

FTY720, a Novel Immunosuppressant, Induces Sequestration of Circulating Mature Lymphocytes by Acceleration of Lymphocyte Homing in Rats. I. FTY720 Selectively Decreases the Number of Circulating Mature Lymphocytes by Acceleration of Lymphocyte Homing

Kenji Chiba,¹ Yoshiki Yanagawa, Yumi Masubuchi, Hirotohi Kataoka, Takafumi Kawaguchi, Makio Ohtsuki, and Yukio Hoshino

FTY720, given i.v. or orally at 0.03 mg/kg or more, significantly prolonged skin allograft survival in a dose-dependent manner and showed more potent immunosuppressive activity than cyclosporin A (CsA) or tacrolimus (FK506) in MHC-incompatible rat strains of WKAH donors and F344 recipients. However, unlike CsA or FK506, FTY720 up to 1000 nM did not affect IL-2 production in allogeneic MLC. Within 3 to 24 h after a single oral administration of FTY720 at 0.1 to 1 mg/kg, the number of lymphocytes in the rats was markedly decreased in the peripheral blood and thoracic duct lymph and partially in spleen. By contrast, the number of lymphocytes in peripheral lymph nodes (PLN), mesenteric lymph nodes (MLN), and Peyer's patches (PP) was significantly increased at the same time. Intravenous transfusion of calcein-labeled rat lymphocytes into rats revealed that FTY720 significantly accelerated lymphocyte homing to PLN, MLN, and PP, dose dependently. Since FTY720-induced lymphocyte homing was completely blocked by simultaneous treatment of the calcein-labeled lymphocytes with mAbs against CD62L, CD49d, and CD11a before the transfusion, the acceleration of lymphocyte homing by FTY720 appears to be mediated by lymphocyte-homing receptors. These findings indicate that FTY720 sequesters circulating mature lymphocytes into PLN, MLN, and PP by acceleration of lymphocyte homing and thereby decreases the number of lymphocytes in peripheral blood, thoracic duct lymph, and spleen. Based on these observations, sequestration of circulating mature-lymphocytes is presumed to be a main mechanism of the immunosuppressive activity of FTY720. *The Journal of Immunology*, 1998, 160: 5037–5044.

Cyclosporin A (CsA)² and tacrolimus (FK506) have made great contributions to the prevention of acute rejection in human organ transplantations (1, 2). Both of these two immunosuppressants are known to exert their immunosuppressive activity by inhibiting the production of Th1-associated cytokines in Ag-stimulated helper T cells (3–5). Although CsA and FK506 bind to different proteins, cyclophilin and FK506 binding protein (FKBP), respectively, both cyclophilin/CsA and FKBP/FK506 complexes inhibit the phosphatase activity of calcineurin, which activates NF-AT involved in the promotion of IL-2 gene transcription (6). Because CsA and FK506 affect the same process of T cell activation, they show quite similar side effects, such as renal and liver toxicities (1, 2). Thus, CsA- or FK506-based multiple drug therapy with steroids or other immunosuppressants has been

widely used to reduce the side effects of individual immunosuppressants in clinical organ transplantation (7, 8).

We previously reported that a potent immunosuppressive compound, ISP-I, and its derivatives, mycostericins, were isolated from the culture broth of *Isaria sinclairii*, a species of vegetative wasp (9, 10). Chemical modification of ISP-I led to a novel synthetic immunosuppressant, 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 (11–14). FTY720, at 0.1 mg/kg or more, significantly prolonged skin or cardiac allograft survival and host survival in lethal graft-vs-host reaction in rats (15–17). In addition, combination treatment with FTY720 and a subtherapeutic dose of CsA resulted in a synergistic effect on canine renal allografts as well as rat skin or cardiac allografts (15, 16, 18, 19). A striking feature of FTY720 is induction of a marked decrease in the number of PBL, especially T cells, at doses that prolong allograft survival (15, 16). It has been hypothesized that the decrease in lymphocyte number is caused by apoptotic cell death of lymphocytes, because FTY720 at 4 μ M (1.4 μ g/ml) or more induces apoptosis of rat spleen cells and human peripheral blood cells in vitro (20, 21). However, the trough level of the blood concentration of FTY720 in dogs given 5 mg/kg is less than 200 ng/ml (21). In addition, the blood concentration range of FTY720 is 0.2 to 20 ng/ml when given to rats at 0.1 mg/kg to 1 mg/kg (unpublished data from our laboratories). Thus, the hypothesis concerning FTY720-induced apoptosis is insufficient to explain the intrinsic mechanism of the decreasing effect on

