Review

Role of Sphingosine 1-Phosphate Receptor Type 1 in Lymphocyte Egress from Secondary Lymphoid Tissues and Thymus

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Circulation of mature lymphocytes between blood and secondary lymphoid tissues plays a central role in the immune system. Homing of lymphocytes from blood into secondary lymphoid tissues beyond high endothelial venules is highly dependent on the interaction between the chemokines CCL19, CCL21, CXCL12, and CXCL13, and their receptors CCR7, CXCR4 and CXCR5. However, the molecular mechanism(s) of lymphocyte egress from secondary lymphoid tissues to lymph remained unclear. We have found a new class of immunomodulator, FTY720 by chemical modification of vegetative wasp-derived natural product, ISP-I (myriocin). FTY720 has been shown to be highly effective in experimental allograft and autoimmune disease models. A striking feature of FTY720 is the induction of a marked decrease in peripheral blood lymphocytes at doses that show immunomodulating activity in these models. The reduction of circulating lymphocytes by FTY720 is caused by sequestration of lymphocytes into secondary lymphoid tissues and thymus. FTY720 is rapidly converted to (*S*)-enantiomer of FTY720-phosphate [(*S*)-FTY720-P] by sphingosine kinase 2 *in vivo*. (*S*)-FTY720-P acting as a potent agonist of S1P receptor type 1 ($S1P_1$), induces long-term down-regulation of $S1P_1$ on lymphocytes, and thereby inhibits the migration of lymphocytes toward S1P. Thus, it is presumed that FTY720-induced lymphocyte sequestration is due to the inhibition of S1P/$S1P_1$-dependent lymphocyte egress from secondary lymphoid tissues and thymus by its active metabolite (*S*)-FTY720-P. Throughout the analysis of the mechanism of action of FTY720, it is clarified that S1P/$S1P_1$ interaction plays an important role for lymphocyte egress from secondary lymphoid tissues and thymus. ***Cellular & Molecular Immunology*****. 2006;3(1):11-19.**

Key Words: FTY720, S1P, $S1P_1$, immunomodulation, lymphocyte egress

Introduction

A potent immunosuppressive natural product, ISP-I (myriocin), and its derivative, mycestericins, were isolated from a culture broth of *Isaria sinclairii*, a kind of vegetative wasp that is an "eternal youth" nostrum in traditional Chinese herbal medicine (1-3). Chemical modification of ISP-I yielded a new synthetic compound, 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride (FTY720), which has more potent immunomodulating activity and less toxicity than ISP-I (4-7). FTY720, at 0.1 mg/kg or higher doses, significantly prolongs skin and cardiac allograft survival and host survival in lethal graft versus host reaction in rats (8-11). In addition, combined treatment with FTY720 and a subtherapeutic dose of cyclosporin A (CsA) or tacrolimus (FK506) results in a synergistic effect on canine renal allograft as well as rat skin and cardiac allografts (8, 9, 11-17). Unlike CsA or FK506, FTY720 does not affect the production of Th1-associated cytokines from antigen-stimulated helper T cells.

A striking feature of FTY720 is the induction of a marked decrease in the number of peripheral blood lymphocytes, especially T cells, at doses that prolong allograft survival (8, 9, 18). The reduction of circulating lymphocytes by FTY720 is mainly caused by the sequestration of circulating mature lymphocytes into secondary lymphoid tissues. FTY720 thereby decreases T cell infiltration into grafted organs

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Abbreviations: FTY720, 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride; FTY720-P, FTY720-phosphate; CsA, cyclosporin A; FK506, tacrolimus; S1P, sphingosine 1-phosphate; $S1P_1$, sphingosine 1-phosphate receptor type 1; MMF, mycophenolate mofetil; AZ, azathiprine; MHC, major histocompatibility complex antigen; CI, combination index; GvHR, graft versus host reaction; IL-2, interleukin 2; IFN-γ, interferon-γ; (*S*)-FTY720-P, (*S*)-enantiomer of FTY720-phosphate.

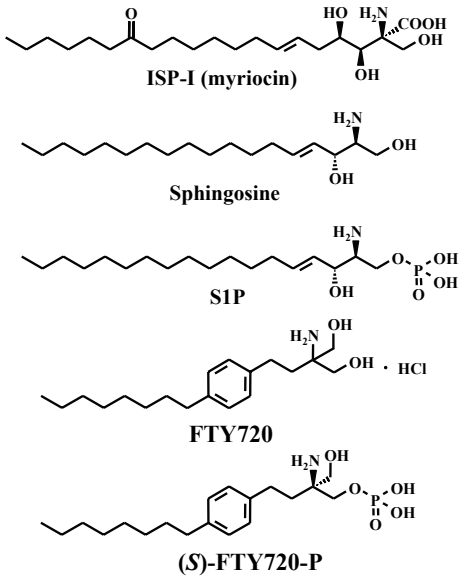

Figure 1.The chemical structures of ISP-I, sphingosine, S1P, FTY720, and (*S*)-FTY720-P.

