REVIEW

Arch. Immunol. Ther. Exp., 2006, **54**, 239–251
PL ISSN 0004-069X

DOI 10.1007/s00005-006-0028-9

The role of the lysophospholipid sphingosine 1-phosphate in immune cell biology

Henrik von Wenckstern*, Karsten Zimmermann* and Burkhard Kleuser

Institute of Pharmacy, Pharmacology and Toxicology, Freie Universität Berlin, Germany

Received: 2006.03.14, **Accepted:** 2006.05.02, **Published online first:** 2006.07.10

Abstract

Sphingosine 1-phosphate (S1P) has been shown to be a bioactive lipid mediator intimately involved in mediating a variety of immunological processes. In particular, S1P regulates lymphocyte cell trafficking between the lymphatic system and the blood. The lysophospholipid signals mainly through five related G protein-coupled receptor subtypes, termed $S1P_1$ to $S1P_5$. $S1P_1$ seems to play an essential role in cell trafficking, as this receptor subtype promotes the egress of T and B cells from secondary lymphatic organs. This $S1P_1$-mediated migratory response is a consequence of different S1P levels in the serum and lymphatic organs. In addition to its direct effects on lymphocyte motility, S1P strengthens cell barrier integrity in sinus-lining endothelial cells, thereby reducing lymphocyte egress out of lymph nodes. Furthermore, S1P modulates cytokine profiles in T and dendritic cells, resulting in an elevated differentiation of T helper-2 cells during the T cell activation process. It is of interest that the mode of molecular action of the novel immunomodulator FTY720 interferes with the signaling of S1P. After phosphorylation, FTY720 shares structural similarity with S1P, but in contrast to the natural ligand, phosphorylated FTY720 induces a prolonged internalization of $S1P_1$, resulting in an impaired S1P-mediated migration of lymphocytes.

Key words: sphingolipids, sphingosine 1-phosphate, FTY720, immunosuppression, T cell, dendritic cell.

Abbreviations: APC – antigen-presenting cell, DC – dendritic cell, Ec – endothelial cell, FTY720-P – phosphorylated FTY720, GPCR – G protein-coupled receptor, GVD – graft vessel disease, HEV – high endothelial venule, IFN – interferon, IL – interleukin, MLR – mixed lymphocyte reaction, MMF – mycophenolate mofetil, NOD – non-obese diabetic mice, regT cell – regulatory T cell, S1P – sphingosine 1-phosphate, SLO – secondary lymphatic organ, SphK – sphingosine kinase, TH cell – T helper cell.

* These authors contributed equally to this work.

Corresponding author: Burkhard Kleuser, Institute of Pharmacy, Pharmacology and Toxicology, Freie Universität Berlin, Königin-Luise-Str. 2+4, D-14195 Berlin, Germany tel.: +49 308 385-45-61, fax: +49 308 385-43-99, e-mail: kleuser@zedat.fu-berlin.de

INTRODUCTION

Sphingolipids were discovered in 1874 by Johann Thudichum [81] by fractional crystallization of ethanolic brain extracts. Although this lipid class shows a great structural diversity and complexity, the characteristic feature of all sphingolipids is the presence of a sphingoid backbone. In mammalian cells this is normally sphingosine ((2S,3R,4E)-2-amino-4-octadecene-1,3-diol). For almost a century, sphingolipids were only regarded as structural components of lipid bilayers, such as biological membranes. However, a milestone was passed with the discovery of high-affinity receptors for the sphingolipid metabolite sphingosine 1-phosphate (S1P) [39]. Moreover, the complexity of S1P signaling was further enhanced as the lysophospholipid S1P was also identified as an intracellularly acting biological mediator. Nonetheless, it is not astonishing that S1P mediates a variety of cellular functions, such as proliferation, migration, survival, adhesion, differentiation, and morphogenesis [11, 76, 100]. These important and tightly regulated functions influence several biological processes, such as angiogenesis, wound healing, neurogenesis, and immune cell regulation. The significance of S1P in especially immune cell regulation became obvious when it was discovered that the novel immunosuppressive drug FTY720 causes lymphopenia via S1P signaling [46]. The rapidly expanding literature on S1P and analogous compounds such as FTY720 on immune cell biology is reviewed here.

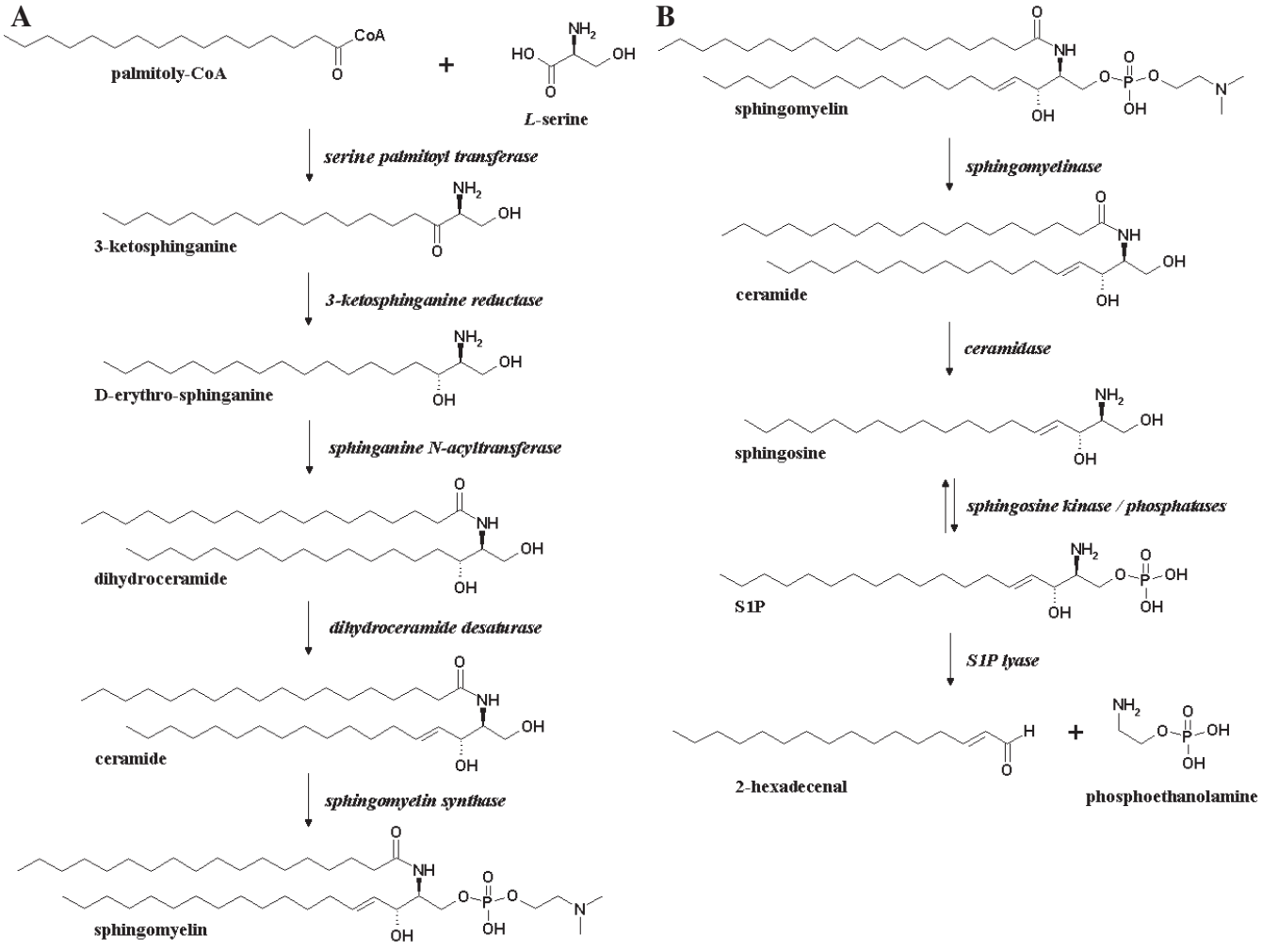

Fig. 1. Biosynthesis (**A**) and degradation (**B**) of sphingomyelin. Sphingosine, the predominant sphingoid base of mammalian sphingolipids, is generated via the intracellular ceramide pathway, where ceramide is formed through *de novo* biosynthesis or degradation of the cell-membrane constituent sphingomyelin. SphKs catalyze the formation of S1P from sphingosine. S1P can then be metabolized by S1P lyase to hexadecenal and phosphoethanolamine. Conversely, S1P-specific and non-specific phosphate phosphohydrolases regenerate sphingosine by dephosphorylating S1P.

