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FTY720, a new class of immunomodulator, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors

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Abstract

FTY720 is the first of a new immunomodulator class: sphingosine 1-phosphate (S1P) receptor agonist. In 1994, an immunosuppressive natural product, ISP-I (myriocin), was isolated from the culture broth of *Isaria sinclairii*, a type of vegetative wasp. The chemical modification of ISP-I yielded a new compound, FTY720, which has more potent immunosuppressive activity and less toxicity than ISP-I does. FTY720 has been shown to be highly effective in experimental allotransplantation models and autoimmune disease models. A striking feature of FTY720 is the induction of a marked decrease in peripheral blood T- and B-cells at doses that show immunosuppressive activity in these models. Reportedly, FTY720 is rapidly converted to FTY720-phosphate (FTY720-P) by sphingosine kinase 2 in vivo, and FTY720-P acts as a potent agonist at S1P receptors. Recently, it has been suggested that FTY720-P internalizes $S1P_1$ on lymphocytes and thereby inhibits the migration of lymphocytes toward S1P. Thus, it is likely that the reduction of circulating lymphocytes by FTY720 is due to the inhibition of S1P/$S1P_1$-dependent lymphocyte egress from secondary lymphoid tissues and thymus. Because FTY720 displays a novel mechanism of action that has not been observed with other immunosuppressive agents and shows a synergism with cyclosporin A (CsA) and tacrolimus, it is presumed that FTY720 provides a useful tool for the prevention of transplant rejection and a new therapeutic approach for autoimmune diseases including multiple sclerosis and rheumatoid arthritis.

Keywords: FTY720; Sphingosine 1-phosphate; $S1P_1$; Immunosuppression; Lymphocyte egress

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

Contents

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1. Introduction

It has been previously reported that a potent immunosuppressive natural product, ISP-I (myriocin), and its derivative, mycestericins, can be isolated from a culture broth of *Isaria sinclairii*, a kind of vegetative wasp that is an "eternal youth" nostrum in traditional Chinese herbal medicine (Fujita et al., 1994a, 1994b; Sasaki et al., 1994). The chemical modification of ISP-I led to a novel synthetic compound, 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720), which has more potent immunosuppressive activity and less toxicity than ISP-I (Adachi et al., 1995; Fujita et al., 1995, 1996; Hirose et al., 1996; Kiuchi et al., 2000). FTY720, at 0.1 mg/kg or higher doses, significantly prolongs skin or cardiac allograft survival and host survival in lethal graft versus host reaction (GvHR) in rats (Chiba et al., 1996; Hoshino et al., 1996; Masubuchi et al., 1996; Chiba & Adachi, 1997). In addition, combination 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 or cardiac allografts (Chiba et al., 1996; Hoshino et al., 1996, 1999; Kawaguchi et al., 1996; Suzuki et al., 1996a, 1996b; Chiba & Adachi, 1997; Yanagawa et al., 1998a, 1998b). 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 (Chiba et al., 1996, 1998; Hoshino et al., 1996). FTY720 does not impair lymphocyte function, including T-cell activation, but instead induces the sequestration of circulating mature lymphocytes into the secondary lymphoid tissues and decreases T-cell infiltration into grafted organs (Chiba et al., 1998, 1999; Yanagawa et al., 1998a, 1998b; Brinkmann et al., 2000). FTY720, unlike ISP-I, does not inhibit serine-palmitoyl-transferase (Fujita et al., 1996), the first enzyme in sphingolipid biosynthesis, but 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 (Brinkmann et al., 2002; Mandala et al., 2002; Paugh et al., 2003). Fig. 1 shows the chemical structures of ISP-1, FTY720, FTY720-P, sphingosine, and S1P. Moreover, it has been suggested that FTY720-P internalizes S1P receptor type 1 ($S1P_1$) on lymphocytes and inhibits S1P/$S1P_1$-dependent lymphocyte egress from secondary lymphoid tissues and thymus (Matloubian et al., 2004; Lo et al., 2005). This paper summarizes the current understanding of the pharmacological actions and the mechanism of action of FTY720.