(16-20). FTY720, unlike ISP-I, does not inhibit serine-palmitoyl-transferase, the first enzyme in sphingolipid biosynthesis; however both molecules are structurally similar to sphingosine. Recently, it has been reported that FTY720 is effectively phosphorylated by sphingosine kinase 2, and that FTY720-phosphate (FTY720-P) is a high affinity agonist for sphingosine 1-phosphate (S1P) receptors (21-23). The chemical structures of ISP-I, sphingosine, S1P, FTY270, and (*S*)-FTY-720-P are shown in Figure 1.

Circulation of mature lymphocytes between blood and secondary lymphoid tissues plays a central role in the establishment of the immune response to foreign antigens. Homing of lymphocyte from blood into secondary lymphoid tissues beyond high endothelial venules is highly dependent on the interaction between the chemokines CCL19/ELC, CCL21/SLC, CXCL12/SDF-1α, and CXCL13/BLC, and their receptors CCR7, CXCR4 and CXCR5 on lymphocytes (24). These chemokines involved in lymphocyte homing are constitutively expressed in secondary lymphoid tissues and can induce migration of T cells, B cells and dendritic cells into lymph nodes and Peyer's patches. Moreover, the precise positioning of lymphocytes (T cells and B cells) within secondary lymphoid tissues is controlled by the responsiveness of these cells towards overlapping gradients of chemokines, including CCL19, CCL21, and CXCL13, that are expressed in separate but adjacent areas of secondary lymphoid tissues. On the other hand, the molecular mechanism(s) of lymphocyte egress from secondary lymphoid tissues to lymph still remained unclear. Based on the analysis of the mechanism of action of FTY720, it is clarified that $S1P/S1P_1$ interaction plays an important role in lymphocyte egress from secondary lymphoid tissues and thymus (25-27) (Figure 2). We summarize the current understanding of FTY720 and discuss the important role of $S1P/S1P_1$ interaction in the lymphocyte recirculation system.

Mechanism of action of FTY720, S1P receptor modulator

FTY720 induces lymphocyte sequestration into secondary lymphoid tissues and thymus

As shown in Table 1, FTY720 has been shown to be highly effective in various experimental allograft and autoimmune disease models (11, 20). A striking feature of FTY720 is the

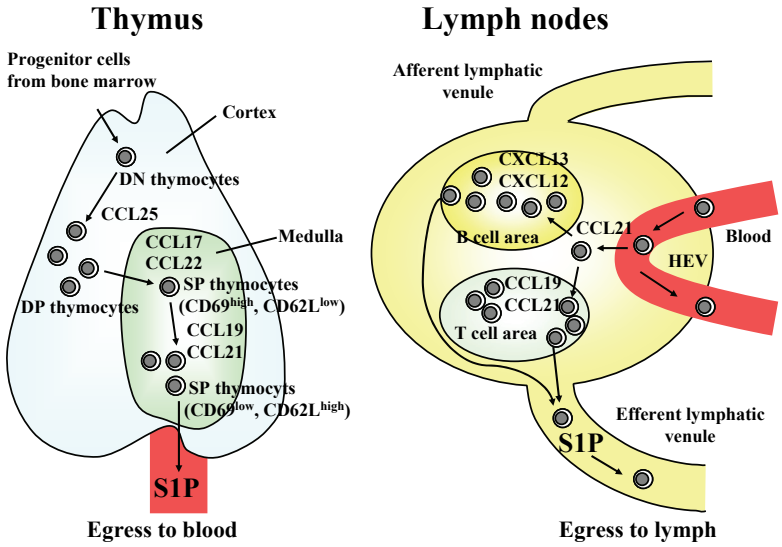

Figure 2. Lymphocytes migrate toward S1P from thymus and secondary lyomphoid tissues.

