FTY720 suppresses CD4^{+}CD44^{high}CD62L^{-} effector memory T cell-mediated colitis


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Inflammatory bowel diseases (IBDs), it is believed that memory T cells are intermittently reactivated in secondary lymphoid organs and thereafter return to inflammatory tissues, such as, in this case, the gut (22). These memory T cells can survive for a long period, and they provide the basis for long-term immunological memory. However, it is little known how memory T cells are controlled by FTY720 in IBDs. In this study, we evaluated the effect of FTY720 using our recently established CD4^{+}CD44^{high}CD62L^{-} effector memory T cell (TEM cell)-mediated chronic colitis model.

MATERIALS AND METHODS

Animals. Female BALB/c and CB-17 severe combined immunodeficiency (SCID) mice were purchased from Japan Clear (Tokyo, Japan). Mice were maintained under specific pathogen-free conditions in the Animal Care Facility of Tokyo Medical and Dental University. The Institutional Committee on Animal Research of Tokyo Medical and Dental University approved the experiments.

Antibodies. The following monoclonal antibodies were used for the purification of cell populations and flow cytometric analysis: RM4-5, CyChrome- or phycoerythrin (PE)-conjugated anti-mouse CD4 (BD PharMingen; San Diego, CA); 16A, FITC-conjugated anti-mouse CD45RB (BD PharMingen); IM7, PE-conjugated anti-mouse CD44, and MEL-14, PE- or FITC-conjugated anti-mouse CD62L.

T cell reconstitution and FTY720 treatment. FTY720 (Novartis Pharma; Basel, Switzerland) was dissolved in sterile distilled water (DW). For in vivo treatment, FTY720 was administered via per os gavage. To exclude the possibility that in vivo FTY720 treatment might be cytotoxic for CD4^{+} T cells, we treated normal BALB/c mice with a single dose of FTY720 (0, 0.3, and 3.0 mg/kg). Seventy-two hours after the treatment, dead and apoptotic cells were detected by annexin V-FITC/propidium iodide (PI) staining (MBL; Nagoya, Japan). As shown in Fig. 1, there were no significant differences in the

2-AMINO-2-[2-(4-OCTYLPHENYL)ETHYL]-1,3-PROPANEDIOL HYDROCHLORIDE (FTY720) is a chemical derivative of myriocin (thermozy-mochin), a substance found in the fungi Myriococcum albo-nyces and Isaria sinclairii (1, 2, 5, 6). FTY720 is a potent immunomodulator that has been shown to prevent graft rejection in various animal allotransplantation models (23, 24), autoimmune disease animal models (11, 16, 20), and viral infection animal models (17, 21). Good synergy with cyclosporine and sirolimus has been reported (3, 28, 30). FTY720 controls the migration property of memory T cells. Here, we demonstrated that FTY720 prevents the development of colitis induced by the adoptive transfer of lamina propria (LP) colitogenic effector memory CD4^{+} T cells (TEM cells; CD45RB^{low}CD44^{high}CD62L^{-}) into several combined immunodeficiency (SCID) mice and suppresses interferon-γ, interleukin-2, and tumor necrosis factor-α production by LP CD4^{+} T cells. The numbers of spleen, peripheral blood, mesenteric lymph node, and LP CD4^{+} T cells in FTY720-treated mice were significantly reduced compared with those in control mice. Notably, LP CD4^{+} TEM cells as well as splenic CD4^{+} CD45RB^{low} T cells expressed several sphingosine-1-phosphate receptors that are targets for FTY720. Furthermore, FTY720 also prevented the development of colitis induced by the adoptive transfer of splenic CD4^{+} CD45RB^{low} T cells into SCID mice. Collectively, the present data indicate that FTY720 treatment may offer the potential not only to prevent the onset of disease but also to treat memory T cell-mediated autoimmune diseases including inflammatory bowel diseases.

therapy; migration; I cell
numbers of dead (PI⁺ and annexin V⁺) or apoptotic (PI⁻ and annexin V⁺) cells between mice treated with each dose. Thus we adopted the 0.3 mg/kg dose of FTY720 in a line of in vivo experiments.