BIOSYNTHESIS OF S1P

The *de novo* synthesis of sphingolipids, shown in Fig. 1, is initiated by the condensation of *L*-serine and palmitoyl-CoA to form 3-ketosphinganine, a process that is catalyzed by the enzyme serine palmitoyl transferase [27]. The product is then reduced by the enzyme 3-ketosphinganine reductase in a NADPH-dependent manner, resulting in *D*-erythro-sphinganine [77]. A further step in the biosynthesis is the acylation of sphinganine to dihydroceramide by sphinganine *N*-acyltransferase [51]. Then desaturation to ceramide takes place in the presence of dihydroceramide desaturase [52]. Complex sphingolipids, such as cerebrosides and gangliosides, can be obtained by the insertion of different substituents at the 1-hydroxyl position of ceramide, these ranking from glucose or galactose to polysaccharide residues, even those containing sialic acid moieties in the carbohydrated group [28]. To form sphingomyelin, a phospholipid with a sphingoid backbone, the phosphocholine group from phosphatidylcholine is transferred to the hydroxyl group of ceramide, this step being catalyzed by sphingomyelin synthase [28].

It is of interest that sphingomyelin can also be hydrolyzed by a variety of biological mediators, leading to intracellular enhanced levels of ceramides. These stimulants have often been identified as inducers of apoptosis and differentiation, cell-damaging substances, chemotherapeutics, and proinflammatory cytokines. A central role of ceramide has been proved in several biological effects, such as cell growth arrest, apoptosis, and differentiation [40]. Although ceramide can be recycled back to sphingomyelin, further degradation and metabolizing occur simultaneously. Ceramidases are the most important enzymes in degrading ceramide, yielding the free sphingoid base. Sphingosine kinases (SphK) are responsible for the phosphorylation of sphingosine, resulting in the formation of the biological mediator S1P. Two isoforms of SphK have been discovered, named type 1 and 2, both

widely expressed and regulating the concentration of S1P [36, 41]. A rapidly growing number of agonists have been identified which stimulate SphK1. Most of them are involved in the regulation of cell growth and survival. Platelet-derived growth factor, epidermal growth factor, vascular endothelial growth factor, and nerve growth factor are typical stimuli activating SphK1 [75]. The degradation of S1P is controlled either by specific and non-specific phosphate phosphohydrolases, which hydrolyze S1P to sphingosine [5, 38], or by S1P lyase, which cleaves S1P into hexadecenal and phosphoethanolamine [82].

Especially the metabolic pathway of S1P by S1P lyase seems to play a pivotal role in adjusting S1P levels in different organs and fluids. The concentrations of S1P in lymphatic organs are usually about 10–30 nM, but inhibition of S1P lyase results in a 20- to 30-fold increase in S1P, leading to concentrations comparable to those which can be found in the blood (150–1000 nM) [72]. Intermediate S1P concentrations are present in the lymph, bridging the gap between the low S1P levels in the lymphatic organs and the high amounts of S1P in the blood. The main source of S1P in the blood are cells of the hematopoietic system, predominantly thrombocytes which, unlike other hematopoietic cells, lack the enzyme S1P lyase and, moreover, possess highly active SphK [97]. Sphingosine, as the essential substrate, is either provided by an enhanced thrombocyte-specific uptake from the plasma or by the degradation of sphingomyelin [79]. Upon activation, thrombocytes release their stored S1P, suggesting a possible role of the lysophospholipid in wound healing [83]. Nevertheless, activated thrombocytes are not the exclusive source of S1P, since other blood cells, such as erythrocytes, neutrophils, and mononuclear cells, release constitutively small amounts of S1P, which contribute to a constant level of S1P in the plasma [96].

REGULATED EXPRESSION OF S1P RECEPTORS ON IMMUNE CELLS

The relevance of S1P in cellular processes was emphasized by the identification of its function as a ligand and on a family of G protein-coupled receptors, originally termed endothelial differentiation gene receptors. According to their dominant ligand, these receptors have been renamed $S1P_{1\text{-}5}$ and the lysophosphatic acid receptors $LPA_{1\text{-}3}$ [30]. S1P exerts diverse cellular effects depending on the expression of the specific S1P receptor subtypes and their coupling to separate G proteins. Furthermore, each receptor subtype signals through at least two different G proteins activating various downstream signaling cascades, which have been reviewed elsewhere [30]. In immune cell biology, $S1P_1$ plays a central role since this receptor is not only dominantly expressed, but also regulates cell migration via inhibitory G protein signaling [23] (Table 1). In contrast, $S1P_2$ is only expressed on the surface of mononuclear cells and, moreover, counteracts $S1P_1$-induced migration of dendritic cells (DCs) [65]. Furthermore, $S1P_2$ initiates mast cell degranulation in response to activated FcεRI [32]. Unlike lymphocytes, whose migratory response towards S1P is completely under the control of $S1P_1$ expression, the chemotaxis of DCs is additionally induced by $S1P_3$ [65]. This receptor subtype is also detectable in other immune cells, but its immunological function remains unknown [24]. Besides $S1P_1$, the immunoregulatory functions of S1P in lymphocytes are also transduced by $S1P_4$, promoting rather cytokine modulation and antiproliferative responses than cell motility [87].

ROLE OF S1P ON IMMUNE CELL TRAFFICKING

Migration of T cells

During the maturation of thymocytes in their dedicated organ, $S1P_1$ is barely expressed on the surface of $CD4^-/CD8^-$ thymocytes [1]. In consequence, these cells fail to respond to the S1P gradient which exists between the thymus and medullary blood vessels. Additionally, the stromal cell-derived chemokine CXCL12, which activates the chemokine receptor CXCR4, contributes to retaining of the cells in the thymus [61]. In the dou-

Table 1. Expressions of the S1P receptors and their dominant effects on immune cells

	T cells	B cells	DCs	Macrophages	Mast cells
$S1P_1$	+ Chemotaxis↑	+ Chemotaxis↑	+ Chemotaxis↑	+ Chemotaxis↑	+ Chemotaxis↑
$S1P_2$	–	–	+ Chemotaxis↓	+	+ Chemotaxis↓ Degranulation↑
$S1P_3$	–	(+)	+ Chemotaxis↑	+	–
$S1P_4$	+ IL-10↑ IL-2↓, IL-4↓ IFN-γ↓	+	+	+	–
$S1P_5$	–	–	–	–	–

+ expressed, – not expressed, (+) only expressed on certain subtypes.

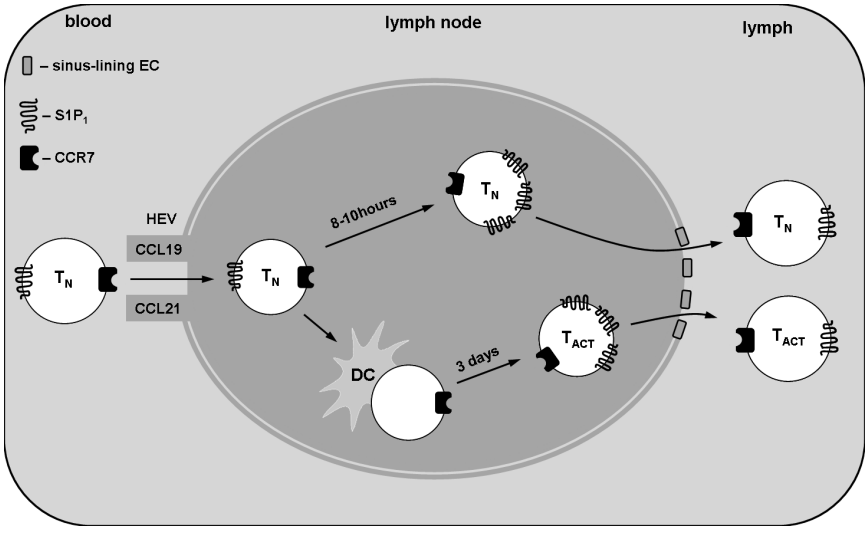

Fig. 2. Involvement of CCL19/CCL21 and S1P on T cell entry to and egress from lymph nodes. Naïve T cells (T_N) continuously circulate between blood and lymphatic tissue. These cells enter lymph nodes via HEVs in response to CCL19/CCL21 and their shared receptor, CCR7. As a result of low S1P levels in the lymph node, $S1P_1$ recaptures on the surface and initiates the egress of T_N through sinus-lining endothelial cells (ECs) into efferent lymph. In case of antigen encounter in the lymph node, T_N become activated and transiently downregulate $S1P_1$. This renders cells unresponsive to the S1P-$S1P_1$ migratory system. As a consequence, activated T cells (T_{ACT}) are retained in the lymph node. At the end of the activation phase, T_{ACT} upregulate $S1P_1$ followed by egress from lymph node.

ble-positive and single-positive states of thymocyte development, $S1P_1$ gets upregulated whereas CXCR4 receptor expression is decreased [34]. This results in a loss of responsiveness to CXCL12 during maturation and shifts the migratory balance to S1P [20]. After major thymocytes have entered the blood via S1P signaling, these naïve T cells circulate between the lymphatic and blood system until they contact their predefined antigen in secondary lymphatic organs (SLOs), initiating activation. Most of the naïve T cells move directly into lymph nodes through high endothelial venules (HEVs) without passing the afferent lymph [55] (Fig. 2). As a result of high S1P levels in the blood, $S1P_1$ undergoes desensitization by internalization [42], allowing T cells to follow the chemokines CCL19 and CCL21. These are either produced or secreted at the luminal surface of HEVs, leading to the movement of T cells into peripheral lymph nodes [55]. CCL19 and CCL21 are both ligands of CCR7, which is upregulated on single-positive thymocytes during terminal differentiation [20, 98]. The relevance of this chemokine system, guiding T cells into lymph nodes, has been demonstrated using both $CCR7^{-/-}$ mice [16] and plt/plt mice [58], which are deficient in CCL19 and CCL21. Indeed, both models showed a reduced amount of naïve T cells in the lymph nodes and an increased number in the blood. In addition, CCL19 and CCL21 are responsible for directing naïve T cells into specific regions of the lymph node, the so-called T cell areas, where they encounter antigen-presenting cells (APCs) [16]. T cells failing to detect their specific antigen are able to leave the lymph node after 8–10 h in response to the S1P concentration gradient, as during this time period $S1P_1$ is recaptured on the surface [42]. Efferent lymph is collected in the thoratic duct, where naïve T cells enter the blood system, rejoining the circulation process between the blood and the lymphatic system.