Table 1. Pharmacological properties of FTY720 and calcineurin inhibitors

Response suppressed	Species	FTY720	CsA	FK506
Allograft rejection	Rat (Skin)	0.1 mg/kg	3 mg/kg	0.3 mg/kg
	Rat (Heart)	0.1 mg/kg	3 mg/kg	0.3 mg/kg
Combination with CsA	Rat (Skin)	0.1 mg/kg		
	Rat (Heart)	0.1 mg/kg		
	Dog (Kidney)	0.03 mg/kg		
	Monkey (Kidney)	0.1 mg/kg		
GvHR	Rat	0.1 mg/kg	3 mg/kg	1 mg/kg
DTH (MeHSA)	Mouse	0.03 mg/kg	3 mg/kg	0.3 mg/kg
Antibody production (to SRBC)	Rat	0.1 mg/kg	3 mg/kg	0.3 mg/kg
Adjuvant-induced arthritis	Rat	0.1 mg/kg	3 mg/kg	1 mg/kg
Collagen-induced arthritis	Rat	0.1 mg/kg	3 mg/kg	1 mg/kg
EAE	Rat	0.1 mg/kg	10 mg/kg	1 mg/kg
Lupus nephritis (MRL/*lpr*)	Mouse	0.1 mg/kg	10 mg/kg	1 mg/kg
Lymphopenia	Mouse	0.1 mg/kg		
	Rat	0.1 mg/kg		
	Dog	0.03 mg/kg		
	Monkey	0.1 mg/kg		

CsA, cyclosporin A; FK506, tacrolimus; GvHR, graft versus host reaction; DTH, delayed-type hyper sensitivity; MeHSA, methylated human serum albmin; SRBC, sheep red blood cells; EAE, experimental autoimmune encephalomyelitis.

induction of a marked decrease in the number of peripheral blood lymphocytes (lymphopenia) at doses that display an immunomodulating activity in these disease models. In rats, the number of lymphocytes (T cells and B cells) in peripheral blood decreased dramatically within 6 hours after oral administration of FTY720 at 0.1 to 1 mg/kg (8, 18, 19). In particular, the reduction in T cell numbers is remarkable. In mice, dogs, and cynomolgus monkeys, marked lymphopenia is also induced by FTY720 administration (11, 14, 28, 29). In the phase 1a study, administration of single oral doses of FTY720, ranging from 0.25 to 3.5 mg/kg, to stable renal transplant patients maintained on a regimen of CsA and corticosteroid, causes a dose-dependent reduction in peripheral blood T cells and B cells (30). At doses greater than 1.0 mg, the mean nadir counts are 30% to 60% below the baseline values. In the phase 1b study on pharmacodynamics, pharmacokinetics, and the safety of multiple FTY720 doses in stable renal transplant patients, FTY720 at 1.0 mg/day or greater significantly reduces the number of peripheral blood lymphocytes by up to 85%, which reverses within 3 days after discontinuation of the study medication (31, 32).

The immunologically mature lymphocytes are known to continuously recirculate in the blood, spleen, lymph nodes, Peyer's patches and lymphatic vessels (24). To clarify the mechanism of FTY720-induced lymphopenia, the lymphocyte distribution in blood, lymph and various lymphoid tissues was analyzed after FTY720 administration in rats (17-19). Within 3 to 24 h after a single oral administration of FTY720 at 0.1 to 1 mg/kg, the number of lymphocytes in rats decreased markedly in the peripheral blood, as well as in thoracic duct lymph, and partially in spleen. In contrast, the number of lymphocytes in peripheral lymph nodes, mesenteric lymph nodes, and Peyer's patches increased significantly at the same time. Intravenous transfusion of fluorescein-labeled rat lymphocytes into rats reveals that the labeled lymphocytes accumulate in lymph nodes and Peyer's patches with FTY720 administration. These data suggest that FTY720 induces sequestration of circulating mature lymphocytes into secondary lymphoid tissues such as lymph nodes and Peyer's patches and thereby decreases the number of lymphocytes in peripheral blood, thoracic duct lymph and spleen. Thus, sequestration of circulating mature lymphocytes is presumed to be the main mechanism of immunomodulating activity of FTY720.