Research Laboratories, Yoshitomi Pharmaceutical Industries, Limited, Iruma, Saitama, Japan

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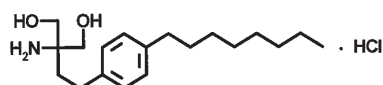
¹ Address correspondence and reprint requests to Dr. Kenji Chiba, Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd 3–7–25, Koyata, Iruma, Saitama, 358-0026 Japan. E-mail address: chiba@yoshitomi.co.jp

² Abbreviations used in this paper: CsA, cyclosporin A; FK506, tacrolimus; TDL, thoracic duct lymph; PLN, peripheral lymph nodes; MLN, mesenteric lymph nodes; PP, Peyer's patches; HEV, high endothelial venules; FTY720, 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride; p.o., by mouth; PE, phycoerythrin

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FTY720

FIGURE 1. The chemical structure of FTY720

PBL number by FTY720, because it is clearly impossible for FTY720 to induce apoptotic cell death of lymphocytes at a dose range of 0.1 to 1 mg/kg *in vivo*.

The immunologically mature lymphocytes are known to continuously recirculate in the peripheral blood, spleen, lymphatic vessels, TDL, PLN, MLN, and PP (22). It has also been reported that lymphocyte recirculation is regulated by lymphocyte trafficking via lymphocyte-homing receptors, including CD62L, CD49d/ β -integrin, and CD11a/CD18 (22–26). We focused on lymphocyte recirculation *in vivo* and hypothesized that, if lymphocyte homing is modulated by FTY720, the number of PBL would decrease without death of lymphocytes. In this paper, we will describe that the marked decrease in the number of PBL induced by FTY720 is due to acceleration of lymphocyte homing to PLN, MLN, and PP via lymphocyte-homing receptors.

Materials and Methods

Animals

Inbred male F344 rats ($RT1^{b/l}$) and WKAH rats ($RT1^k$) were purchased from Japan Charles River (Atsugi, Kanagawa, Japan) and Japan SLC (Hamamatsu, Shizuoka, Japan), respectively. All rats were used at 4 to 12 wk of age.

Cell lines

A mouse IL-2-dependent cytotoxic T cell line, CTLL-2 (27), was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and maintained in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10 mM HEPES, 100 U/ml penicillin, 60 μ g/ml kanamycin sulfonate, 50 μ M 2-ME, 10% FCS (Boehringer Mannheim, Mannheim, Germany) and 20% Con A-stimulated rat spleen cell culture supernatant prepared by the method previously described (28).

Reagents

FTY720 was synthesized according to the method previously described (13). The chemical structure of FTY720 is shown in Figure 1. For *i.v.* injection, FTY720 was dissolved in 1% α -cyclodextrin and 5% mannitol solution. CsA (Sandimmun, for *i.v.* injection; Sandoz, Basel, Switzerland) and FK506 (Prograf, for *i.v.* injection, Fujisawa Pharmaceutical, Osaka, Japan) was diluted with saline. For oral administration, FTY720 was dissolved in distilled water. CsA (Sandimmun, for oral solution) and FK506 (Prograf, for *i.v.* injection) were diluted with olive oil (Sigma Chemicals, St. Louis, MO) and with distilled water, respectively. Control animals received the vehicle only. For *in vitro* treatments, FTY720, CsA (Sandimmun, for *i.v.* injection) and FK506 (Prograf, for *i.v.* injection) were dissolved in saline and diluted to the appropriate concentrations with RPMI 1640 medium containing 10% FCS.

Monoclonal Abs

FITC-conjugated anti-rat CD3 mAb (1F4; Ref. 29) was obtained from Caltag Laboratories (South San Francisco, CA). Biotinylated anti-rat CD3 mAb (G4 18; Ref. 30) and phycoerythrin (PE)-conjugated anti-rat CD45RA or A/B mAb (OX-33; Ref. 31), FITC-conjugated anti-rat CD4 mAb (OX-38; Ref. 32), PE-conjugated anti-rat CD8 mAb (OX-8; Ref. 33) and streptavidin-Cy-chrome conjugate were obtained from PharMingen (La Jolla, CA). Hamster anti-rat CD62L mAb (HRL3; Ref. 34), mouse anti-rat CD49d mAb (TA-2; Ref. 35), mouse anti-rat CD11a mAb (clone WT 1; Ref. 36), and biotinylated TA-2 were purchased from Seikagaku-kogyo (Tokyo, Japan). PE-conjugated HRL-3, PE-conjugated WT 1, and isotype-matched control IgGs were obtained from PharMingen.