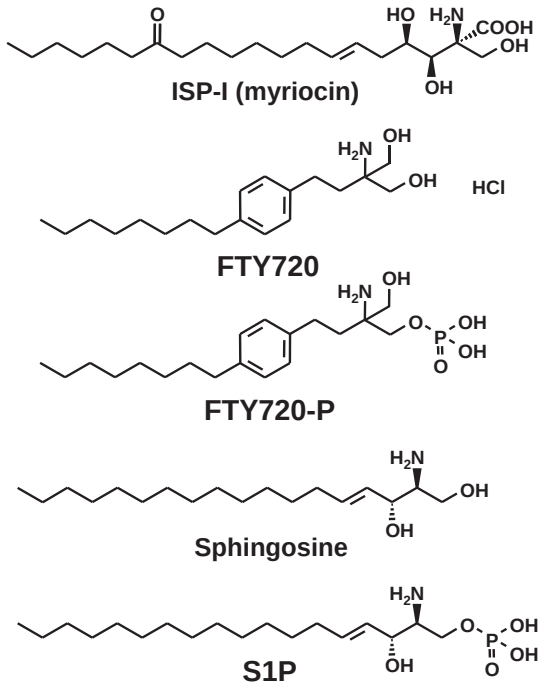

Fig. 1. The chemical structures of ISP-I, FTY720, FTY720-P, sphingosine, and S1P.

2. Effect of FTY720 in experimental allograft models

FTY720 has been shown to be highly effective in prolonging allograft survival in various experimental allotransplantation models (Chiba & Adachi, 1997; Brinkmann et al., 2000). To clarify the efficacy and potency of the immunosuppressive 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 (Chiba et al., 1996, 1998, 2005; Fig. 2A). The immunosuppressants were administered orally for 14 days from the day of the transplantation. FTY720 at 0.03 mg/kg or higher doses significantly prolongs allograft survival in a dose-dependent manner. CsA and FK506 are also effective at doses of 3 mg/kg or more and 0.3 mg/kg or more, respectively, in this model. MMF and AZ show a marginal immunosuppressive effect only at high doses, and all

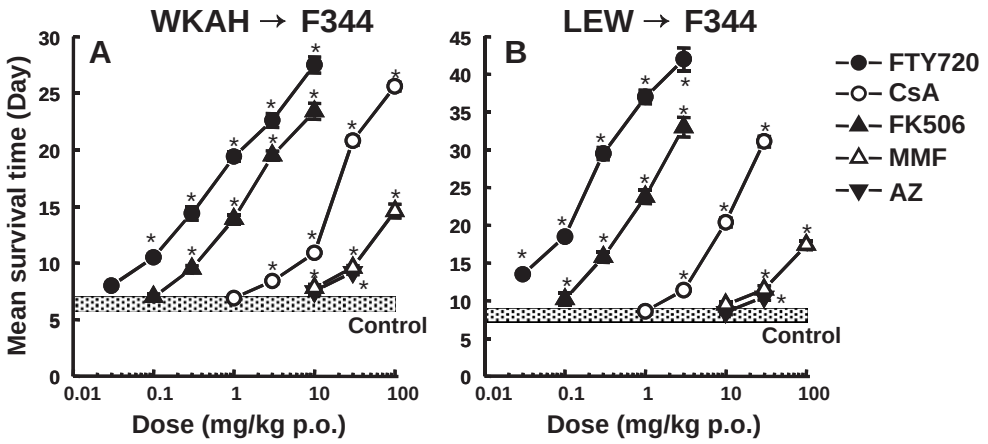

Fig. 2. Dose–response relationship between FTY720, CsA, FK506, MMF, and AZ and skin allograft survival in MHC-incompatible and MHC-compatible rat strain systems. (**A**) MHC-incompatible rat skin allograft was performed using WKAH rats ($RT1^k$) as donors and F344 rats ($RT1^{lvI}$) as recipients. (**B**) MHC-compatible rat skin allograft was performed using LEW rats ($RT1^l$) as donors and F344 rats ($RT1^{lvI}$) as recipients. Full-thickness skin grafts (2.0×2.0 cm^2) from donor rats were transplanted to the lateral thorax of the recipient rats. The grafts were inspected daily until rejection, which was defined as more than 90% necrosis of the graft epithelium. FTY720, CsA, FK506, MMF, and AZ were orally administered to the grafted recipients for 14 days after the transplantation. Each symbol represents the mean±SE of the 8 animals. The statistical differences in allograft survival time compared with the vehicle-treated control group were calculated by generalized Wilcoxon test with Hommel's multiple comparison test (*$P<0.05$).

animals died in a group given AZ at 100 mg/kg. In MHC-compatible rat strains of LEW donor and F344 recipient, data are similar (Fig. 2B; Chiba et al., 1996, 1999, 2005).