Several mouse models have proved the importance of $S1P_1$ on lymphocyte trafficking. Since $S1P_1^{-/-}$ mice die at a prenatal stage, a number of approaches were considered to evaluate the function of $S1P_1$ on lymphocyte motility. Such a model is the reconstitution of lethally irradiated wild-type mice with fetal hepatic precursors of lymphocytes from $S1P_1^{-/-}$ mice. Flow cytometric analysis of peripheral blood lymphocytes in this model revealed a complete loss of T cells in the blood, whereas an accumulation of mature thymocytes was detectable in the thymus, demonstrating a specific role of $S1P_1$ on thymocyte egress. Further analysis of lymphatic tissues showed a deficiency of all T cell stages in SLOs as a result of the decreased supply of mature thymocytes out of the thymus. A similar result was obtained when a selective depletion of early $S1P_1^{+}$ thymocytes was performed by LCK-CRE conditional knockout technology [47]. Moreover, the use of labeled $S1P_1^{-/-}$ T cells which were adoptively transferred into irradiated wild-type mice indicated that $S1P_1^{-/-}$ and wild-type T cells were able to enter SLOs and that they even homed to T cell compartments. Nevertheless, $S1P_1^{-/-}$ T cells disappeared from the blood and were not detectable in the lymph. These results are consistent with a reverse approach using a transgene $S1P_1$ mouse model. Indeed the main proportion of $S1P_1$-overexpressing T cells was localized in the blood, and just a minor fraction of T cells was able to enter lymph nodes and Peyer's patches [47].

T cells undergoing activation by APCs normally remain up to 3 days in SLOs. Interestingly, during this time period T cells seem to be resistant to the S1P migratory system. This effect can be explained by the different expressions of $S1P_1$ in distinct phases of the activation process. This has been shown in an ovalbumin-immunized T cell model. In the beginning of the T cell activation process, measurement of mRNA transcripts revealed a significant downregulation of $S1P_1$. Three days after immunization, the receptor downregulation of $S1P_1$ was reversed and fully activated T cells reacquired their S1P responsiveness, initiating lymphocyte egress [47].

Migration of DCs

DCs are the main subtype of APCs that contribute to the activation of T cells in SLOs. Depending on their differentiation state, DCs possess opposite properties in immune cell responses. In the immature state, DCs initiate immunosuppression by activation of regulatory (reg) T cells, preventing autoimmune diseases. In contrast, immunostimulatory signals are transmitted by either cytotoxic $CD8^+$ T cells or T helper (TH) cells which have been in contact with fully matured DCs [2]. Since interaction with T cells occurs in the lymph nodes, mature and immature DCs have to emigrate from peripheral tissue sites to T cell areas in SLOs. In contrast to naïve T cells, which enter lymph nodes through HEVs, most DC subtypes use the afferent lymph for SLO entrance [9].

The mechanisms involved in guiding DCs from peripheral sites through afferent lymph into the lymph nodes have not been clarified in detail. A central role possesses the CCL21/CCL19 chemokine system and the corresponding receptor, CCR7, which is upregulated during maturation of DCs [95]. In plt/plt mice lacking CCL21 and CCL19, DCs were absent in the lymph node T cell area, whereas a minor fraction of DCs was detectable in the lymph node subcapsular sinus [26, 56]. Although CCR7 expression is induced by diverse stimuli independent of their maturation state, the majority of immature DCs does not express CCR7 and fails to respond to the CCL19/CCL21 chemokine system [66]. Nevertheless, immature DCs are present in lymph nodes to fulfil their immunoregulatory functions, indicating the involvement of other migratory mechanisms [43]. *In vitro* cell culture assays on human monocyte-derived DCs revealed S1P as a migratory stimulus of immature DCs, but when these cells were matured by treatment with lipopolysaccharides, they lost their S1P migratory capability [29]. These results are in contradiction to experiments on murine DCs, where S1P was able to induce migration regardless of the maturation state [67]. Other studies implied that immature murine DCs were resistant to a migratory S1P stimulus and first became responsive to S1P when maturation occurred. In these studies, maturation was accompanied by an upregulation of $S1P_1$ and $S1P_3$ [14]. The importance of these receptors on DC migration had already been demonstrated on the murine DC line XS52 by migratory experiments in the presence of $S1P_1$- and $S1P_3$-antisense oligodesoxynucleotides [65]. The diverse observations of S1P-induced migration on different DC maturation states might be explained by distinct DC phenotypes as a result of various differentiation procedures, complicating comparison between these reports. In addition, species-dependent discrepancies between humans and mice underline the complexity of this cell type.

Migration of B cells

Unlike T cells, $S1P_1$ expression is not necessary for the egress of fully differentiated B cells out of the bone marrow. This was demonstrated by the reconstitution of lethally irradiated wild-type mice with fetal hepatic $S1P_1$-deficient B cell precursors [47]. During late stages of B cell development, expressions of the chemokine receptors CXCR5 and CCR7 are upregulated, inducing accumulation of B cells in SLOs in response to their ligands CXCL13 and CCL19/CCL21, respectively [4, 55]. However, in contrast to the case of bone marrow, S1P plays a central role in the egress of B cells from lymph nodes and the spleen. Indeed, reconstitution of $S1P_1$--deficient B cell precursors in irradiated wild-type mice resulted in the disappearance of B cells from the blood and a slightly altered proportion of B cells in SLOs. It should be mentioned, however, that the total peripheral number of B cells was comparable to that of the wild--type mice. Similar results were obtained in a further model, where labeled, $S1P_1$-deficient B cells were transferred into wild-type mice, emphasizing the importance of $S1P_1$ in B cell function [47].

Moreover, the localization of B cells in specific compartments within the spleen differed in $S1P_1^{-/-}$ mice models compared with wild-type mice. Normally, lymphocytes enter the spleen directly with the blood through terminal arterioles without passing the afferent lymph or HEVs. The major proportion of the blood is collected in the marginal zone of the spleen. In this area, a specific B cell subtype resides which is continuously exposed to the blood, assuring high contact to blood--borne antigens. One activated, these marginal zone B cells relocate either to the lymphoid follicle in response to CXCL13 or to T cell/B cell boundaries located in the splenic white pulp in response to CXCL13 and the CCR7 ligands [13]. Under homeostatic conditions these B cells are retained in the marginal zone by the S1P--$S1P_1$ axis, thereby counteracting the migratory effect of the chemokine systems [10].

MIGRATION-INDEPENDENT ACTIONS OF S1P ON IMMUNE CELLS

Besides its role on lymphocyte trafficking, S1P exerts anti-apoptotic effects on lymphocytes by inhibiting the expressions of the proapoptotic mitochondrial protein BAX (B cell lymphoma-2-associated X protein) and certain caspases [12, 22]. Furthermore, S1P modulates cytokine secretion of T cells by downregulation of the immunostimulatory cytokines interleukin (IL)-2, IL-4, and interferon (IFN)-γ and upregulation of the immunosuppressive cytokine IL-10. In contrast to $S1P_1$, which predominantly induces migration of T cells, $S1P_4$ is the receptor subtype mainly responsible for these non-migratory effects [87]. Moreover, S1P is also required for the functioning of reg T cells, as the optimal suppressive activity declines in the absence of S1P. This can be demonstrated in co-culture experiments of activated, IL-2-producing $CD4^+CD25^-$ T cells and $CD4^+CD25^+$ reg T cells, which transduced their antiproliferative and IL-2-suppressing effects in a S1P-

-dependent manner [86]. Furthermore, in TH_2 cells, S1P impairs the secretion of IL-4, a cytokine that mainly promotes the generation of TH_2 cells in an autocrine fashion. Nevertheless, the general outcome of S1P on T cell differentiation during activation is characterized by a decreased TH_1 and an enhanced TH_2 cell immune response. This S1P-induced TH_2 switch is caused by a modified cytokine secretion profile of mature DCs in response to S1P during the T cell activation process. DCs, matured in the presence of S1P, showed reduced levels of tumor necrosis factor-α and IL-12, the major stimuli initiating TH_1 differentiation [29].