Moreover, FTY720 reportedly inhibits mature thymocyte emigration from the thymus to the periphery in mice (29). In the thymus, long-term FTY720 administration causes a three- to four-fold increase in the number of mature medullary thymocytes ($CD4^+CD8^-$ and $CD4^-CD8^+$) as well as a slight decrease in the double-positive immature thymocyte ($CD4^+$ $CD8^+$) ratio. An intrathymic fluorescein-labeling technique confirms that only one fourth of the labeled cells are detected in the lymph nodes and in the spleen of FTY720-treated mice compared to control mice. These results suggest that the immunomodulating activity of FTY720, at least in part, could be due to its inhibitory effect on the egress of mature

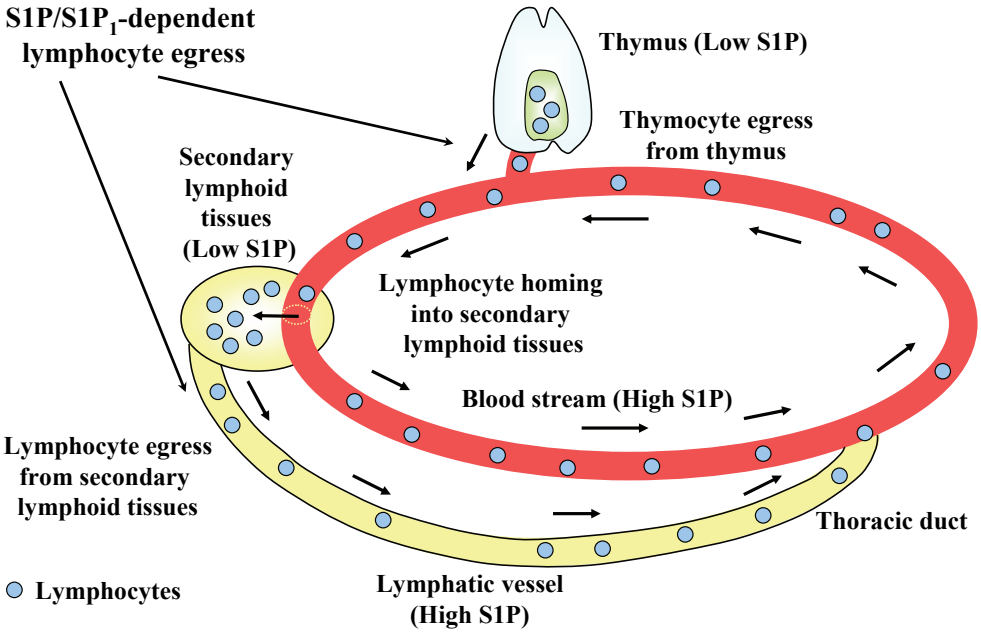

Figure 3. Role of S1P/$S1P_1$ interaction for lymphocyte egress from thymus and secondary lyomphoid tissues.

thymocytes from the thymic medulla into peripheral blood.

(S)-FTY720-phosphate induces long-term down-regulation of $S1P_1$ and inhibits lymphocyte egress from secondary lymphoid tissues and thymus

It has been demonstrated that a phosphorylated form of FTY720 acts as an agonist of S1P receptors (21, 22). S1P, a pleiotropic lysophospholipid mediator is converted primarily by the phosphorylation of sphingosine by sphingosine kinase 1 and stimulates multiple signaling pathways resulting in calcium mobilization from intracellular stores, polymerization of actin, chemotaxis/migration, and escape from apoptosis (33, 34). S1P is released by platelets during inflammatory processes and is found in significant amounts (100 to 400 nM) in serum (35). S1P binds with nanomolar (nM) affinities to five related G-protein-coupled receptors (GPCRs), termed $S1P_{1\text{-}5}$ (formerly Edg-1, -5, -3, -6 and -8). It is confirmed that FTY720 is a substrate for sphingosine kinase 1a, and sphingosine kinase 2 (21-23, 36); however, S1P is mainly converted from sphingosine by sphingosine kinase 1 whereas FTY720, unlike sphingosine, is effectively phosphorylated by sphingosine kinase 2 rather than sphingosine kinase 1a. After oral or intravenous FTY720 administration, the plasma concentration of FTY720-P was 2 to 6 times higher than FTY720 and FTY720-P is a high affinity agonist at four out of five S1P receptors (21, 22).

Recently, it has been reported that $S1P_1$ is essential for lymphocyte recirculation and that $S1P_1$ regulates lymphocyte egress from thymus and secondary lymphoid tissues (25, 26, 37). In mice whose hematopoietic cells lack a single S1P receptor, $S1P_1$, there are no T cells in the periphery because mature T cells are unable to exit thymus and secondary lymphoid tissues. Moreover, $S1P_1$-dependent chemotactic responsiveness is strongly up-regulated in T cell development before exit from the thymus, whereas $S1P_1$ is down-regulated during peripheral lymphocyte activation, and this is associated with retention in lymphoid tissues (25, 38). FTY720 treatment down-regulates $S1P_1$, creating a temporary pharmacological $S1P_1$-null state in lymphocytes, providing an explanation for the mechanism of FTY720-induced lymphocyte sequestration. Since $S1P_1$ surface expression on lymphocytes highly depends on the extracellular concentration of S1P, $S1P_1$ on lymphocytes down-regulated in the blood, up-regulated in secondary lymphoid tissues, and down-regulated again in the lymph. Thus, it is proposed that cyclical modulation of $S1P_1$ surface expression on circulating lymphocytes by S1P contributes to establishing their transit time in secondary lymphoid tissues (26) (Figure 3).