FTY720 ACCELERATES LYMPHOCYTE HOMING IN RATS

Rat skin allograft

MHC-incompatible rat skin allograft was performed by the method described previously, with WKAH rats ($RT1^k$) as donors and F344 rats ($RT1^{b/l}$) as recipients (15). Briefly, full-thickness skin grafts (2.0×2.0 cm² pieces) from donor rats were transplanted to the lateral thorax of the recipient rats and covered with sterile bactericidal gauze. The entire chest was then wrapped with an elastic bandage. The dressings were removed on day 5 and the grafts were inspected daily until rejection, which was defined as more than 90% necrosis of the graft epithelium. FTY720, CsA, or FK506 was administered daily to the allografted recipients for 14 days from the day of transplantation.

IL-2 production in allogeneic MLC in rats

Effects on IL-2 production in rat allogeneic MLC were evaluated according to the method previously reported (28). Allogeneic MLC was performed by using nylon nonadherent splenic lymphocytes from F344 rats as responder cells and mitomycin C (Kyowa Hakko, Tokyo, Japan)-pretreated spleen cells from WKAH rats as stimulator cells. Responder cells at 5×10^6 cells/well were cocultured with an equal number of stimulator cells in 2.0 ml of RPMI 1640 medium containing 50 μ M 2-ME and 10% FCS. After culturing for 48 h at 37°C in 5% CO₂, the culture supernatants were collected, and their IL-2 activities were determined by CTLL-2 proliferation assay (27). Briefly, CTLL-2 cells (10^4 cells/well) were cultured in the presence of serial twofold dilution of culture supernatants for 20 h at 37°C, pulsed with 0.5 μ Ci of [³H]thymidine (TdR) (Amersham, Tokyo, Japan) for 4 h at 37°C in 5% CO₂ and then harvested onto glass fiber filters using an automatic cell harvester. The radioactivity incorporated into the cells was determined by a scintillation counter (1450 MicroBeta; Pharmacia Biotech, Uppsala, Sweden). IL-2 activity in the supernatants is expressed as U/ml in comparison with recombinant rat IL-2 (Genzyme, Cambridge MA) as a standard (27).

Flow cytometry

Peripheral blood was collected from the tail veins of F344 rats. Spleen, PLN (axillary lymph nodes were used as PLN in this study), MLN, and PP were removed from rats, and single cell suspensions were prepared by mincing and passing through stainless mesh. Lymphocytes in TDL were collected by cannulation of thoracic duct under anesthesia according to the method described previously (37). Flow cytometry analysis was performed by using EPICS XL-MCL (Coulter, Miami, FL). Lymphocytes from rat peripheral blood, TDL, spleen, PLN, MLN, or PP were stained with FITC-anti-rat CD3 mAb (1F4) and PE-anti-rat CD45RA or A/B mAb (OX-33), which is reported to bind B cells only (31). The number of total lymphocytes was determined by the lymphocyte gating method. The numbers of T

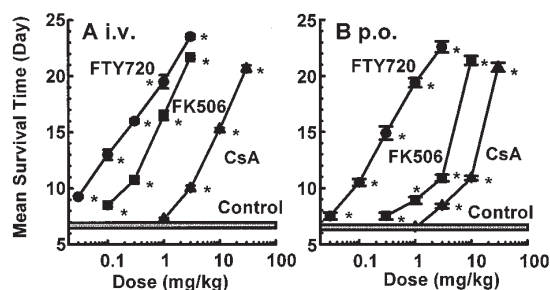


FIGURE 2. Dose-response relationship between FTY720, CsA, and FK506 and skin allograft survival in an MHC-incompatible rat strain system. *A*, *i.v.* administration; *B*, oral administration. MHC-incompatible rat skin allograft was performed using WKAH rats ($RT1^k$) as donors and F344 rats ($RT1^{b/l}$) as recipients. Full-thickness skin grafts (2.0×2.0 cm²) from donor rats were transplanted to the lateral thorax of recipient rats. The grafts were inspected daily until rejection, which was defined as more than 90% necrosis of the graft epithelium. FTY720, CsA, or FK506 was administered to the allografted recipients for 14 days after the transplantation. Each symbol represents the mean \pm SE of eight animals. The statistical differences in allograft survival time compared with vehicle-treated control group were calculated by the generalized Wilcoxon test with Hommel's multiple comparison test (* $p < 0.05$).