Transient elevated levels of S1P appear in mast cells in response to IgE stimulation due to activation of SphK and a subsequent release of S1P. Since TH_2 cell immune responses correlate with a raised differentiation of IgE-producing plasma cells, S1P might enlarge its function in mast cell biology by an extended TH_2 polarization, augmenting future IgE-dependent mast cell activities. In addition, S1P exerts its effects directly by enhancing migration of mast cells towards low concentrations of antigens via $S1P_1$. In contrast, high levels of antigens lead to an upregulation of $S1P_2$, enabling S1P to transduce antimigratory effects on IgE-crosslinked mast cells [32]. Furthermore, $S1P_2$ is involved in mast cell degranulation, indicating that S1P contributes to IgE-dependent mast cell responses by different mechanisms, thus questioning the importance of S1P during allergic processes [32].

THE IMMUNOSUPPRESSIVE AGENT FTY720 AND S1P SIGNALING

The importance of S1P on immune cell functioning has been emphasized by the discovery of the signaling mechanism of the immunosuppressant agent FTY720 (2-amino-2[2-(4-octylphenyl) ethyl]propane-1,3-diol). In contrast to classical immunosuppressant drugs, FTY720 does not interfere with T cell proliferation or apoptosis, but induces a severe loss of lymphocytes in the blood due to modification of S1P signaling. FTY720 was developed in order to obtain more potent substances to prevent skin allograft rejection by modifying the natural compound myoriscin. This metabolite of the ascomycete *Isaria sinclairii* had been discovered during a screening of biological substances able to reduce the mixed lymphocyte reaction (MLR) [18]. FTY720 differs from myoriscin as it did not affect MLR and inhibited neither T cell activation nor proliferation, but reduced the number of circulating lymphocytes within a few hours and caused an accumulation of lymphocytes in lymph nodes [8, 64]. Although apoptotic effects could be observed in several cancer and T cell lines at micromolar FTY720-concentrations, no clinical relevance of this has so far been indicated, as FTY720 mediates its immunosuppressive properties in the low-nanomolar range [7, 48].

FTY720 shares structural characteristics with sphingosine and is also a substrate for SphK2 and, to a lesser extent, for SphK1 [63, 99]. Once phosphorylated, FTY720 (FTY720-P) acts as an agonist on four of the five known S1P receptors, namely $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$ [7]. In contrast to the natural ligand S1P, FTY720-P is not degraded by S1P lyase and is therefore only metabolized by phosphatases [3] (Fig. 3). This discrepancy is a crucial factor in the altered behavior of FTY720-P towards S1P receptors, resulting in a distinct immune modulation by FTY720-P compared with S1P.

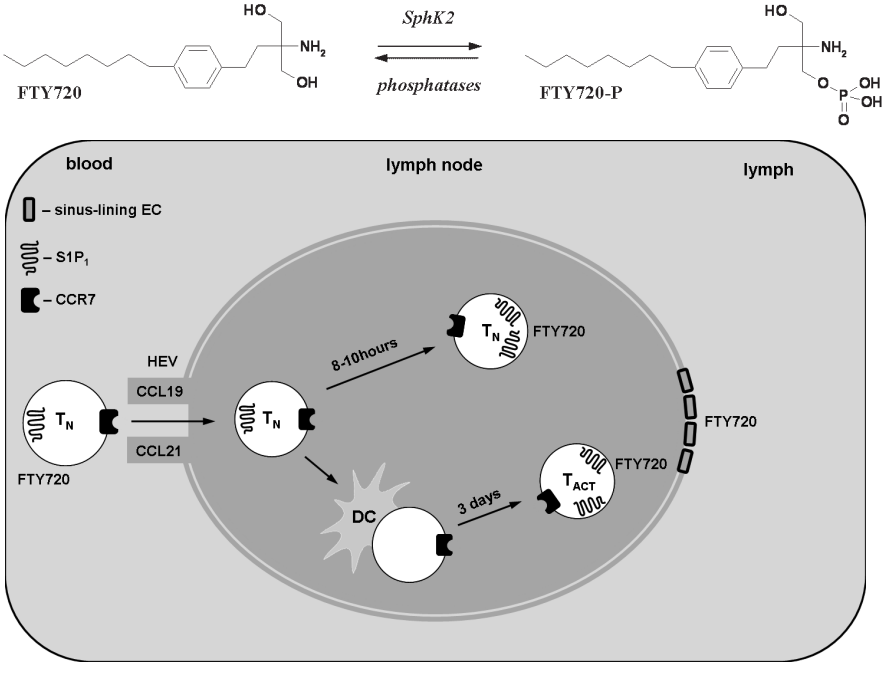

Fig. 3. Metabolism of FTY720 and its modulation of S1P signaling. FTY720 is a structural analog of sphingosine and also a substrate for SphK2. In contrast to the natural ligand S1P, FTY720-P is only degraded by phosphatases. FTY720-P induces an aberrant internalization of $S1P_1$ on T cells. As a consequence, T cells do not respond to the S1P migratory system and fail to egress from lymph nodes. Furthermore, FTY720 enhances sinus-lining endothelial cell (EC) barrier integrity, thereby additionally reducing lymphocyte egress.

FTY720-INDUCED MODULATION OF LYMPHOCYTE TRAFFICKING

A key result in explaining the effects of FTY720 was the discovery that FTY720 induces a persistent internalization of $S1P_1$, which is in contrast to the transient receptor internalization mediated by the natural ligand, S1P [25, 47]. The resistance of FTY720-P to S1P lyase, thereby decelerating FTY720-P degradation, might be responsible for the prolonged downregulation of $S1P_1$. Pharmacological knockdown of $S1P_1$ by FTY720 in wild-type mice exhibited an immunomodulatory pattern similar to that of lethally irritated wild-type mice reconstituted with $S1P_1^{-/-}$ lymphocytes. In both models the egress of lymphocytes out of SLOs was decreased, whereas chemokine-induced entry of lymphocytes into SLOs was not affected [47].

FTY720-treated mice showed a reduced emigration of mature thymocytes into the blood and a diminished egress of naïve T cells out of lymph nodes, while T cells in the spleen were not restrained [64]. Effector T cells partially escaped FTY720-transmitted sequestration as they were mainly located in peripheral tissue compartments and entered the peripheral lymph nodes only to a minor extent [70, 89]. In consistence with T cells, FTY720 retained B cells in peripheral lymph nodes, whereas no sequestration was observed in the spleen. In accordance with the importance of $S1P_1$ on B cell localization within the spleen, as demonstrated in lethally irritated wild-type mice reconstituted with $S1P_1^{-/-}$ B cells, pharmacological knockdown of $S1P_1$ by FTY720 induced a similar displacement of marginal-zone B cells towards the splenic follicles [47, 84].

In addition to the direct effect on lymphocyte migration out of the lymph nodes, FTY720 might interact with sinus-lining endothelial cells (ECs), promoting increased barrier integrity, thereby blocking lymphocyte egress. Administration of FTY720 resembles the effect of S1P as it inhibits vascular endothelial growth factor-induced vascular permeability, as shown in mice and in cell culture experiments [68]. This may be caused by translocation of vascular endothelial cadherin to the contact site, resulting in a tightened layer of ECs. Regulation of EC morphology by S1P is mainly transduced via $S1P_1$ and $S1P_3$ [68]. Recent data revealed that $S1P_1$ is responsible for EC cytoskeletal rearrangement and barrier integrity in human pulmonary artery ECs [73]. In contrast, splenic marginal-zone EC alignment is dependent on the expression of $S1P_3$, which was demonstrated in $S1P_3^{-/-}$ mice as these ECs did not arrange properly and were unable to restrict entry of marginal-zone B cells into splenic follicles [21]. These data indicate that S1P and FTY720 exhibit similar effects on ECs, whereas in lymphocytes both molecules mediate partially divergent actions. A possible explanation is a different internalization, especially of $S1P_1$, depending on the cell type.

To prove more rigorously whether long-term internalization of $S1P_1$ is required, SEW2871, a selective agonist on $S1P_1$ which induces only a transient internalization of $S1P_1$, comparable to the natural ligand S1P, was used in several studies [31]. Mice treated with SEW2871 developed acute lymphopenia which was reversed when SEW2871-levels in the plasma declined [69]. Recently, the involvement of $S1P_1$ in lymphatic EC barrier integrity and arrangement on lymphocyte egress was analyzed by a new approach on isolated lymph nodes by visualizing the movement of fluorescence-labeled T cells in specific lymph node compartments. Indeed, in this model, treatment with SEW2871 resulted in an inhibition of lymphocyte transendothelial migration from the medulla to the sinus and a reduced motility of lymphocytes in the entire lymph node. These effects were rapidly reversed by either $S1P_1$ antagonists or washout of SEW2871, indicating that an agonistic activity of $S1P_1$ contributes to the migratory-inhibiting effect of sinus-lining ECs [88]. With respect to these findings, the crucial step of FTY720-induced immunosuppression remains unclear, since FTY720 receptor signaling differs from that of SEW2871 and S1P. Therefore it would be of interest to analyze FTY720 in this model to indicate the role of EC involvement clearly.