We successfully established an effective method for asymmetric synthesis of both (*S*)- and (*R*)-enantiomers of FTY720-P with respective high eantio-selectivity, and confirmed that (*S*)-FTY720-P binds to $S1P_{1,\ 3,\ 4,\ 5}$, but not $S1P_2$, at nano-molar concentrations (39). On the contrary, the binding affinities of (*R*)-enantiomer of FTY720-P for S1P receptors were more than 100-fold weaker than those of (*S*)-FTY720-P. After administration to rats, FTY720 is metabolized by omega-oxidation of the octyl side chain, and subsequent β-oxidation, or phosphorylation to (*S*)-FTY720-P by sphingosine kinase 2 rather than sphingosine kinase 1 (23, 40). We then clarified the contribution of FTY720-metabolites including an active metabolite, (*S*)-FTY720-P to the induction of lymphopenia and immunomodulating activity by FTY720 *in vivo*, because only (*S*)-FTY720-P was detected in blood from rats administered with FTY720.

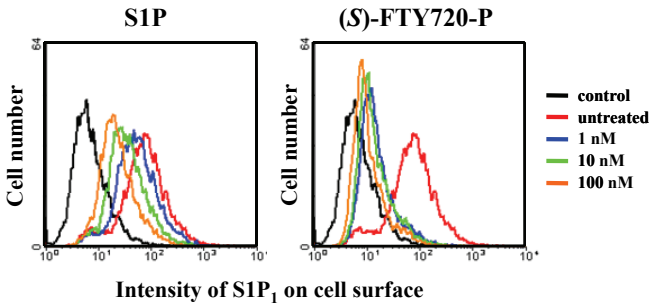

Figure 4. Effect of (*S*)-FTY720-P and S1P on the surface expression of $S1P_1$ on transfected CHO cells. Human $S1P_1$ stably expressed CHO cells was stained with FITC-conjugated anti-human $S1P_1$ monoclonal antibody and expression of $S1P_1$ on cell surface was analyzed by flow cytometry.

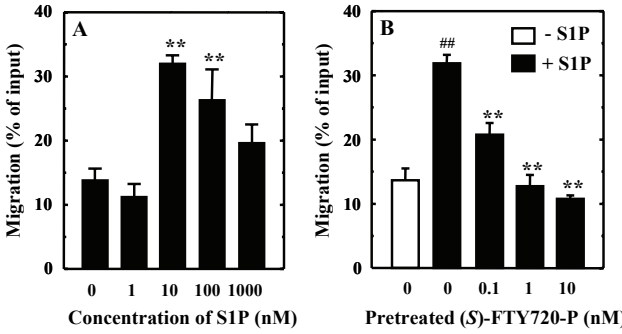

Figure 6. (*S*)-FTY720-P inhibited the migration of $CD4^+$ T cells toward S1P. Mouse $CD4^+$ T cells (5×10^5 cells) were added to the upper wells of 5 μm-pore polycarbonate tissue culture inserts with S1P dilution in bottom wells. Migration toward S1P was performed at 37°C for 180 min and migrated cells were counted by flow cytometry (A). (*S*)-FTY720-P was added to upper well just before the migration assay toward S1P at 10 nM (B). Each column represents the mean ± SE of triplicate determination. Statistical significance was calculated by Dunnett's multiple-comparison test (**$p < 0.01$ *vs* control migration toward S1P at 10 nM, $^{\#\#}p < 0.01$ *vs* migration in the absence of S1P).

(*S*)-FTY720-P at 0.1 and 1 mg/kg intravenously induces a marked lymphopenia, significantly prolonged the survival of rat skin allograft, and inhibited graft versus host reaction in mice (40, 41). On the other hand, ϖ- and β-oxidized 4 metabolites (M1, M2, M3 and M4) show neither lymphopenia nor immunomodulating activity at an intravenous dose of 10 mg/kg in the rat skin allograft (40). In addition, M1, M2, M3, and M4 as well as FTY720 up to 10,000 nM do not bind S1P receptors. These results suggest the lymphopenia and the immunomodulating activity induced by FTY720 administration are due to the agonistic activity against S1P receptors of the active metabolite, (*S*)-FTY720-P.