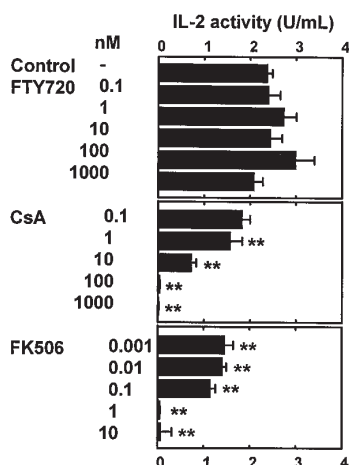


FIGURE 3. Effect of FTY720, CsA, and FK506 on IL-2 production in rat allogeneic MLC. Allogeneic MLC was performed by using nylon non-adherent splenic lymphocytes from F344 rats (*RT1^{lv1}*) as responder cells and mitomycin C-pretreated spleen cells from WKAH rats (*RT1^k*) as stimulator cells. Responder cells (5×10^6 cells/well) were cocultured with an equal number of stimulator cells in 2.0 ml of RPMI 1640 medium containing 50 μ M 2-ME and 10% FCS. After culturing for 48 h, the supernatants were collected and were assessed for IL-2 activity by CTLL-2 proliferation assay. IL-2 activity is expressed as U/ml (mean \pm SE of triplicate determinations), with recombinant rat IL-2 as a standard. Statistical differences were calculated by Dunnett's test (**: $p < 0.01$ vs culture with medium alone).

cells and B cells were determined by two-color flow cytometry. The proportions of CD4⁺ T cells and CD8⁺ T cells were determined by three-color flow cytometry using biotinylated-anti-rat CD3 mAb (G4 18), FITC-anti-rat CD4 (OX-38), PE-anti-rat CD8 (OX-8), and streptavidin-Cy-chrome conjugate. Expression of lymphocyte-homing receptors on rat T cells was determined by two-color flow cytometry using FITC-anti-rat CD3 (IF4), PE-anti-rat CD62L mAb (HRL3), biotinylated-anti-rat CD49d mAb (TA-2), PE-anti-rat CD11a mAb (WT 1), and streptavidin-Cy-Chrome conjugate.

Analysis of lymphocyte homing with calcein-labeled lymphocytes

Lymphocytes (1×10^8 cells) from PLN and MLN of F344 rats were labeled by incubation for 30 min on ice in 10 ml of RPMI 1640 medium containing 0.2 μ M calcein-AM (Molecular Probes, Eugene, OR) (38). After labeling with calcein, the viability of lymphocytes was more than 94% by trypan blue dye exclusion test. The calcein-labeled lymphocytes (5×10^7 cells/0.5 ml) were *i.v.* transfused at 2.5 h after FTY720 administration to F344 rats. Then, the rats were sacrificed 30 min after the transfusion, and the peripheral blood, spleen, PLN, MLN, and PP were collected. The number of calcein-labeled lymphocytes in these tissues was determined by flow cytometry. To examine the effect of mAbs against lymphocyte-homing receptors, the calcein-labeled lymphocytes were treated with 60 μ g/ml of hamster anti-rat CD62L mAb (HRL3), mouse anti-rat CD49d mAb (TA-2), mouse anti-rat CD11a mAb (WT 1), or all three mAbs at 4°C for 30 min. As a control, isotype-matched irrelevant IgGs were used under the same conditions. After the treatment of mAbs, the calcein-labeled lymphocytes were transfused *i.v.* to the rats, and their tissue distribution was determined as described above.

Statistical analysis

The statistical differences in allograft survival time compared with vehicle-treated control group were calculated by the generalized Wilcoxon test with Hommel's multiple comparison test. In other experiments, statistical differences compared with the vehicle-treated control were calculated by Dunnett's test. Differences between groups were considered significant at $p < 0.05$.