In accordance to SEW2871, the natural ligand S1P also induced lymphopenia when S1P levels were increased about 2-fold. This effect was reversed when S1P levels returned to physiological conditions [46]. Moreover, inhibition of S1P lyase, either pharmacologically by treatment with 2-acetyl-4-tetrahydroxybutylimidazole or genetically using the siRNA technique, not only increased S1P levels in lymphatic tissue, but also promoted thymocyte sequestration and lymphopenia [72]. Most interestingly, FTY720 has also been indicated to inhibit the S1P-lyase activity and to increase S1P levels in thymus and spleen [3]. This effect might additionally contribute to FTY720 function.

FTY720-INDUCED MODULATION OF DC FUNCTION

DCs possess a pivotal role in the immune system since they initiate immune-cell responses by presenting antigens and regulate T cell differentiation, as described above. In correlation with lymphocytes, FTY720 is not able to evoke migration of *in vitro* differentiated bone marrow-derived DCs isolated from mice. In contrast to lymphocytes, FTY720 reduced S1P-induced motility of DCs only to a minor extent [14]. These data suggest that either $S1P_1$ is not completely internalized after treatment with FTY720, or $S1P_3$, which is not desensitized by FTY720 [25], contributes to the S1P-induced migration of DCs. Indeed, in murine DCs both receptor subtypes have been identified to mediate the migratory response of S1P [65]. Contradictory data exist on the influence of FTY720 on chemokine-induced migration which might be explained by different maturation states or distinct isolation and differentiation procedures of DCs [14, 57]. *In vivo* experiments on FITC-labeled skin-resident DCs

enlarged the relevance of FTY720 on DC function, as the amount of labeled cells in lymph nodes was reduced after administration of FTY720 [14]. These data are in line with a recent report on mice where FTY720 treatment resulted in an increased number of DCs in the blood and a reduced number in lymph nodes and the spleen [37]. This increase of DCs within the blood was accompanied by a downregulation of CCR7, which may contribute to the altered migration towards SLOs. The disability of FTY720 to induce migration of DCs in cell culture experiments indicates that the impaired translocation of DCs into SLOs is dominantly initiated by an augmented response towards the CCR7-CCL19/CCL21 chemokine system [37].

FTY720 also modulates the interaction of DCs with lymphocytes and the balance between TH_1 and TH_2 cells. Treatment of DCs with FTY720 resulted in a pronounced secretion of the immunosuppressive IL-10 and a reduced production of IL-12. Moreover, when T cells were coincubated with FTY720-stimulated DCs, higher amounts of IL-4 and decreased levels of IFN-γ were observed compared with coculture experiments of T cells with unstimulated DCs. These data indicate that FTY720-stimulated DCs induce a switch of T cells to the TH_2 cell phenotype [57]. Therefore the effect of FTY720 on allergic processes should be further examined.

FTY720 IN AUTOIMMUNE DISEASES

Since FTY720 exerts its effects by mechanisms other than apoptosis or inhibition of lymphocyte proliferation, it has emerged as a promising therapeutic agent in autoimmune diseases and on preventing allograft rejections. Several animal models substantiated the efficacy of FTY720 in experimentally induced autoimmune or inflammatory diseases. A mouse model of acute myocarditis proved the benefit of FTY720 on the course of disease, as tissue infiltration and necrosis were reduced whereas calcineurin inhibitors failed to function [54]. FTY720 reduced the development of adjuvant- or collagen-induced arthritis comparably to therapeutic doses of prednisolone or mizoribine [49].

In a non-obese diabetic (NOD) mice model of autoimmune diabetes mellitus type I, it has been reported that the use of FTY720 was beneficial in prediabetic individuals to prevent the development of overt diabetes. Moreover, administration of FTY720 to euglycemic NOD mice with advanced stages of insulitis or overtly diabetic NOD mice prevented or cured spontaneous diabetes [44, 45]. In an autoimmune encephalitis model of multiple sclerosis, FTY720 reduced the progression of the acute and chronic-relapsing forms [7]. Data from the extension of a phase II study confirmed the beneficial effects of FTY720 in the treatment of patients with relapsing multiple sclerosis. In this study, the effect of FTY720 on disease activity as well as tolerability and safety were evaluated. Indeed, more than 80% of patients who received FTY720 for up to 12 months were free from lesions, showing no inflammation on magnetic resonance imaging [62]. In contrast, classical multiple sclerosis therapeutic regimens such as IFN-β only led to a relapse reduction of 30% [60].

FTY720 IN TRANSPLANTATION

Various preclinical studies revealed the efficiency of FTY720 in preventing allograft rejection. Indeed, allograft survival was prolonged by FTY720 in grafts of skin [94], heart [85], and small bowels [35] in rats, pancreatic islet cells [17] and small bowels [91] in mice, liver [19] and kidney [78] in dogs, and kidney [71] in monkeys. FTY720 is effective in a concentration-dependent manner alone and has been shown to extend the survival time induced by classical immunosuppressive agents such as cyclosporine, FK506, rapamycin, and SDZ RAD (40-O-(2-hydroxyethyl)-rapamycin). This effect is rather synergistic than additive. Activation of graft-associated T cells and infiltration of T cells into grafts was reduced by FTY720 administration, providing a potential benefit to the host-versus-graft and graft-versus-host reaction following organ transplantation [53, 93]. Most organ rejections are a result of chronic processes characterized by perivascular inflammation and allograft arteriosclerosis, also termed graft vessel diseases (GVD). In contrast to classical immunosuppressive agents, which only prevent GVD to a small extend, FTY720 markedly reduced all symptoms of GVD in a rat carotid artery model [59]. The efficacy of a FTY720 regimen has also been demonstrated in a mouse intestinal transplantation model which is resistant against standard immunosuppressive agents [92].

Clinical phase I and phase II trials on renal transplant patients proved the efficacy of FTY720 in combination with a standard therapeutic regimen of cyclosporine and glucocorticoids. Furthermore, administration of FTY720 enabled patients to reduce cyclosporine doses up to 50%. Pharmacokinetic data propose an oral once daily application to be sufficient for constant FTY720 plasma levels without the necessity for monitoring blood levels or dose titration. The toxicity of classical immunosuppressive drugs was not enhanced, indicating the potential use of FTY720 in combination with glucocorticoids and calcineurin inhibitors [33, 74, 80]. Current phase III studies will clarify the efficacy of the promising immunosuppressive agent FTY720 in organ transplantation.

ADVERSE SIDE EFFECTS OF FTY720

Preclinical studies and first clinical trials revealed that FTY720 is well reconcilable without the occurrence of side effects normally observed after treatment with classical immunosuppressive substances. There has been no evidence that FTY720 negatively affects renal

or hepatic functions, as indicated by the failure of tissue infiltration or any sclerotic or fibrotic processes or histological changes within the organs. Nevertheless, FTY720 was able to upregulate the expressions of connective tissue growth factor, matrix metalloproteinase 9, and collagen in renal mesangium cells, which are considered as profibrotic markers, indicating a possible relevance of FTY720 in fibrotic processes during long-term administration [90].

In a clinical phase II trial on renal transplant patients, initiation of FTY720 treatment was associated with a transient reduction in the heart rate from baseline by 12–15 beats on average. In most of the cases the decrease in heart rate was asymptotic and resolved without medical intervention [74, 80]. These side effects are consistent with data obtained from several organ models where S1P-receptor agonists induced bradycardia in response to an activation of $G_{\alpha i}$-dependent inward-rectifying potassium channels that promote membrane hyperpolarization and subsequent reduction in heart rate [6]. $S1P_3$ appears to be the dominant receptor subtype for this effect since $S1P_3^{-/-}$ mice were resistant to FTY720-induced bradycardia compared with wild-type mice [15, 69]. Nevertheless, in humans an involvement of $S1P_1$ on heart rate regulation cannot be excluded, as this receptor subtype is dominantly expressed in the atrium and ventricle [50]. Besides heart rate reduction, no relevant adverse reactions were observed in the above-mentioned phase II trial on renal transplant patients in comparison with a standard therapeutic regimen with mycophenolate mofetil (MMF). The incidence of infections did not alter significantly between the FTY720- and MMF-group and no malignancies were reported during FTY720 treatment over a time period of a 6-month study [80].

SUMMARY AND FUTURE PERSPECTIVES

FTY720 has emerged as a new immunosuppressive agent and possesses the potential of expanding the therapeutic range of drug regimens for autoimmune diseases and the prevention of graft rejection since it suppresses immune responses by a mechanism completely different from classical immunosuppressive agents. Treatment with FTY720 causes lymphopenia by sequestration of lymphocytes in peripheral lymph nodes through interaction with S1P-receptors, dominantly $S1P_1$, whose natural ligand, S1P, plays a central role in maintaining the homeostasis of lymphocyte motility and contributes to the control of allergic responses and T cell differentiation procedures. Preclinical studies and clinical trials have proved the benefit of FTY720 in the course of autoimmune diseases such as multiple sclerosis and in the prevention of graft rejections and outlined the possibility of a combination with calcineurin inhibitors and glucocorticoids without enhancing their toxic effects.