We also confirmed that (*S*)-FTY720-P shows agonist activity for $S1P_1$ at nano-molar concentrations using extracellular signal regulated kinase 1/2 (ERK1/2) phosphorylation assay and subsequently induces long-term down-regulation of $S1P_1$ in Chinese hamster ovary (CHO) cells stably expressing human $S1P_1$ (Figure 4). The down-regulation of $S1P_1$ by (*S*)-FTY720-P appeared to be maintained longer than that by S1P (27) (Figure 5). It is still unclear why (*S*)-FTY720-P can induce long-term down-regulation of $S1P_1$ compared with S1P; however, the difference between S1P and (*S*)-FTY720-P might be due to the stability of (*S*)-FTY720-P for degradation by S1P lyase. S1P at concentrations of 10 to 100 nM induces migration of lymph node $CD4^+$ T cells and $CD4^+$ thymocytes in mice. By contrast, (*S*)-FTY720-P effectively inhibits the migration of $CD4^+$ T cells toward S1P (27) (Figure 6). Based on these results, it is presumed that (*S*)-FTY720-P converted from FTY720 acts as an agonist at $S1P_1$, induces long-term down-regulation of $S1P_1$ on lymphocytes, and shows immunomodulating activity by inhibition of $S1P/S1P_1$-dependent lymphocyte egress from secondary lymphoid tissues and thymic medulla (Figure 7).

Pharmacological effect of FTY720

Immunomodulating effect of FTY720 in experimental allograft models

Pharmacological properties of FTY720 as compared with

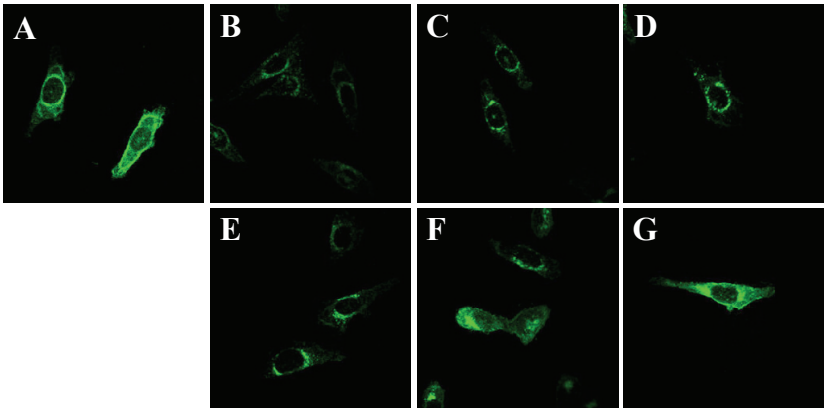

Figure 5. (*S*)-FTY720-P induced a long-term down-regulation of $S1P_1$ as compared S1P. Hemagglutinin (HA)-tagged human $S1P_1$ stably expressed in transfected CHO cells was visualized by FITC-conjugated anti-HA antibody under fluorescence microscopy. (A) Confocal microscopy of HA-$S1P_1$ control. (B) (*S*)-FTY720-P 100 nM for 30 min. (C) (*S*)-FTY720-P 100 nM for 3 h. (D) (*S*)-FTY720-P 100 nM for 8 h. (E) S1P at 100 nM for 30 min. (F) S1P at 100 nM for 3 h. (G) S1P at 100 nM for 8 h.

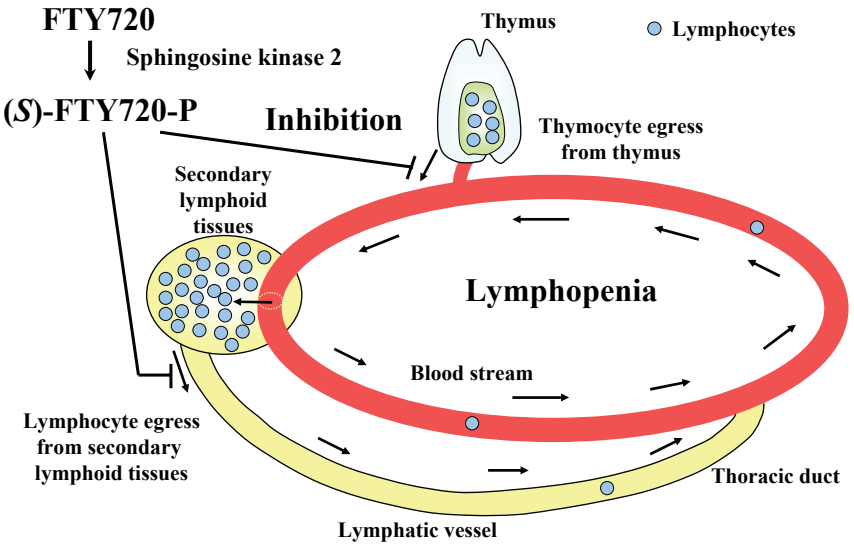

Figure 7. (*S*)-FTY720-P converted from FTY720 inhibits S1P/$S1P_1$-dependent lymphocyte egress from secondary lymphoid tissues and thymus.

