:2960-2967.
- 37 Walzer T, Chiossone L, Chaix J, Calver A, Carozzo C, Garrigue-Antar L, Jacques Y, Baratin M, Tomasello E, Vivier E: Natural killer cell trafficking in vivo requires a dedicated sphingosine 1-phosphate receptor. *Nat Immunol* 2007;8:1337-1344.
- 38 Czeloth N, Schippers A, Wagner N, Muller W, Kuster B, Bernhardt G, Forster R: Sphingosine-1 phosphate signaling regulates positioning of dendritic cells within the spleen. *J Immunol* 2007;179:5855-5863.
- 39 Idzko M, Hammad H, van Nimwegen M, Kool M, Muller T, Soullie T, Willart MA, Hijdra D, Hoogsteden HC, Lambrecht BN: Local application of fty720 to the lung abrogates experimental asthma by altering dendritic cell function. *J Clin Invest* 2006;116:2935-2944.
- 40 Jenne CN, Enders A, Rivera R, Watson SR, Bankovich AJ, Pereira JP, Xu Y, Roots CM, Beilke JN, Banerjee A, Reiner SL, Miller SA, Weinmann AS, Goodnow CC, Lanier LL, Cyster JG, Chun J: T-bet-dependent s1p5 expression in nk cells promotes egress from lymph nodes and bone marrow. *J Exp Med* 2009;206:2469-2481.
- 41 Shio LR, Rosen DB, Brdickova N, Xu Y, An J, Lanier LL, Cyster JG, Matloubian M: Cd69 acts downstream of interferon-alpha/beta to inhibit s1p1 and lymphocyte egress from lymphoid organs. *Nature* 2006;440:540-544.
- 42 Hla T, Maciag T: An abundant transcript induced in differentiating human endothelial cells encodes a polypeptide with structural similarities to g-protein-coupled receptors. *J Biol Chem* 1990;265:9308-9313.
- 43 Windh RT, Lee MJ, Hla T, An S, Barr AJ, Manning DR: Differential coupling of the sphingosine 1-phosphate receptors edg-1, edg-3, and h218/edg-5 to the g(i), g(q), and g(12) families of heterotrimeric g proteins. *J Biol Chem* 1999;274:27351-27358.
- 44 Sensken SC, Stäubert C, Keul P, Levkau B, Schöneberg T, Gräler MH: Selective activation of g alpha i mediated signaling of s1p3 by fty720-phosphate. *Cell Signal* 2008;20:1125-1133.

- 45 Gonzalez E, Nagiel A, Lin AJ, Golan DE, Michel T: Small interfering rna-mediated down-regulation of caveolin-1 differentially modulates signaling pathways in endothelial cells. *J Biol Chem* 2004;279:40659-40669.
- 46 Weis N, Weigert A, von Knethen A, Brune B: Heme oxygenase-1 contributes to an alternative macrophage activation profile induced by apoptotic cell supernatants. *Mol Biol Cell* 2009;20:1280-1288.
- 47 Gräler M, Goetzl EJ: Activation-regulated expression and chemotactic function of sphingosine 1-phosphate receptors in mouse splenic t cells. *Faseb J* 2002;16:1874-1878.
- 48 Sanchez T, Thangada S, Wu MT, Kontos CD, Wu D, Wu H, Hla T: Pten as an effector in the signaling of antimigratory g protein-coupled receptor. *Proc Natl Acad Sci U S A* 2005;102:4312-4317.
- 49 Cattoretti G, Mandelbaum J, Lee N, Chaves AH, Mahler AM, Chadburn A, Dalla-Favera R, Pasqualucci L, MacLennan AJ: Targeted disruption of the slp2 sphingosine 1-phosphate receptor gene leads to diffuse large b-cell lymphoma formation. *Cancer Res* 2009;69:8686-8692.
- 50 Herr DR, Grillet N, Schwander M, Rivera R, Muller U, Chun J: Sphingosine 1-phosphate (slp) signaling is required for maintenance of hair cells mainly via activation of slp2. *J Neurosci* 2007;27:1474-1478.
- 51 Kono M, Belyantseva IA, Skoura A, Frolenkov GI, Starost MF, Dreier JL, Lidington D, Bolz SS, Friedman TB, Hla T, Proia RL: Deafness and stria vascularis defects in slp2 receptor-null mice. *J Biol Chem* 2007;282:10690-10696.
- 52 Sinha RK, Park C, Hwang IY, Davis MD, Kehrl JH: B lymphocytes exit lymph nodes through cortical lymphatic sinusoids by a mechanism independent of sphingosine-1-phosphate-mediated chemotaxis. *Immunity* 2009;30:434-446.
- 53 Girkontaite I, Sakk V, Wagner M, Borggreffe T, Tedford K, Chun J, Fischer KD: The sphingosine-1-phosphate (slp) lysophospholipid receptor slp3 regulates madcam-1+ endothelial cells in splenic marginal sinus organization. *J Exp Med* 2004;200:1491-1501.
- 54 Nofer JR, van der Giet M, Tolle M, Wolinska I, von Wnuck Lipinski K, Baba HA, Tietge UJ, Godecke A, Ishii I, Kleuser B, Schafers M, Fobker M, Zidek W, Assmann G, Chun J, Levkau B: Hdl induces no-dependent vasorelaxation via the lysophospholipid receptor slp3. *J Clin Invest* 2004;113:569-581.
- 55 Gräler MH, Bernhardt G, Lipp M: Edg6, a novel g-protein-coupled receptor related to receptors for bioactive lysophospholipids, is specifically expressed in lymphoid tissue. *Genomics* 1998;53:164-169.
- 56 Gräler MH, Grosse R, Kusch A, Kremmer E, Gudermann T, Lipp M: The sphingosine 1-phosphate receptor slp4 regulates cell shape and motility via coupling to gi and g12/13. *J Cell Biochem* 2003;89:507-519.
- 57 Malek RL, Toman RE, Edsall LC, Wong S, Chiu J, Letterle CA, Van Brocklyn JR, Milstien S, Spiegel S, Lee NH: Nrg-1 belongs to the endothelial differentiation gene family of g protein-coupled sphingosine-1-phosphate receptors. *J Biol Chem* 2001;276:5692-5699.