Results

Effects of FTY720, CsA, and FK506 on skin allograft survival in MHC-incompatible strain combination in rats

To clarify the efficacy and potency of the immunosuppressive activity of FTY720, the prolonging effect of FTY720, CsA, and FK506 on rat skin allograft survival was examined in MHC-incompatible rat strains of WKAH donors and F344 recipients. The immunosuppressants were administered *i.v.* or orally for 14 days from the day of the transplantation. In this skin allograft models, all grafts in the control (vehicle-treated) group were rejected 6 to 7 days after transplantation. As shown in Figure 2, FTY720 at 0.03 mg/kg or higher doses significantly prolonged allograft survival in a dose-dependent manner by either *i.v.* or oral administration. CsA and FK506 were also effective at doses of 3 mg/kg or more and 0.3 mg/kg or more, respectively, in this model. These results indicate that FTY720 possesses more potent immunosuppressive activity than CsA or FK506 on allograft rejection in an MHC-incompatible combination.

Effects of FTY720, CsA, and FK506 on IL-2 production in rat allogeneic MLC

The effect of FTY720 on IL-2 production in allogeneic MLC in rats was examined in comparison with that of CsA and FK506. The results are shown in Figure 3. CsA and FK506 dose-dependently inhibited IL-2 production in rat allogeneic MLC, consistent with the results of previous studies in mice and humans (3–5). The IC₅₀ values (concentrations that inhibit 50%) of CsA and FK506 for IL-2 production were 3.5 nM and 0.043 nM, respectively. By contrast, FTY720 up to 1000 nM did not affect IL-2 production in rat allogeneic MLC (Fig. 3) and failed to inhibit T cell proliferation by

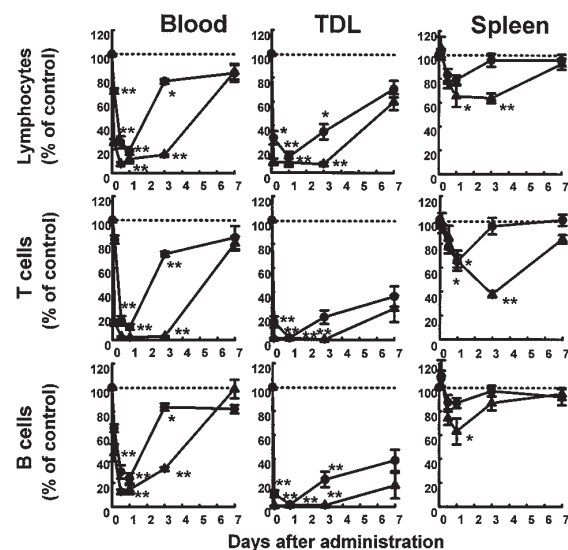


FIGURE 4. Time course of numbers of lymphocytes, T cells, and B cells in blood, TDL, and spleen in rats following a single oral administration of FTY720. FTY720 was administered orally to F344 rats, and peripheral blood, TDL, and spleen were periodically collected. Lymphocytes of blood, TDL, and spleen were stained with FITC-anti-rat CD3 mAb (IF4) and PE-anti-rat CD45RA or A/B mAb (OX-33). The numbers of total lymphocytes, T cells, and B cells were determined by two-color flow cytometry. \bullet , FTY720 at 0.1 mg/kg *p.o.*; \blacktriangle , FTY720 at 1 mg/kg *p.o.* Each symbol represents the mean \pm SE of four animals. Statistical significance was calculated by Dunnett's test (*: $p < 0.05$, **: $p < 0.01$ vs vehicle-treated control group).

