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FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats, III. Increase in frequency of CD62L-positive T cells in Peyer's patches by FTY720-induced lymphocyte homing

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SUMMARY

FTY720, a novel immunosuppressant, sequesters circulating mature lymphocytes, especially T cells, within lymph nodes and Peyer's patches by accelerating lymphocyte homing, and thereby causes lymphocyte depletion in the blood. The FTY720-induced acceleration of lymphocyte homing appears to be mediated by lymphocyte homing receptors including CD62L, CD49d/β7, and CD11a/CD18. In this study, expressions of CD62L, CD49d and CD11a on T cells in the peripheral blood, lymph nodes and Peyer's patches were analysed by flow cytometry in rats given FTY720 (1 mg/kg) orally. FTY720 markedly decreased the number of peripheral blood T cells, while not affecting CD62L, CD49d and CD11a expressions at 1–3 hr after administration. In contrast, both the frequency of CD62L-positive T cells and intensity of CD62L expression on T cells were increased in Peyer's patches but not lymph nodes at 3 hr after administration of FTY720. CD49d and CD11a expressions on T cells were unaffected by FTY720 in both Peyer's patches and lymph nodes at the same point in time. On the other hand, analysis of lymphocyte homing with calcein-labelled lymphocytes and anti-CD62L monoclonal antibody (mAb) confirmed that FTY720 predominantly increased CD62L-dependent lymphocyte homing to Peyer's patches. These findings indicate that FTY720 increases the frequency of CD62L-positive T cells by accelerating CD62L-predominant homing in Peyer's patches.

INTRODUCTION

FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride), a novel immunosuppressant, was created by chemical modification of ISP-I, a derivative of *Isaria sinclairii*.[1,2] FTY720 shows more potent immunosuppressive activity than cyclosporin A (CsA) and FK506 in skin and cardiac allograft models while, unlike CsA and FK506, unaffecting lymphocyte proliferation and interleukin-2 (IL-2) production in rat allogeneic mixed lymphocyte culture *in vitro*.[3–5] In addition, FTY720 combined with CsA displays a marked synergistic effect in prolonging skin, cardiac and renal allograft survival in rats and dogs, suggesting that FTY720 possesses a unique mechanism of action.[3–9] A striking feature of FTY720 is induction of a marked decrease in the number of circulating lymphocytes, especially T cells, at the doses prolonging allograft survival.[5,8] The marked decrease in circulating T cells appears to cause a reduction of T-cell recruitment into allografts.[9] Recently, we reported a mechanism underlying the disappearance of circulating lymphocytes in FTY720-treated rats.[5] As described in our previous report, while markedly decreasing the circulating lymphocytes in the bloodstream, FTY720 significantly increased the lymphocyte number in lymph nodes (LN) and Peyer's patches (PP) at 3–24 hr after administration to rats.[5] In addition, analysis of lymphocyte trafficking using calcein-labelled lymphocytes revealed that FTY720 accelerated lymphocyte homing to LN and PP, and this acceleration was almost completely inhibited by simultaneous treatment with anti-CD62L, CD49d, and CD11a monoclonal antibodies (mAbs).[5] Therefore, we concluded that FTY720 sequesters circulating lymphocytes within LN and PP by accelerating lymphocyte homing through lymphocyte-homing receptors, including CD62L, CD49d/β7 and CD11a/CD18. In this study, to elucidate the relation between the acceleration of lymphocyte homing and expressions of lymphocyte-homing receptors, we analysed CD62L, CD49d and CD11a expressions on T cells in peripheral blood, LN and PP by flow cytometry in FTY720-treated rats.

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Abbreviations: PBL, peripheral blood lymphocytes; LN, lymph nodes; PLN, peripheral lymph nodes; MLN, mesenteric lymph nodes; PP, Peyer's patches; MFI, mean fluorescence intensity; mAb, monoclonal antibody.

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MATERIALS AND METHODS

Animals

Inbred strain male F344 rats ($RT1^{lv1}$) were purchased from Japan Charles River Inc. (Atsugi, Kanagawa, Japan). All rats were used at 5–7 weeks of age.

Reagents and monoclonal antibodies

FTY720 was synthesized by Taito Co., Ltd.[1] For oral administration, FTY720 was dissolved in distilled water. Control animals received the vehicle only. Calcein-AM was obtained from Molecular Probes (Eugene, OR). Fluoroscein isothiocyanate (FITC)-conjugated anti-rat CD3 mAb (1F4)[10] was obtained from Caltag Laboratories (South San Francisco, CA). Hamster anti-rat CD62L mAb (HRL-3)[11] and biotinylated mouse anti-rat CD49d (TA-2)[12] were purchased from Seikagaku-kougyou Ltd. (Tokyo, Japan). Phycoerythrin (PE)-conjugated HRL-3, PE-conjugated mouse antirat CD11a mAb (WT.1),[13] isotype-matched control immunoglobulin G (IgG), and streptavidin-Cy-Chrome™ were obtained from PharMingen (La Jolla, CA).