In the future, ongoing phase III studies will evaluate the potential benefit of FTY720 more precisely, including a broader range of indications. The signaling events of FTY720 on lymphatic cells and ECs remain to be elucidated in order to estimate the involvement of EC integrity and the loss of responsiveness of lymphocytes to S1P.

REFERENCES

1. Allende M. L., Dreier J. L., Mandala S. and Proia R. L. (2004): Expression of the sphingosine 1-phosphate receptor, $S1P_1$, on T-cells controls thymic emigration. J. Biol. Chem., **279**, 15396–15401.
2. Banchereau J. and Steinman R. M. (1998): Dendritic cells and the control of immunity. Nature, **392**, 245–252.
3. Bandhuvula P., Tam Y. Y., Oskouian B. and Saba J. D. (2005): The immune modulator FTY720 inhibits sphingosine-1-phosphate lyase activity. J. Biol. Chem., **280**, 33697–33700.
4. Bowman E. P., Campbell J. J., Soler D., Dong Z., Manlongat N., Picarella D., Hardy R. R. and Butcher E. C. (2000): Developmental switches in chemokine response profiles during B cell differentiation and maturation. J. Exp. Med., **191**, 1303–1318.
5. Brindley D. N., English D., Pilquil C., Buri K. and Ling Z. C. (2002): Lipid phosphate phosphatases regulate signal transduction through glycerolipids and sphingolipids. Biochim. Biophys. Acta, **1582**, 33–44.
6. Brinkmann V., Cyster J. G. and Hla T. (2004): FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. Am. J. Transplant., **4**, 1019–1025.
7. Brinkmann V., Davis M. D., Heise C. E., Albert R., Cottens S., Hof R., Bruns C., Prieschl E., Baumruker T., Hiestand P., Foster C. A., Zollinger M. and Lynch K. R. (2002): The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J. Biol. Chem., **277**, 21453–21457.
8. Brinkmann V., Pinschewer D., Chiba K. and Feng L. (2000): FTY720: a novel transplantation drug that modulates lymphocyte traffic rather than activation. Trends Pharmacol. Sci., **21**, 49–52.
9. Cavanagh L. L. and Von Andrian U. H. (2002): Travellers in many guises: the origins and destinations of dendritic cells. Immunol. Cell Biol., **80**, 448–462.
10. Cinamon G., Matloubian M., Lesneski M. J., Xu Y., Low C., Lu T., Proia R. L. and Cyster J. G. (2004): Sphingosine 1-phosphate receptor 1 promotes B cell localization in the splenic marginal zone. Nat. Immunol., **5**, 713–720.
11. Cuvillier O., Pirianov G., Kleuser B., Vanek P. G., Coso O. A., Gutkind S. and Spiegel S. (1996): Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. Nature, **381**, 800–803.
12. Cuvillier O., Rosenthal D. S., Smulson M. E. and Spiegel S. (1998): Sphingosine 1-phosphate inhibits activation of caspases that cleave poly(ADP-ribose) polymerase and lamins during Fas- and ceramide-mediated apoptosis in Jurkat T lymphocytes. J. Biol. Chem., **273**, 2910–2916.
13. Cyster J. G. (2005): Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. Annu. Rev. Immunol., **23**, 127–159.

14. Czeloth N., Bernhardt G., Hofmann F., Genth H. and Forster R. (2005): Sphingosine-1-phosphate mediates migration of mature dendritic cells. J. Immunol., **175**, 2960–2967.
15. Forrest M., Sun S. Y., Hajdu R., Bergstrom J., Card D., Doherty G., Hale J., Keohane C., Meyers C., Milligan J., Mills S., Nomura N., Rosen H., Rosenbach M., Shei G. J., Singer II, Tian M., West S., White V., Xie J., Proia R. L. and Mandala S. (2004): Immune cell regulation and cardiovascular effects of sphingosine 1-phosphate receptor agonists in rodents are mediated via distinct receptor subtypes. J. Pharmacol. Exp. Ther., **309**, 758–768.
16. Forster R., Schubel A., Breitfeld D., Kremmer E., Renner-Muller I., Wolf E. and Lipp M. (1999): CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell, **99**, 23–33.
17. Fu F., Hu S., Li S., DeLeo J., Hoover J., Wang S., Lake P. and Shi V. (2001): FTY720, a novel immunosuppressive agent with insulinotropic activity, prolongs graft survival in a mouse islet transplantation model. Transplant. Proc., **33**, 672–673.
18. Fujita T., Hirose R., Yoneta M., Sasaki S., Inoue K., Kiuchi M., Hirase S., Chiba K., Sakamoto H. and Arita M. (1996): Potent immunosuppressants, 2-alkyl-2-aminopropane-1,3-diols. J. Med. Chem., **39**, 4451–4459.
19. Furukawa H., Suzuki T., Jin M. B., Yamashita K., Taniguchi M., Magata S., Ishikawa H., Ogata K., Masuko H., Shimamura T., Fukai M., Hayashi T., Fujita M., Nagashima K., Omura T., Kishida A. and Todo S. (2000): Prolongation of canine liver allograft survival by a novel immunosuppressant, FTY720: effect of monotherapy and combined treatment with conventional drugs. Transplantation, **69**, 235–241.
20. Gill J., Malin M., Sutherland J., Gray D., Hollander G. and Boyd R. (2003): Thymic generation and regeneration. Immunol. Rev., **195**, 28–50.
21. Girkontaite I., Sakk V., Wagner M., Borggrefe T., Tedford K., Chun J. and Fischer K. D. (2004): The sphingosine-1-phosphate (S1P) lysophospholipid receptor S1P3 regulates MAdCAM-1+ endothelial cells in splenic marginal sinus organization. J. Exp. Med., **200**, 1491–1501.
22. Goetzl E. J., Kong Y. and Mei B. (1999): Lysophosphatidic acid and sphingosine 1-phosphate protection of T cells from apoptosis in association with suppression of Bax. J. Immunol., **162**, 2049–2056.
23. Goetzl E. J., Wang W., McGiffert C., Huang M. C. and Graler M. H. (2004): Sphingosine 1-phosphate and its G protein-coupled receptors constitute a multifunctional immunoregulatory system. J. Cell. Biochem., **92**, 1104–1114.
24. Graler M. H. and Goetzl E. J. (2002): Lysophospholipids and their G protein-coupled receptors in inflammation and immunity. Biochim. Biophys. Acta, **1582**, 168–174.
25. Graler M. H. and Goetzl E. J. (2004): The immunosuppressant FTY720 down-regulates sphingosine 1-phosphate G-protein-coupled receptors. FASEB J., **18**, 551–553.
26. Gunn M. D., Kyuwa S., Tam C., Kakiuchi T., Matsuzawa A., Williams L. T. and Nakano H. (1999): Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. J. Exp. Med., **189**, 451–460.
27. Hannun Y. A., Luberto C. and Argraves K. M. (2001): Enzymes of sphingolipid metabolism: from modular to integrative signaling. Biochemistry, **40**, 4893–4903.
28. Huwiler A., Kolter T., Pfeilschifter J. and Sandhoff K. (2000): Physiology and pathophysiology of sphingolipid metabolism and signaling. Biochim. Biophys. Acta, **1485**, 63–99.
29. Idzko M., Panther E., Corinti S., Morelli A., Ferrari D., Herouy Y., Dichmann S., Mockenhaupt M., Gebicke-Haerter P., Di Virgilio F., Girolomoni G. and Norgauer J. (2002): Sphingosine 1-phosphate induces chemotaxis of immature dendritic cells and modulates cytokine-release in mature human dendritic cells for emergence of Th2 immune responses. FASEB J., **16**, 625–627.
30. Ishii I., Fukushima N., Ye X. and Chun J. (2004): Lysophospholipid receptors: signaling and biology. Annu. Rev. Biochem., **73**, 321–354.
31. Jo E., Sanna M. G., Gonzalez-Cabrera P. J., Thangada S., Tigyi G., Osborne D. A., Hla T., Parrill A. L. and Rosen H. (2005): S1P(1)-selective *in vivo*-active agonists from high-throughput screening: off-the-shelf chemical probes of receptor interactions, signaling, and fate. Chem. Biol., **12**, 703–715.
32. Jolly P. S., Bektas M., Olivera A., Gonzalez-Espinosa C., Proia R. L., Rivera J., Milstien S. and Spiegel S. (2004): Transactivation of sphingosine-1-phosphate receptors by FcepsilonRI triggering is required for normal mast cell degranulation and chemotaxis. J. Exp. Med., **199**, 959–970.
33. Kahan B. D., Karlix J. L., Ferguson R. M., Leichtman A. B., Mulgaonkar S., Gonwa T. A., Skerjanec A., Schmouder R. L. and Chodoff L. (2003): Pharmacodynamics, pharmacokinetics, and safety of multiple doses of FTY720 in stable renal transplant patients: a multicenter, randomized, placebo-controlled, phase I study. Transplantation, **76**, 1079–1084.
34. Kim C. H., Pelus L. M., White J. R. and Broxmeyer H. E. (1998): Differential chemotactic behavior of developing T cells in response to thymic chemokines. Blood, **91**, 4434–4443.
35. Kimura T., Hasegawa T., Nakai H., Azuma T., Usui N., Sasaki T. and Okada A. (2003): FTY720 reduces T-cell recruitment into murine intestinal allograft and prevents activation of graft-infiltrating cells. Transplantation, **75**, 1469–1474.
36. Kohama T., Olivera A., Edsall L., Nagiec M. M., Dickson R. and Spiegel S. (1998): Molecular cloning and functional characterization of murine sphingosine kinase. J. Biol. Chem., **273**, 23722–23728.
37. Lan Y. Y., De Creus A., Colvin B. L., Abe M., Brinkmann V., Coates P. T. and Thomson A. W. (2005): The sphingosine-1-phosphate receptor agonist FTY720 modulates dendritic cell trafficking *in vivo*. Am. J. Transplant., **5**, 2649–2659.
38. Le Stunff H., Peterson C., Liu H., Milstien S. and Spiegel S. (2002): Sphingosine-1-phosphate and lipid phosphohydrolases. Biochim. Biophys. Acta, **1582**, 8–17.
39. Lee M. J., Van Brocklyn J. R., Thangada S., Liu C. H., Hand A. R., Menzeleev R., Spiegel S. and Hla T. (1998): Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. Science, **279**, 1552–1555.
40. Levade T. and Jaffrezou J. P. (1999): Signalling sphingomyelinases: which, where, how and why? Biochim. Biophys. Acta, **1438**, 1–17.
41. Liu H., Sugiura M., Nava V. E., Edsall L. C., Kono K., Poulton S., Milstien S., Kohama T. and Spiegel S. (2000): Molecular cloning and functional characterization of a novel mammalian sphingosine kinase type 2 isoform. J. Biol. Chem., **275**, 19513–19520.