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 (Hoshino et al., 1996, 1999). All cardiac allografts in control group are rejected within 14 days after the transplantation: median survival time (MST) is 12.0 days. 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. The MST of FTY720 administration with 0.1, 0.3, 1, 3, and 10 mg/kg for 14 days is 20.0, 21.0, 25.5, 29.5, and 58.5 days, respectively. FTY720 at 10 mg/kg induces long-term graft survival for more than 100 days in 3 out of 8 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 immunosuppressive activity than do other immunosuppressive drugs on graft rejection in rat allograft models.

3. Synergistic effect of FTY720 in combination with calcineurin inhibitors

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 combined use of both 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 (Chiba et al., 1996, 1998, 2005; Hoshino et al., 1999). As shown in Table 1, 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

Table 1
Concomitant effect of FTY720 and calcineurin inhibitors in rat skin allograft

Treatment	Mean survival time	*P* value
WKAH to F344 control (vehicle)	6.6±0.2	
FTY720, 0.1 mg/kg p.o.	10.5±0.3	<0.05 vs. control
FTY720, 1 mg/kg p.o.	19.4±0.4	<0.05 vs. control
CsA, 3 mg/kg p.o.	8.4±0.2	<0.05 vs. control
FTY720, 0.1 mg/kg p.o. +CsA, 3 mg/kg p.o.	20.8±2.0	<0.05 vs. CsA alone
FK506, 1 mg/kg p.o.	11.3±0.3	<0.05 vs. control
FTY720, 0.1 mg/kg p.o. +FK506, 1 mg/kg	28.8±2.8	<0.05 vs. FK506 alone
AZ, 30 mg/kg p.o.	9.6±0.2	<0.05 vs. control
AZ, 30 mg/kg p.o. +CsA, 3 mg/kg p.o.	10.8±0.3	<0.05 vs. CsA alone
MMF, 100 mg/kg p.o.	14.6±0.6	<0.05 vs. control
MMF, 100 mg/kg p.o. +CsA, 3 mg/kg p.o.	16.4±0.8	<0.05 vs. CsA alone
LEW to F344 control (vehicle)	8.8±0.3	
FTY720, 0.1 mg/kg p.o.	18.5±0.7	<0.05 vs. control
CsA, 3 mg/kg p.o.	11.4±0.3	<0.05 vs. control
FTY720, 0.1 mg/kg p.o. +CsA, 3 mg/kg p.o.	35.1±2.4	<0.05 vs. CsA alone

Rat skin allograft was performed between WKAH or LEW donors and F344 recipients. FTY720, CsA, FK506, AZ, and MMF were administered orally for 14 days from the day of transplantation. The results were expressed as mean±SE of 8 animals, and statistical significance was calculated by generalized Wilcoxon test.

FTY720 with CsA or FK506 is stronger than that of AZ with CsA or MMF with CsA. To clarify the concomitant effects, the combination index (CI) values of these combination therapy groups were calculated according to the method of median effect analysis (Hoshino et al., 1999; Chiba et al., 2005). Since the CI values are less than 0.2 in the concomitant groups with FTY720 and CsA or FK506, it confirms that the combination therapy with FTY720 and CsA or FK506, 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 MHC-compatible rat strains of LEW donors and F344 recipients, FTY720 at an oral dose of 0.1 mg/kg significantly prolongs the allograft survival and shows a synergistic effect in combination with CsA at 3 mg/kg (Table 1).

In 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 (Table 2; Hoshino et al., 1996, 1999; Chiba et al., 2005). 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 of FTY720 at 0.03 and 1 mg/kg with CsA at 10 mg/kg compared with the monotherapy with FTY720 or CsA (Table 3; Kawaguchi et al., 1996; Suzuki et al., 1998,;Chiba et al., 2005). With combination treatment of FTY720 and CsA, there is no severe toxicity in kidney and liver functions and the blood concentration of FTY720 or CsA did not affect each other. On the contrary, there is no clear effect in the group given AZ at 1 mg/kg in combination with CsA at 10 mg/kg compared with the CsA monotherapy. The combination therapy with AZ at 2.5 mg/kg and CsA at 10 mg/kg has a significant prolonging effect on renal allograft survival; however, 2 dogs died during the administration period without showing increased serum creatinine and blood urea nitrogen, suggesting AZ toxicity. Moreover, it was previously reported that FTY720, in combination with a subtherapeutic dose of CsA, displays a synergistic effect on the prolongation of renal allograft survival in cynomolgus monkeys (Troncoso et al., 1999). 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.