Flow cytometry

Peripheral blood was collected from tail vain of F344 rats. Peripheral lymph nodes (PLN; axillary lymph nodes), mesenteric lymph nodes (MLN) and PP were removed from rats, and single cell suspensions were prepared by mincing and passing through stainless mesh. The cells were stained with FITC-anti-rat CD3 mAb (1F4), and PE-anti-rat CD62L mAb (HRL3) or PE-anti-rat CD11a mAb (WT.1). For analysis of CD49d expression, the cells were stained with FITC-anti-rat CD3 mAb (1F4), biotinylated-anti-rat CD49d mAb (TA-2), and streptavidin-Cy-Chrome™. CD62L, CD49d and CD11a expressions on rat T cells were determined by two-colour flow cytometry with EPICS® XL-MCL (Coulter Co. Miami, FL). The percentage of positive cells was determined by setting the lower limit above the non-specific fluorescence with isotype-matched control IgG. The mean fluorescence intensity (MFI) was recorded in the linear amplification mode of EPICS® XL.

Analysis of lymphocyte-homing with calcein-labelled lymphocytes

The *in vivo* lymphocyte-homing was analysed as previously described.[5] Lymphocytes (1×10^8 cells) from the PLN and MLN of F344 rats were labelled by incubation for 30 min on ice in 10 ml of RPMI-1640 medium containing 0·2 μM calcein-AM, as described in previous reports.[5,14,15] The calcein-labelled lymphocytes were treated with 60 μg/ml of hamster antirat CD62L mAb (HRL3) or isotype-matched control IgG. After treatment with the mAbs, the calcein-labelled lymphocytes (5×10^7 cells/0·5 ml) were intravenously transfused to the rats at 2·5 hr after FTY720-administration. The rats were killed at 30 min after the transfusion, and all PP were collected. The number of calcein-labelled lymphocytes in PP was determined by flow cytometry.

RESULTS

Effect of FTY720 on T-cell number and CD62L, CD49d and CD11a expressions of T cells in peripheral blood

The number of T cells and expressions of CD62L, CD49d, and CD11a on T cells in peripheral blood was analysed by flow cytometry at 1–3 hr after oral administration of FTY720 (1 mg/kg) to rats. The number of T cells in peripheral blood

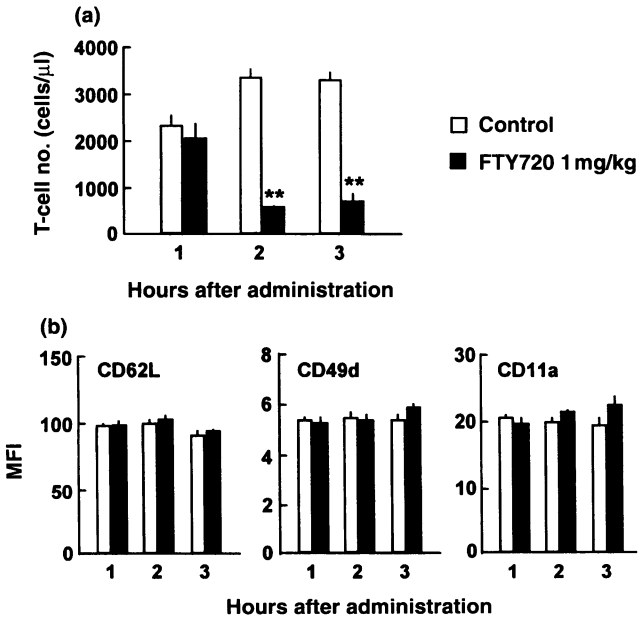

Figure 1. The number of T cells (a) and expressions of CD62L, CD49d, and CD11a on T cells (b) in the peripheral blood of control and FTY720-treated rats at 1, 2 and 3 hr after administration. Blood was collected from the tail vein of the rats at 1, 2 and 3 hr after oral administration of FTY720 (1 mg/kg). The number of $CD3^+$ T cells and expressions of CD62L, CD49d and CD11a on $CD3^+$ T cells was determined by flow cytometry. Each column represents the mean ± SE of three animals. (**$P < 0{\cdot}01$, t-test as compared with control.)