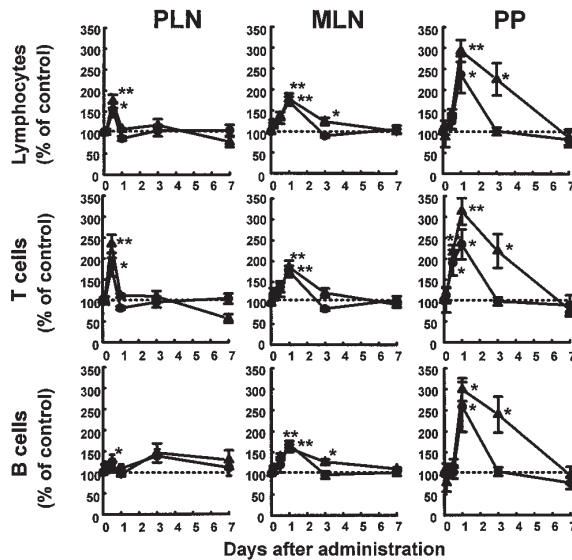


FIGURE 5. Time course of numbers of lymphocytes, T cells, and B cells in PLN, MLN, and PP in rats following a single oral administration of FTY720. FTY720 was administered orally to F344 rats, and PLN, MLN, and PP were periodically collected. Lymphocytes of PLN, MLN, and PP were stained with FITC-anti-rat CD3 mAb (1F4) and PE-anti-rat CD45RA or A/B mAb (OX-33). The numbers of total lymphocytes, T cells, and B cells were determined by two-color flow cytometry. ●, FTY720 at 0.1 mg/kg; ▲, FTY720 at 1 mg/kg. Each symbol represents the mean \pm SE of four animals. Statistical significance was calculated by Dunnett's test (*: $p < 0.05$, **: $p < 0.01$ vs vehicle-treated control group).

alloantigen stimulation or IL-2-dependent proliferation of CTLL-2 cells (data not shown). These findings suggest that FTY720 exerts a potent immunosuppressive effect by a mechanism distinct from that of CsA and FK506.

Lymphocytes were selectively decreased by FTY720 in blood, TDL, and spleen, but increased in PLN, MLN, and PP

As described in our previous papers, FTY720 significantly decreases the number of PBLs in allografted rats, especially the number of T cells (15, 16). To clarify the mechanism of the decrease in the number of lymphocytes by FTY720, the tissue distribution of lymphocytes was analyzed in the peripheral blood, TDL, spleen, PLN, MLN, and PP of F344 rats, following a single oral administration of FTY720 (0.1 and 1 mg/kg). The proportions of lymphocytes, T cells, T cell subsets ($CD4^+$ or $CD8^+$ T cells), and B cells were determined by flow cytometry. Figure 4 shows the time course of lymphocyte, T cell, and B cell numbers in blood, TDL, and spleen after a single oral administration of FTY720. The numbers of total lymphocytes, T cells, and B cells in blood and TDL dramatically decreased to less than 10% of the control values within 3 to 24 h after administration. The decrease in the number of lymphocytes by FTY720 was more marked in TDL than in peripheral blood. The numbers of splenic lymphocytes, T cells, and B cells were also significantly decreased by FTY720 treatment to 40% to 80% of the control with a time course similar to that of the PBLs. Lymphocyte numbers in blood and spleen had recovered to the control level on day 7 after administration. Although the number of lymphocytes in TDL was still decreased to 20% to 40% of the control by FTY720 administration, there was almost com-

plete recovery within 2 wk (data not shown). By contrast, the numbers of lymphocytes in PLN, MLN, and PP were significantly increased in a dose-dependent manner after administration of FTY720 (Fig. 5). The number of PLN lymphocytes reached a maximum at 12 h after FTY720 administration and returned to the control level by 24 h. Lymphocytes in MLN and PP also increased to 180% and 300%, respectively, of the controls at 24 h after FTY720 administration and then returned to the control level within 5 days. The time courses of the numbers of T cells and B cells in PLN, MLN, and PP were similar to that of the total lymphocyte number. The increase in numbers of T cells was especially marked in PLN, MLN, and PP. These findings indicate that FTY720 modulates the tissue distribution of lymphocytes in blood, TDL, spleen, PLN, MLN, and PP in rats. Figure 6, A and B, shows the proportions of T cells, B cells, or T cell subsets ($CD4^+$ or $CD8^+$ T cells) in these lymphoid tissues 12 h after administration of FTY720. The numbers of T cells, B cells, $CD4^+$ T cells or $CD8^+$ T cells showed changes similar to the total numbers of lymphocytes in all tissues tested. By contrast, FTY720 did not