Table 2
Concomitant effect of FTY720 and calcineurin inhibitors in rat heterotopic cardiac allograft

Treatment	Mean survival time	*P* value
Control (vehicle)	11.3±0.8	
FTY720, 0.1 mg/kg p.o.	30.6±10.9	<0.05 vs. control
CsA, 3 mg/kg p.o.	26.4±11.4	<0.05 vs. control
FTY720, 0.1 mg/kg p.o. +CsA, 3 mg/kg p.o.	63.7±14.7	<0.05 vs. CsA alone
FK506, 1 mg/kg p.o.	20.2±0.8	<0.05 vs. control
FTY720, 0.1 mg/kg +FK506, 1 mg/kg p.o.	47.9±12.2	<0.05 vs. FK506 alone
AZ, 10 mg/kg p.o.	12.3±0.4	NS vs. control
AZ, 10 mg/kg p.o. +CsA, 3 mg/kg p.o.	36.1±11.4	NS vs. CsA alone

Rat heterotopic cardiac allograft was performed between WKAH donors and ACI recipients. FTY720, CsA, FK506, and AZ were administered orally for 14 days from the day of transplantation. The results were expressed as mean±SE of 8 animals, and statistical significance was calculated by generalized Wilcoxon test. NS: not significant.

Table 3
Concomitant effect of FTY720 and CsA in canine renal allograft

Treatment	Mean survival time	*P* value
Control (vehicle)	7.3±0.4	
FTY720, 3 mg/kg p.o.	11.7±1.9	NS vs. control
CsA, 10 mg/kg p.o.	10.2±1.0	NS vs. control
FTY720, 0.03 mg/kg p.o. +CsA, 10 mg/kg p.o.	59.6±11.8	<0.05 vs. CsA alone
FTY720, 1 mg/kg p.o. +CsA, 10 mg/kg p.o.	59.8±13.2	<0.05 vs. CsA alone
AZ, 1 mg/kg p.o. +CsA, 10 mg/kg p.o.	11.5±0.7	NS vs. CsA alone
AZ, 2.5 mg/kg p.o. +CsA, 10 mg/kg p.o.	37.3±16.4	<0.05 vs. CsA alone

Canine renal allograft was performed between mongrel donors and beagle recipients. FTY720 and AZ were orally administered from 1 day before transplantation and CsA orally from the day of transplantation. These agents were given daily until the death of the recipient dogs. The results were expressed as mean±SE of 5 to 8 animals, and statistical significance was calculated by generalized Wilcoxon test. NS: not significant.

4. Effect of FTY720 on graft versus host disease models

When spleen cells from LEW rats are injected into the foot pad of (LEW × BN)F_1 rats, local graft versus host reaction (GvHR) is induced and the popliteal lymph node increases to its maximum weight after 7 days. FTY720 significantly inhibits the popliteal lymph node enlargement at doses of 0.1 mg/kg or more in a dose-dependent manner (Masubuchi et al., 1996). To examine the effect of FTY720 in preventing lethal GvHR, splenic lymphocytes from LEW donor rats were injected intravenously into cyclophosphamide-pretreated (LEW × BN)F_1 recipients. In the control group, all rats develop severe GvHR-associated symptoms, including redness of skin and hair loss, within 15 days after the injection of LEW spleen cells and die with a MST of 22.0 days (Masubuchi et al., 1996). The oral administration of FTY720 at 0.1 mg/kg p.o. for 30 days prevents the development of GvHR-associated symptoms and prolongs host survival significantly (MST: 50.0 days). Treatment with FTY720 at a dose of 0.3 mg/kg induces survival for more than 60 days in 4 out of 5 rats without GvHR-associated symptoms. Thus, FTY720 induces long-lasting unresponsiveness by treatment with low doses (0.1 to 0.3 mg/kg) in a

rat lethal GvHR model. Similar results are obtained in mouse lethal GvHR model using C57BL/6 donors and (C57BL/6 × DBA/2)F_1 recipients (Kataoka et al., 2005).