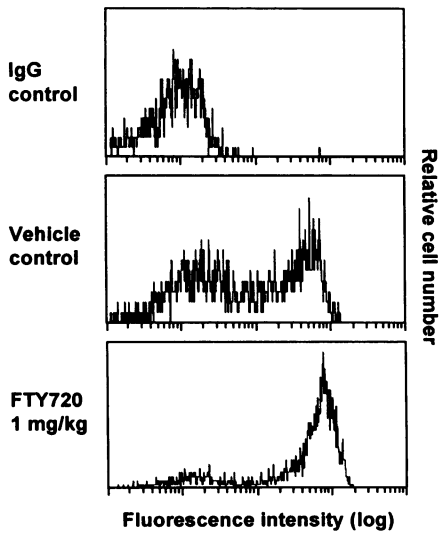

Figure 2. Effect of FTY720 on CD62L expression on T cells in PP. PP were separated at 3 hr after a single oral administration of FTY720 (1 mg/kg) to rats. Lymphocytes from PP were stained with FITC-anti-rat CD3 (1F4) and PE-anti-rat CD62L mAb (HRL3). As a negative control, lymphocytes were treated with isotype-matched control IgG. CD62L expression on T cells was determined by two-colour flow cytometry. Results are representative of data obtained in four pairs of rats.

was significantly decreased at 2–3 hr (Fig. 1a). In contrast, FTY720 did not affect CD62L, CD49d and CD11a expressions on peripheral blood T cells at 1–3 hr (Fig. 1b).

Effect of FTY720 on CD62L, CD49d and CD11a expressions of T cells in PP and LN

Expressions of CD62L, CD49d and CD11a on T cells in PP and LN was analysed by flow cytometry at 3 hr after administration of FTY720 (1 mg/kg). In the control rats, the frequency of CD62L-positive T cells in PP was lower than in PLN and MLN. FTY720 significantly increased the frequency of CD62L-positive T cells in PP but not PLN and MLN (Figs 2 and 3). Although the data are not shown, all T cells in the PLN, MLN and PP were CD49d-and CD11a-positive in both the control and FTY720-treated rats. The intensity of CD62L expression on CD62L-positive T cells was also significantly increased in PP by FTY720-treatment, while slightly decreasing in PLN and MLN (Fig. 4). On the other hand, FTY720 did not affect the intensity of CD49d and CD11a expression on T cells in PLN, MLN, and PP (Fig. 4).

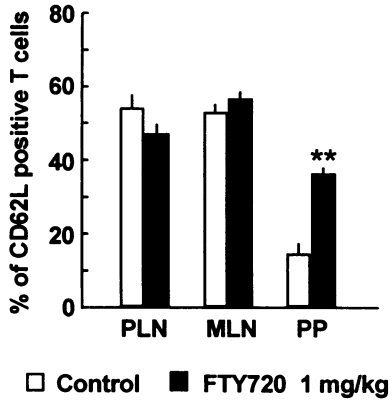

Figure 3. The percentage of $CD62L^+$ T cells in lymphocytes from PLN, MLN and PP of control and FTY720-treated rats at 3 hr after oral administration of FTY720. PLN, MLN and PP were separated at 3 hr after single oral administration of FTY720 (1 mg/kg) to rats. CD62L expressions on $CD3^+$ T cells were determined by two-colour flow cytometry. Each column represents the mean ± SE of four animals. (**$P<0{\cdot}01$, *t*-test as compared with control.)

Effect of anti-CD62L mAb on lymphocyte homing into PP in control and FTY720-treated rats

Lymphocyte homing was assessed by flow cytometry with calcein-labelled lymphocytes in rats. Calcein-labelled lymphocytes treated with anti-CD62L mAb were intravenously transfused into rats 2·5 hr after oral administration of FTY720 (1 mg/kg). The rats were sacrificed 30 min after the transfusion, and the number of calcein-labelled lymphocytes in the PP was determined by flow cytometry. As shown Fig. 5, FTY720 markedly increased lymphocyte homing to PP. When treated with anti-CD62L mAb, lymphocyte homing into PP was only slightly increased by FTY720. On the other hand, anti-CD62L mAb partially decreased lymphocyte homing to PP in control rats (by 48%), and markedly decreased it in FTY720-trated rats (by 72%). Thus, FTY720 appeared to predominantly induce CD62L-dependent lymphocyte-homing to PP.

DISCUSSION

Circulating lymphocytes in the blood home to LN and PP through interaction of lymphocyte homing receptors including

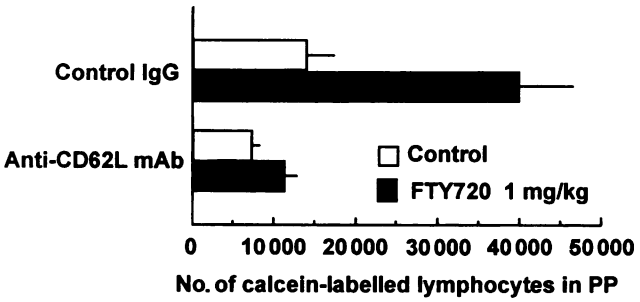

Figure 5. Effect of anti-CD62L mAb on lymphocyte homing into PP in control and FTY720-treated rats. Calcein-labelled lymphocytes were treated with 60 μg/ml of hamster anti-rat CD62L mAb or isotype-matched control IgG. The calcein-labelled lymphocytes were intravenously transfused into the rats at 2·5 hr after administration of FTY720 (1 mg/kg). Peyer's patches were collected at 30 min after the transfusion, and the number of calcein-labelled lymphocytes in the PP was determined by flow cytometry. Each column represents the mean ± SE of four animals.