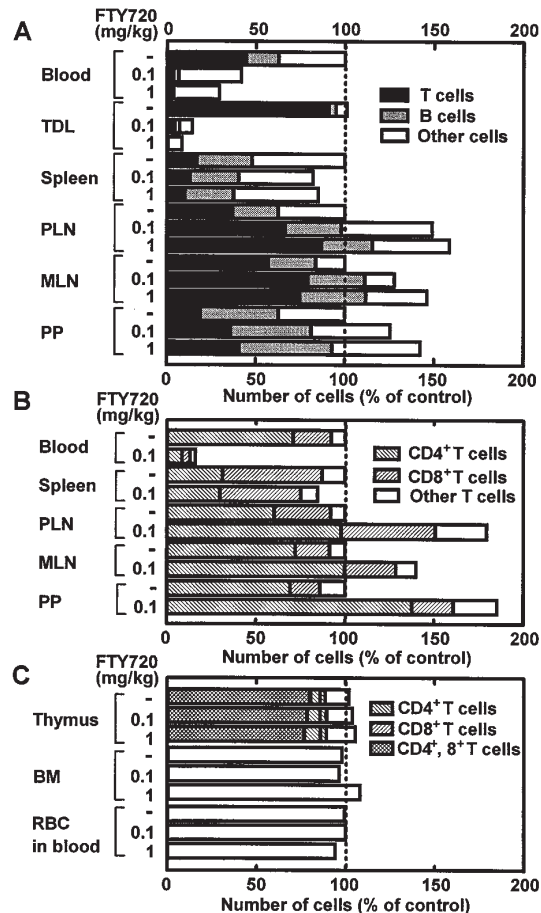


FIGURE 6. Effect of FTY720 on the numbers of various cells in blood and lymphoid tissues in rats 12 h after a single oral administration of FTY720. A, T cells and B cells in blood, TDL, spleen, PLN, MLN, and PP; B, $CD4^+$ T cells and $CD8^+$ T cells in blood, spleen, PLN, MLN, and PP; C, red blood cells (RBC) in blood, thymocyte subpopulations, and bone marrow (BM) cells. Each column represents the mean of four animals.

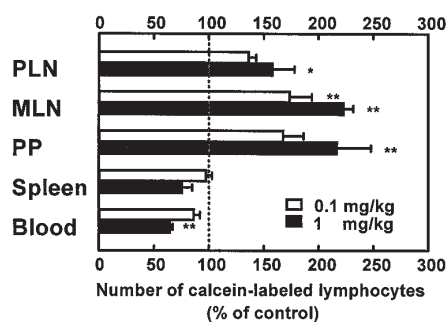


FIGURE 7. Effect of FTY720 on lymphocyte homing of calcein-labeled lymphocytes in rats. The calcein-labeled lymphocytes (5×10^7 cells/0.5 ml) were transfused i.v. into F344 rats 2.5 h after oral administration of FTY720. The rats were sacrificed 30 min later, and peripheral blood, spleen, PLN, MLN, and PP were collected. The number of calcein-labeled lymphocytes in these tissues was determined by flow cytometry. Each column represents the mean \pm SE of four animals. Statistical significance was calculated by Dunnett's test (*: $p < 0.05$, **: $p < 0.01$ vs vehicle-treated control group).

cause any clear changes in the number of red blood cells, thymocytes ($CD4^+$, $CD8^+$ or $CD4^+CD8^+$ subpopulation), or bone marrow cells 12 h after administration of FTY720 (Fig. 6C). Thus, the changes in lymphocyte distribution induced by FTY720 appear to be specific for mature lymphocytes but do lack selectivity for T cells, B cells, or T cell subsets.

FTY720 accelerates lymphocyte homing to PLN, MLN, and PP

To determine the effect of FTY720 on lymphocyte trafficking between blood to various lymphoid tissues in rats, calcein-labeled lymphocytes were i.v. transfused to the strain- and sex-matched F344 rats 2.5 h after administration of FTY720. The rats were sacrificed 30 min later, and the tissue distribution of calcein-labeled lymphocytes was determined by flow cytometry. Figure 7 shows the number of calcein-labeled lymphocytes found in the PLN, MLN, PP, spleen, and blood of rats given FTY720 (0.1 and 1 mg/kg) orally as compared with vehicle-treated control rats. FTY720 significantly increased the number of calcein-labeled lymphocytes in PLN, MLN, and PP in a dose-dependent manner but decreased in spleen and blood. These results indicate that FTY720 accelerates lymphocyte homing from blood or spleen to PLN, MLN, and PP. In addition, the involvement of lymphocyte-homing receptors in FTY720-induced acceleration of lymphocyte homing was assessed by pretreatment with mAb against CD62L, CD49d, or CD11a with calcein-labeled lymphocytes. With a similarity to the results in previous studies (39, 40), anti-CD62L mAb and CD49d mAb prevented normal lymphocyte homing to PLN and MLN, whereas CD11a mAb partially inhibited homing to PLN, MLN, and PP (Fig. 8A). Anti-CD62 mAb inhibited normal lymphocyte homing by 90% in PLN, 70% in MLN, and 50% in PP. Treatment with CD49d mAb, on the other hand, resulted in inhibition of normal lymphocyte homing by 20% in PLN, 60% in MLN, and 80% in PP. Treatment with anti-CD11a mAb displayed a partial inhibition of normal lymphocyte homing by 35% to 60% in these lymphoid tissues. Simultaneous treatment with these mAbs resulted in marked inhibition (>90%) of normal lymphocyte homing in these lymphoid tissues. As shown in Figure 8B, FTY720-induced lymphocyte homing, as well as normal lymphocyte homing, was prevented by treatment with