calcineurin inhibitors are summarized in Table 1. To clarify the efficacy and potency of the immunomodulating activity of FTY720, the prolonging effect of FTY720, CsA, FK506, mycophenolate mofetil (MMF), and azathioprine (AZ) on rat skin allograft survival was compared in major histocompatibility complex antigen (MHC)-incompatible rat strains of WKAH donors and F344 recipients (8, 18, 40). FTY720 and the other immunosuppressants were administered orally for 14 days from the day of trans- plantation. FTY720 at 0.03 mg/kg or higher doses significantly prolongs allograft survival in a dose-dependent manner (8, 18, 19, 25). CsA and FK506 are also effective at doses of 3 mg/kg or more and 0.3 mg/kg or more in this model, respectively. MMF and AZ show a marginal immunosuppressive effect only at high doses, and all animals in a group given AZ at 100 mg/kg died.

The effect of FTY720 on heterotopic cardiac allograft survival was compared with those of CsA and FK506 in MHC-incompatible rat strains of WKAH donors and ACI recipients (9, 15). All cardiac allografts in the control group are rejected within 14 days after transplantation. Treatment with FTY720 at 0.1 mg/kg *p.o.* or higher doses for 14 days significantly prolonged the cardiac allograft survival in a dose-dependent manner. FTY720 at 10 mg/kg induced long-term graft survival for more than 100 days in three out of eight recipient rats. CsA and FK506 also significantly prolong the cardiac allograft survival at doses of 10 mg/kg or more and 1 mg/kg or more, respectively. These results indicate that FTY720 possesses more potent immunomodulating activity than other immunosuppressive drugs on graft rejection in rat allograft models.

In clinical organ transplantations, CsA- or FK506-based combination therapy with prednisolone or other immunosuppressants is widely used to reduce the side effects of individual drugs. Therefore, it is important to investigate whether the combined use of FTY720 and CsA or FK506 produces a synergistic effect on experimental allograft models. We evaluated the concomitant effect of FTY720 with CsA or FK506 in comparison with those of AZ or MMF with CsA on acute rejection in rat skin allograft models (8, 15, 18, 19, 27, 40). The combination therapy of FTY720 with CsA or FK506 has a marked prolonging effect on allograft survival as compared with the monotherapy of FTY720, CsA or FK506. The concomitant effect of FTY720 with CsA or FK506 is stronger than that of AZ with CsA or MMF with CsA. To clarify the concomitant effects, median effect analysis was used to calculate the combination index (CI) values of these combination therapy groups (15, 40). Since the CI values are less than 0.2 in the concomitant groups with FTY720 and CsA, it confirms that the combination therapy with FTY720 and CsA exerts a synergistic effect. On the other hand, the concomitant treatment of AZ and CsA or MMF and CsA shows only an additive effect, because the CI values of these groups are 0.9 to 1.1. In the rat heterotopic cardiac allograft model using WKAH donors and ACI recipients, the combination therapy with FTY720 and CsA or FK506 shows a more marked prolonging effect compared with that in concomitant treatment with AZ and CsA (9, 15, 40). The CI values are less than 0.2 in the concomitant group with FTY720 and CsA or FK506, indicating a synergistic effect, whereas those in the group with AZ plus CsA are 0.5 to 0.9. Canine renal allograft survival is significantly prolonged by combination therapy with FTY720 at 0.03 and 1 mg/kg plus CsA at 10 mg/kg compared to the monotherapy with FTY720 or CsA (12-14). In combined treatment with FTY720 and CsA, there is no severe toxicity in kidney and liver, and the blood concentrations of FTY720 and CsA did not affect each other. Moreover, FTY720 in combination with

a subtherapeutic dose of CsA displays a synergistic effect on prolongation of renal allograft survival in cynomolgus monkeys (42). From these results, it is presumed that the combination therapy with FTY720 and calcineurin inhibitors provides a more beneficial tool for human organ transplantation compared with the conventional combination therapies, including calcineurin inhibitors plus AZ or MMF.