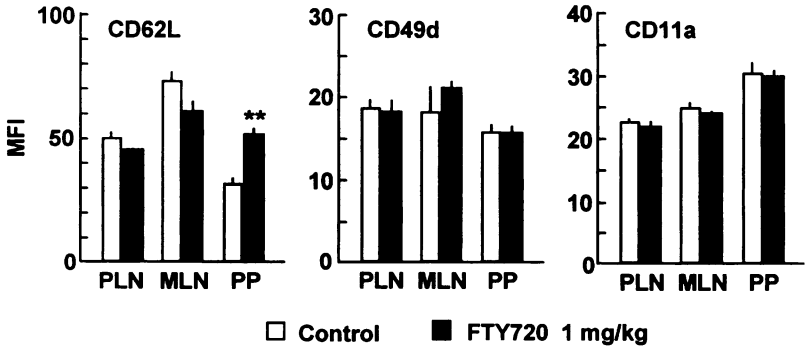

Figure 4. Expressions of CD62L, CD49d and CD11a on T cells in PLN, MLN and PP of control and FTY720-treated rats at 3 hr after administration of FTY720. Peripheral lymph nodes, MLN and PP were separated at 3 hr after a single oral administration of FTY720 (1 mg/kg) to rats. CD62L, CD49d and CD11a expressions on $CD3^+$ T cells were determined by two-colour flow cytometry. Each column represents the mean ± SE of four animals. (**$P<0{\cdot}01$, *t*-test as compared with control.)

CD62L, CD49d/β7 and CD11a/CD18 to their ligands on high endothelial venule (HEV), and then subsequently return to the blood again.[16,17] The involvement of respective lymphocyte-homing receptors in lymphocyte trafficking is thought to be closely related to the expression pattern of their ligands on HEV. glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1), the CD62L ligand, is expressed on PLN-HEV at high level, and on PP-HEV at low level,[18] while anti-CD62L mAb almost completely inhibits lymphocyte entry into PLN, but only partially into PP.[19] Lymphocyte homing into LN is more dependent on interaction through CD62L than homing into PP. Mucosal addressin cell adhesion molecule-1 (MAdCAM-1), the CD49d/β7 ligand is expressed on PP-HEV but not PLN,[20,21] while lymphocyte entry into PP but not PLN is markedly inhibited by treatment with anti-CD49d mAb or anti-β7-integrin mAb.[19] Therefore, lymphocyte homing into PP is predominantly mediated by CD49d/β7 rather than CD62L. Consequently, the frequency of CD62L-positive lymphocytes in PP is lower than that in LN.[11] In this study, FTY720 increased the frequency of CD62L-positive T cells in PP (Figs 2 and 3). In addition, FTY720 predominantly enhanced CD62L-mediated lymphocyte homing to PP when compared with CD62L-independent homing (Fig. 5), indicating that FTY720 increased the involvement of CD62L in lymphocyte homing to PP. These observations indicate that FTY720 increases CD62L-positive T cells in PP by accelerating CD62L-predominant lymphocyte homing. On the other hand, FTY720 did not affect CD62L expression on T cells, while markedly decreasing T-cell numbers in blood (Fig. 1). It is possible that the entry of CD62L-positive and CD62L high-expressing T cells into PP is enhanced by increase in expression of CD62L-ligands on PP-HEV but not CD62L in FTY720-treated rats. In contrast to PP, there was no notable change in lymphocyte homing receptor expressions on T cells in the PLN and MLN of FTY720-treated rats (Figs 3 and 4). FTY720 may not affect the involvement of respective lymphocyte-homing receptors in lymphocyte trafficking to PLN and MLN, while accelerating lymphocyte homing to these organs.

Peyer's patches are thought to play a central role in immune defence and oral immune tolerance in gut.[22,23] In this study, we demonstrated that FTY720 modulates the frequency of CD62L-possitive T cells in PP, raising the possibility that FTY720 affects the immune responses in the gut. The effect of FTY720 on T-cell functions in PP are currently being analysed. Finally, we believe that FTY720, which has a unique mechanism of action, is useful as an immunosuppressive drug for organ transplantation and as a tool for investigating immune responses.

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