anti-CD49d or CD11a mAb. Unlike normal lymphocyte homing, CD62L mAb inhibited the FTY720-induced lymphocyte homing by 85% in PP, 70% in MLN, and 50% in PLN. In addition, FTY720-induced lymphocyte homing is almost completely blocked (>90% inhibition) by simultaneous treatment with mAbs against CD49d, CD62L, and CD11a in PLN, MLN, and PP. These results suggest that FTY720-induced acceleration of lymphocyte homing, as well as normal lymphocyte homing, is mediated by lymphocyte-homing receptors, including CD62L, CD49d/ β_7 integrin, and CD11a/CD18.

Effect of FTY720 on expression of CD62L, CD49d, and CD11a on lymphocytes in rats following single oral administration

Since the acceleration of lymphocyte homing by FTY720 appears to be mediated by lymphocyte-homing receptors, there is a possibility that FTY720 up-regulates the expression of lymphocyte-homing receptors on lymphocytes. To determine whether FTY720 induces up-regulation, the expression of CD62L, CD49d, and CD11a on T cells was analyzed in rats at 1 h in blood and at 3 h in MLN after single oral administration of FTY720. The expression of these receptors could not be determined in blood at 3 h after

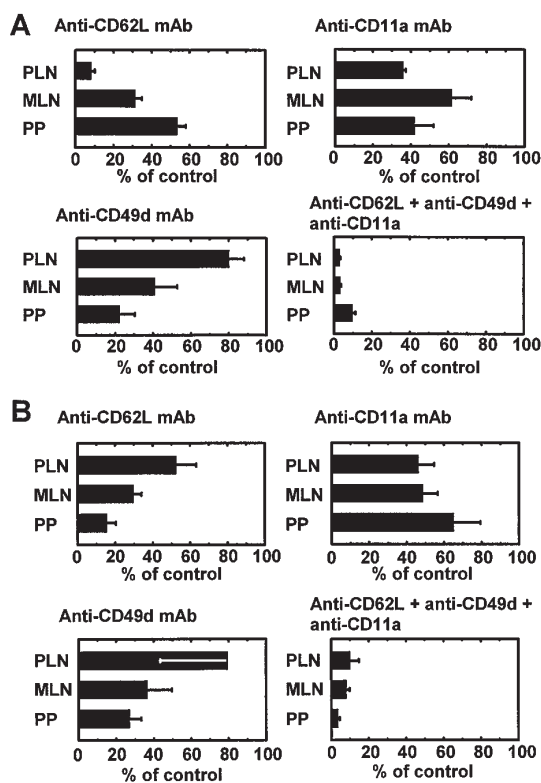


FIGURE 8. The influence of anti-lymphocyte homing-receptor mAbs on FTY720-induced lymphocyte homing in rats. The calcein-labeled lymphocytes were treated with 60 μ g/ml of hamster anti-rat CD62L mAb (HRL3), mouse anti-rat CD49d mAb (TA-2), mouse anti-rat CD11a mAb (WT 1), or all three mAbs at 4°C for 30 min. As a control, isotype-matched irrelevant IgGs were used under the same conditions. After treatment with mAbs, calcein-labeled lymphocytes were transfused i.v. into the rats, and tissue distribution of lymphocytes was determined by flow cytometry. A, normal lymphocyte homing; B, FTY720 (1 mg/kg p.o.)-induced lymphocyte homing. Each column represents the mean \pm SE of four animals.

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