5. 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 (Teshima et al., 1995; Chiba & Adachi, 1997; Fujino et al., 2003). Therapeutic treatment with FTY720 inhibits EAE relapse induced by myelin proteolipid protein immunization in SJL mice (Brinkmann et al., 2002; Kataoka et al., 2004). The therapeutic potential of FTY720 is more markedly compared with recombinant mouse interferon-β (rm-IFN-β; Fig. 3A) and the area of demyelination and the infiltration of $CD4^+$ T-cells into the spinal cord are reduced by FTY720 treatment (Kataoka et al., 2004). 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 (Chiba & Adachi, 1997; Matsuura et al., 2000a, 2000b). Moreover, FTY720 shows a marked prophylactic and therapeutic effect on lupus nephritis in autoimmune MRL/*lpr* mice (Okazaki et al., 2002; Sugahara et al., 2004). Fig. 3B shows the therapeutic effect of FTY720 on proteinuria in aged MRL/*lpr* mice with lupus nephritis. 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 the therapy of autoimmune diseases including multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus.

6. FTY720 sequesters circulating lymphocytes into secondary lymphoid tissues

A striking feature of FTY720 is the induction of a marked decrease in the number of peripheral blood lymphocytes (lymphopenia) at doses that display an

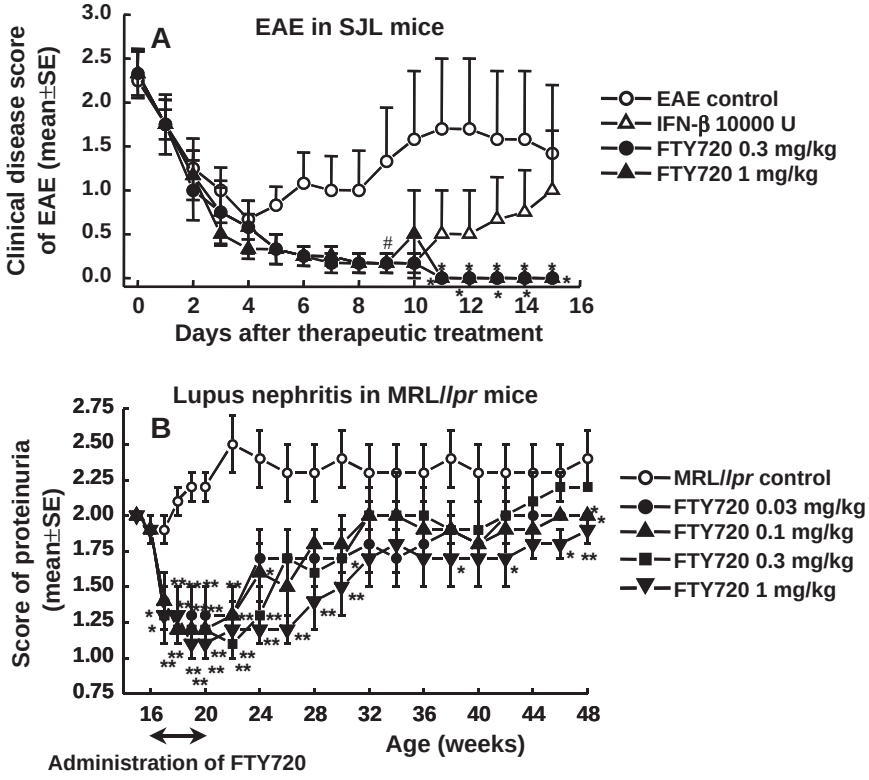

Fig. 3. Therapeutic effect of FTY720 on EAE in SJL mice and lupus nephritis in MRL/*lpr* mice. (**A**) EAE was induced by the immunization of myelin proteolipid protein and Freund's complete adjuvant in SJL mice. EAE-developed mice were pooled and evaluated for the relapse of EAE. FTY720 was orally administered daily and rm-IFN-β at 10,000 IU was given intraperitoneally 3 times a week to EAE-developed mice. Each symbol represents the mean ± SE of 6 animals. Statistical significance was calculated by nonparametric Dunnett's test (FTY720 vs. control, $^*P<0.05$) and Mann–Whitney *U* test (rm-IFN-β vs. control, $^{\#}P<0.05$). (**B**) FTY720 was orally administrated to 16-week-old, proteinuria-developed MRL/*lpr* mice for 4 weeks, and the urinary protein levels were assessed weekly. Each symbol represents the mean ± SE of 10 animals. The statistical significance was calculated by nonparametric Dunnett's test as compared with the control ($^*P<0.05$, $^{**}P<0.01$).

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