42. Lo C. G., Xu Y., Proia R. L. and Cyster J. G. (2005): Cyclical modulation of sphingosine-1-phosphate receptor 1 surface expression during lymphocyte recirculation and relationship to lymphoid organ transit. J. Exp. Med., **201**, 291–301.
43. Mahnke K., Schmitt E., Bonifaz L., Enk A. H. and Jonuleit H. (2002): Immature, but not inactive: the tolerogenic function of immature dendritic cells. Immunol. Cell Biol., **80**, 477–483.
44. Maki T., Gottschalk R. and Monaco A. P. (2002): Prevention of autoimmune diabetes by FTY720 in nonobese diabetic mice. Transplantation, **74**, 1684–1686.
45. Maki T., Gottschalk R., Ogawa N. and Monaco A. P. (2005): Prevention and cure of autoimmune diabetes in nonobese diabetic mice by continuous administration of FTY720. Transplantation, **79**, 1051–1055.
46. Mandala S., Hajdu R., Bergstrom J., Quackenbush E., Xie J., Milligan J., Thornton R., Shei G. J., Card D., Keohane C., Rosenbach M., Hale J., Lynch C. L., Rupprecht K., Parsons W. and Rosen H. (2002): Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science, **296**, 346–349.
47. Matloubian M., Lo C. G., Cinamon G., Lesneski M. J., Xu Y., Brinkmann V., Allende M. L., Proia R. L. and Cyster J. G. (2004): Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature, **427**, 355–360.
48. Matsuda S., Minowa A., Suzuki S. and Koyasu S. (1999): Differential activation of c-Jun NH2-terminal kinase and p38 pathways during FTY720-induced apoptosis of T lymphocytes that is suppressed by the extracellular signal-regulated kinase pathway. J. Immunol., **162**, 3321–3326.
49. Matsuura M., Imayoshi T. and Okumoto T. (2000): Effect of FTY720, a novel immunosuppressant, on adjuvant- and collagen-induced arthritis in rats. Int. J. Immunopharmacol., **22**, 323–331.
50. Mazurais D., Robert P., Gout B., Berrebi-Bertrand I., Laville M. P. and Calmels T. (2002): Cell type-specific localization of human cardiac S1P receptors. J. Histochem. Cytochem., **50**, 661–670.
51. Merrill A. H. Jr. and Wang E. (1986): Biosynthesis of long--chain (sphingoid) bases from serine by LM cells. Evidence for introduction of the 4-trans-double bond after *de novo* biosynthesis of N-acylsphinganine(s). J. Biol. Chem., **261**, 3764–3769.
52. Michel C., van Echten-Deckert G., Rother J., Sandhoff K., Wang E. and Merrill A. H. Jr. (1997): Characterization of ceramide synthesis. A dihydroceramide desaturase introduces the 4,5-trans-double bond of sphingosine at the level of dihydroceramide. J. Biol. Chem., **272**, 22432–22437.
53. Mitsusada M., Suzuki S., Kobayashi E., Enosawa S., Kakefuda T. and Miyata M. (1997): Prevention of graft rejection and graft-versus-host reaction by a novel immunosuppressant, FTY720, in rat small bowel transplantation. Transpl. Int., **10**, 343–349.
54. Miyamoto T., Matsumori A., Hwang M. W., Nishio R., Ito H. and Sasayama S. (2001): Therapeutic effects of FTY720, a new immunosuppressive agent, in a murine model of acute viral myocarditis. J. Am. Coll. Cardiol., **37**, 1713–1718.
55. Miyasaka M. and Tanaka T. (2004): Lymphocyte trafficking across high endothelial venules: dogmas and enigmas. Nat. Rev. Immunol., **4**, 360–370.
56. Mori S., Nakano H., Aritomi K., Wang C. R., Gunn M. D. and Kakiuchi T. (2001): Mice lacking expression of the chemokines CCL21-ser and CCL19 (plt mice) demonstrate delayed but enhanced T cell immune responses. J. Exp. Med., **193**, 207–218.
57. Muller H., Hofer S., Kaneider N., Neuwirt H., Mosheimer B., Mayer G., Konwalinka G., Heufler C. and Tiefenthaler M. (2005): The immunomodulator FTY720 interferes with effector functions of human monocyte-derived dendritic cells. Eur. J. Immunol., **35**, 533–545.
58. Nakano H., Mori S., Yonekawa H., Nariuchi H., Matsuzawa A. and Kakiuchi T. (1998): A novel mutant gene involved in T-lymphocyte-specific homing into peripheral lymphoid organs on mouse chromosome 4. Blood, **91**, 2886–2895.
59. Nikolova Z., Hof A., Baumlin Y. and Hof R. P. (2001): Combined FTY720/cyclosporine treatment promotes graft survival and lowers the peripheral lymphocyte count in a murine cardiac allotransplantation model. Transplantation, **72**, 168–171.
60. Ebers G. C. (1998): Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) Study Group. Lancet, **352**, 1498–1504.
61. Norment A. M. and Bevan M. J. (2000): Role of chemokines in thymocyte development. Semin. Immunol., **12**, 445–455.
62. O'Connor P. (2005) Phase II study with oral FTY720 in relapsing MS: Results of the dose-blinded active drug extension phase at 12 months. 21st Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS)/10th Annual Meeting of the Americas Committee for Treatment and Research in Multiple Sclerosis (ACTRIMS), Thessaloniki, Greece.
63. Paugh S. W., Payne S. G., Barbour S. E., Milstien S. and Spiegel S. (2003): The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2. FEBS Lett., **554**, 189–193.
64. Pinschewer D. D., Ochsenbein A. F., Odermatt B., Brinkmann V., Hengartner H. and Zinkernagel R. M. (2000): FTY720 immunosuppression impairs effector T cell peripheral homing without affecting induction, expansion, and memory. J. Immunol., **164**, 5761–5770.
65. Radeke H. H., von Wenckstern H., Stoidtner K., Sauer B., Hammer S. and Kleuser B. (2005): Overlapping signaling pathways of sphingosine 1-phosphate and TGF-beta in the murine Langerhans cell line XS52. J. Immunol., **174**, 2778–2786.
66. Randolph G. J., Sanchez-Schmitz G. and Angeli V. (2005): Factors and signals that govern the migration of dendritic cells via lymphatics: recent advances. Springer Semin. Immunopathol., **26**, 273–287.
67. Renkl A., Berod L., Mockenhaupt M., Idzko M., Panther E., Termeer C., Elsner P., Huber M. and Norgauer J. (2004): Distinct effects of sphingosine-1-phosphate, lysophosphatidic acid and histamine in human and mouse dendritic cells. Int. J. Mol. Med., **13**, 203–209.
68. Sanchez T., Estrada-Hernandez T., Paik J. H., Wu M. T., Venkataraman K., Brinkmann V., Claffey K. and Hla T. (2003): Phosphorylation and action of the immunomodulator FTY720 inhibits vascular endothelial cell growth factor-induced vascular permeability. J. Biol. Chem., **278**, 47281–47290.
69. Sanna M. G., Liao J., Jo E., Alfonso C., Ahn M. Y., Peter-

son M. S., Webb B., Lefebvre S., Chun J., Gray N. and Rosen H. (2004): Sphingosine 1-phosphate (S1P) receptor subtypes S1P1 and S1P3, respectively, regulate lymphocyte recirculation and heart rate. J. Biol. Chem., **279**, 13839–13848.