The phase 2a, multicenter, open-label, dose-finding study was performed to evaluate the efficacy and safety of FTY720 compared with MMF in combination with CsA and corticosteroid in *de novo* renal transplant patients (43). The incidence of biopsy-confirmed acute rejection is 23.3%, 34.9%, 17.5%, and 9.8%, respectively, with FTY720 at doses of 0.25, 0.5, 1.0, and 2.5 mg, versus 17.1% with MMF. Thus, FTY720 at 2.5 mg is as effective as MMF in combination with CsA for the prevention of acute rejection after human renal transplantation.

FTY720 decreases T cell infiltration into inflammatory sites

Unlike calcineurin inhibitors, FTY720 up to 1,000 nM does not affect T cell proliferation and Th1-associated cytokine production induced by antigen stimulation. To elucidate the mechanism of the synergistic effect of FTY720 in combination with CsA, we analyzed mRNA expression of interleukin 2 (IL-2) and interferon-γ (IFN-γ), and that of CD3, which reflects T cell infiltration in rat skin allograft (16). In the skin allograft, mRNA levels of IL-2, IFN-γ, and CD3 increase, peaking on day 4 to 5 after transplantation. CsA at 10 mg/kg significantly inhibits the elevation of IL-2 and IFN-γ mRNA. On the contrary, FTY720 at 0.1 mg/kg markedly inhibits the elevation of CD3 mRNA, while slightly inhibits that of IL-2 and IFN-γ mRNA. FTY720 combined with CsA almost completely suppresses the intragraft expression of mRNA for IL-2, IFN-γ, and CD3. Immunohistochemical staining and flow cytometric analysis also confirm that FTY720 decreases T cell infiltration into the allograft (16, 44, 45). These findings suggest that unlike calcineurin inhibitors, FTY720 prolongs allograft survival by decreasing T cell infiltration into grafts but not Th1-associated cytokine production. It is highly probable that the decreasing effect of FTY720 on T cell infiltration into inflammatory sites is due to a reduction in the number of circulating T cells by the sequestration of lymphocytes into secondary lymphoid tissues. Thus, it is presumed that the synergistic effect of FTY720 combined with calcineurin inhibitors on the prolongation of allograft survival is based on the respective inhibitions of T cell infiltration and cytokine production in allografts.

Effect of FTY720 on experimental autoimmune disease models

FTY720 at 0.1 mg/kg *p.o.* or higher doses almost completely prevents paralysis in experimental autoimmune encephalomyelitis (EAE) induced by myelin basic protein in LEW rats (11, 27, 46, 47). Therapeutic treatment with FTY720 inhibits EAE relapse induced by myelin proteolipid protein immunization in SJL mice (48-50). The therapeutic potential of FTY720 is more marked as compared with recombinant mouse interferon-β (rm-IFN-β), and the area of demyelination and the infiltration of $CD4^+$ T cells into the spinal cord are reduced by FTY720 treatment (49, 50). In the same dose range, FTY720 almost completely inhibits joint destruction as well as paw edema in adjuvant arthritis and type II collagen-induced arthritis in LEW rats (11, 51, 52). Moreover, FTY720 shows a markedly prophylactic and therapeutic effect on lupus nephritis in autoimmune MRL/*lpr* mice (53, 54). With only 4 weeks of FTY720 treatment at low doses, long-term improvement of lupus nephritis is observed in this autoimmune model. Moreover, therapeutic FTY720 administration decreases the number of $CD4^+$ Th1 cells infiltrated into lupus kidney. Based on these findings, it is presumed that FTY720 can provide a new approach not only for the prevention of transplant rejection, but also for therapy in autoimmune diseases including multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus.

Recent published data suggest that FTY720, after phosphorylation, acts as an agonist of the $S1P_1$ receptor, down-regulates $S1P_1$ on lymphocytes, and inhibits $S1P/S1P_1$-dependent lymphocyte egress from secondary lymphoid tissues and thymus (25-27). Thus, FTY720 causes the sequestration of circulating mature lymphocytes into lymphoid tissues and modulates the recirculation of lymphocytes between blood and lymphoid tissues. Consequently, it is presumed that FTY720 decreases the trafficking and the infiltration of antigen-specific T cells into grafted organs or inflammatory sites in autoimmune diseases, thereby exerting immunomodulating activity. Since FTY720 possesses a completely new mechanism of action, FTY720 may be a useful tool for the prevention of transplant rejection and a new therapeutic approach for autoimmune diseases including multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus.

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