70. Sawicka E., Zuany-Amorim C., Manlius C., Trifilieff A., Brinkmann V., Kemeny D. M. and Walker C. (2003): Inhibition of Th1- and Th2-mediated airway inflammation by the sphingosine 1-phosphate receptor agonist FTY720. J. Immunol., **171**, 6206–6214.
71. Schuurman H. J., Menninger K., Audet M., Kunkler A., Maurer C., Vedrine C., Bernhard M., Gaschen L., Brinkmann V. and Quesniaux V. (2002): Oral efficacy of the new immunomodulator FTY720 in cynomolgus monkey kidney allotransplantation, given alone or in combination with cyclosporine or RAD. Transplantation, **74**, 951–960.
72. Schwab S. R., Pereira J. P., Matloubian M., Xu Y., Huang Y. and Cyster J. G. (2005): Lymphocyte sequestration through S1P lyase inhibition and disruption of S1P gradients. Science, **309**, 1735–1739.
73. Singleton P. A., Dudek S. M., Chiang E. T. and Garcia J. G. (2005): Regulation of sphingosine 1-phosphate-induced endothelial cytoskeleton rearrangement and barrier enhancement by S1P1 receptor, PI3 kinase, Tiam1/Rac1, and alpha-actinin. FASEB J., **19**, 1646–1656.
74. Skerjanec A., Tedesco H., Neumayer H. H., Cole E., Budde K., Hsu C. H. and Schmouder R. (2005): FTY720, a novel immunomodulator in *de novo* kidney transplant patients: pharmacokinetics and exposure-response relationship. J. Clin. Pharmacol., **45**, 1268–1278.
75. Spiegel S. and Milstien S. (2002): Sphingosine 1-phosphate, a key cell signaling molecule. J. Biol. Chem., **277**, 25851–25854.
76. Spiegel S. and Milstien S. (2003): Sphingosine-1-phosphate: an enigmatic signalling lipid. Nat. Rev. Mol. Cell Biol., **4**, 397–407.
77. Stoffel W., LeKim D. and Sticht G. (1968): Metabolism of sphingosine bases. 8. Distribution, isolation and properties of D-3-oxosphinganine reductase. Stereospecificity of the NADPH-dependent reaction of 3-oxodihydrospingosine (2-amino-1-hydroxyoctadecane-3-one). Hoppe Seylers Z. Physiol. Chem., **349**, 1637–1644.
78. Suzuki T., Jin M. B., Shimamura T., Yamashita K., Taniguchi M., Nomura M., Yokota R., Fukai M., Magata S., Horiuchi H., Fujita M., Nagashima K., Furukawa H. and Todo S. (2004): A new immunosuppressant, FTY720, in canine kidney transplantation: effect of single-drug, induction and combination treatments. Transpl. Int., **17**, 574–584.
79. Tani M., Sano T., Ito M. and Igarashi Y. (2005): Mechanisms of sphingosine and sphingosine 1-phosphate generation in human platelets. J. Lipid Res., **46**, 2458–2467.
80. Tedesco-Silva H., Mourad G., Kahan B. D., Boira J. G., Weimar W., Mulgaonkar S., Nashan B., Madsen S., Charpentier B., Pellet P. and Vanrenterghem Y. (2005): FTY720, a novel immunomodulator: efficacy and safety results from the first phase 2A study in *de novo* renal transplantation. Transplantation, **79**, 1553–1560.
81. Thudichum J. L. (1884) A treatise on the chemical constitution of brain. Bailliere, Tindall and Cox, London, 149–411.
82. Van Veldhoven P. P. and Mannaerts G. P. (1994): Sphinganine 1-phosphate metabolism in cultured skin fibroblasts: evidence for the existence of a sphingosine phosphatase. Biochem. J., **299**, 597–601.
83. Vogler R., Sauer B., Kim D. S., Schafer-Korting M. and Kleuser B. (2003): Sphingosine-1-phosphate and its potentially paradoxical effects on critical parameters of cutaneous wound healing. J. Invest. Dermatol., **120**, 693–700.
84. Vora K. A., Nichols E., Porter G., Cui Y., Keohane C. A., Hajdu R., Hale J., Neway W., Zaller D. and Mandala S. (2005): Sphingosine 1-phosphate receptor agonist FTY720-phosphate causes marginal zone B cell displacement. J. Leukoc. Biol., 78, 471–480.
85. Wang M. E., Tejpal N., Qu X., Yu J., Okamoto M., Stepkowski S. M. and Kahan B. D. (1998): Immunosuppressive effects of FTY720 alone or in combination with cyclosporine and/or sirolimus. Transplantation, **65**, 899–905.
86. Wang W., Graeler M. H. and Goetzl E. J. (2004): Physiological sphingosine 1-phosphate requirement for optimal activity of mouse CD4+ regulatory T Cells. FASEB J., **18**, 1043–1045.
87. Wang W., Graeler M. H. and Goetzl E. J. (2005): Type 4 sphingosine 1-phosphate G protein-coupled receptor (S1P4) transduces S1P effects on T cell proliferation and cytokine secretion without signaling migration. FASEB J., **19**, 1731–1733.
88. Wei S. H., Rosen H., Matheu M. P., Sanna M. G., Wang S. K., Jo E., Wong C. H., Parker I. and Cahalan M. D. (2005): Sphingosine 1-phosphate type 1 receptor agonism inhibits transendothelial migration of medullary T cells to lymphatic sinuses. Nat. Immunol., **6**, 1228–1235.
89. Xie J. H., Nomura N., Koprak S. L., Quackenbush E. J., Forrest M. J. and Rosen H. (2003): Sphingosine-1-phosphate receptor agonist impairs the efficiency of the local immune response by altering trafficking of naive and antigen-activated CD4(+) T cells. J. Immunol., **170**, 3662–3670.
90. Xin C., Ren S., Eberhardt W., Pfeilschifter J. and Huwiler A. (2006): The immunomodulator FTY720 and its phosphorylated derivative activate the Smad signalling cascade and upregulate connective tissue growth factor and collagen type IV expression in renal mesangial cells. Br. J. Pharmacol., **147**, 164–174.
91. Yan S., Rodriguez-Barbosa J. I., Pabst O., Beckmann J. H., Brinkmann V., Forster R. and Hoffmann M. W. (2005): Control of intestinal allograft rejection by FTY720 and costimulation blockade. Transplant. Proc., **37**, 114–115.
92. Yan S., Rodriguez-Barbosa J. I., Pabst O., Beckmann J. H., Brinkmann V., Forster R. and Hoffmann M. W. (2005): Protection of mouse small bowel allografts by FTY720 and costimulation blockade. Transplantation, **79**, 1703–1710.
93. Yanagawa Y., Hoshino Y., Kataoka H., Kawaguchi T., Ohtsuki M., Sugahara K. and Chiba K. (1999): FTY720, a novel immunosuppressant, prolongs rat skin allograft survival by decreasing T-cell infiltration into grafts. Transplant. Proc., **31**, 1227–1229.
94. Yanagawa Y., Sugahara K., Kataoka H., Kawaguchi T., Masubuchi Y. and Chiba K. (1998): FTY720, a novel immunosuppressant, inuces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. II. FTY720 prolongs skin allograft survival by decreasing T cell infiltration into grafts but not cytokine production *in vivo*. J. Immunol., **160**, 5493–5499.
95. Yanagihara S., Komura E., Nagafune J., Watarai H. and Yamaguchi Y. (1998): EBI1/CCR7 is a new member of dendritic cell chemokine receptor that is up-regulated upon maturation. J. Immunol., **161**, 3096–3102.

96. Yang L., Yatomi Y., Miura Y., Satoh K. and Ozaki Y. (1999): Metabolism and functional effects of sphingolipids in blood cells. Br. J. Haematol., **107**, 282–293.
97. Yatomi Y., Ozaki Y., Ohmori T. and Igarashi Y. (2001): Sphingosine 1-phosphate: synthesis and release. Prostaglandins Other Lipid Mediat., **64**, 107–122.
98. Yoshida R., Nagira M., Kitaura M., Imagawa N., Imai T. and Yoshie O. (1998): Secondary lymphoid-tissue chemokine is a functional ligand for the CC chemokine receptor CCR7. J. Biol. Chem., **273**, 7118–7122.
99. Zemann B., Kinzel B., Muller M., Reuschel R., Mechtcheriakova D., Urtz N., Bornancin F., Baumruker T. and Billich A. (2006): Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720. Blood, **107**, 1454–1458.
100. Zhang H., Desai N. N., Olivera A., Seki T., Brooker G. and Spiegel S. (1991): Sphingosine-1-phosphate, a novel lipid, involved in cellular proliferation. J. Cell Biol., **114**, 155–167.