Colitis was induced in CB-17 SCID mice by the adoptive transfer of syngeneic CD4⁺CD45RB⁺high T cells as described previously (10). Briefly, CD4⁺ T cells were isolated from splenocytes from normal BALB/c mice using the anti-CD4 (L3T4) MACS magnetic separation system (Miltenyi Biotec; Auburn, CA). Enriched CD4⁺ T cells were labeled with PE-conjugated anti-mouse CD4 monoclonal antibodies and FITC-conjugated anti-CD45RB monoclonal antibodies and the isolated CD45RB⁺high (highest staining: 30%) fraction on a FACS Aria (BD PharMingen) and hamster anti-mouse CD28 monoclonal antibodies (37.51, BD PharMingen) and 0.01% DNase (Worthington Biomedical; Freehold, NJ) and 0.01% DNase (Worthington Biomedical; Freehold, NJ) for 2 h. Cells were pelleted two times through a 40% isotonic Percoll solution and then subjected to Ficoll-Hypaque density gradient centrifugation (40%/75%). Enriched CD4⁺ LP T cells were obtained by positive selection using anti-CD4 (L3T4) MACS magnetic beads. The resultant cells, when analyzed by FACS Calibur, contained >96% CD4⁺ cells. To investigate LP memory CD4⁺ T cell migration properties and the preventive effect by FTY720, we next induced a colitogenic LP memory CD4⁺ T cell-mediated colitis as previously described (25). In brief, seven SCID mice from each group were injected intraperitoneally with 200 μl PBS containing 3 × 10⁶ colitic LP CD4⁺ T cells and were treated with DW or FTY720 (0.3 mg/kg) daily starting 1 day before the transfer over a period of 4 wk. In another experiment, seven SCID mice from each group were injected intraperitoneally with 200 μl PBS containing 3 × 10⁵ normal splenic CD4⁺CD45RB⁺high T cells and were treated with DW or FTY720 (0.3 mg/kg) daily starting 1 day before T cell transfer over a period of 5 wk. SCID mice after transfer were weighed initially and then three times per week thereafter. They were observed for the following clinical signs of illness: hunched over appearance, piloerection of the coat, diarrhea, and blood in the stool. The mice were then killed and assessed for a clinical score, that is, the sum of the following four parameters: hunching and wasting, 0 or 1; colon thickening, 0–3 (0, no colon thickening; 1, mild thickening; 2, moderate thickening; and 3, extensive thickening); and stool consistency, 0–3 (0, normal beaded stool; 1, soft stool; and 2, diarrhea; and an additional point was added if gross blood was noted) (29).

Histological examination and immunohistology. Tissue samples were fixed in PBS containing 6% neutral buffered formalin. Paraffin-embedded sections (5 μm) were stained with hematoxylin and eosin. Three tissue samples from the middle part of the colon were prepared. Sections were analyzed without prior knowledge of the type of T cell reconstitution or treatment. The area most affected was graded by the number and severity of lesions. The mean degree of inflammation in the colon was calculated using a modification of the previously described scoring system (29).

Flow cytometry. Flow cytometry three-color analysis was performed. Isolated peripheral blood, spleen, mesenteric lymph node (MLN), peripheral lymph node (PLN; inguinal, axillary, and lateral axillary), and LP cells obtained from FTY720- and DW-treated mice were preincubated with Fcy receptor-blocking monoclonal antibodies for 20 min followed by an incubation with CyChrome-conjugated anti-mouse CD4, PE-conjugated anti-CD44, and FITC-conjugated anti-CD62L monoclonal antibodies for 30 min on ice. After the cells had been stained, flow cytometry and data analysis were performed using FACS Calibur and CELLQUEST software (BD Biosciences; San Jose, CA).

Cytokine production assay. To measure cytokine production, 1 × 10⁶ LP CD4⁺ T cells were cultured in 200 μl culture medium at 37°C in a humidified atmosphere containing 5% CO₂ in 96-well plates (Costar; Cambridge, MA) precoated with 5 μg/ml hamster anti-mouse CD3e monoclonal antibodies (145-2C11, BD PharMingen) and hamster 2 μg/ml anti-mouse CD28 monoclonal antibodies (37.51, BD PharMingen) in PBS overnight at 4°C. Culture supernatants were removed after 48 h and assayed for cytokine production. Cytokine concentrations were determined by a specific ELISA [interleukin (IL)-10] (R&D; Minneapolis, MN) or a mouse T helper (Th)1/Th2 cytokine bead array kit [IL-2, IL-4, IL-5, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ] (BD Biosciences) per the manufacturer’s recommendation.

RT-PCR. Total cellular RNA was extracted from 7 × 10⁶ cells using the RNeasy Mini Kit (Qiagen; Valencia, CA). Five micrograms of total RNA were reverse transcribed using SuperScript reverse transcriptase (Invitrogen). S1P receptor (S1P1, S1P2, S1P3, S1P4, and S1P5) levels were measured by a QuantiTect SYBER green PCR kit using an ABI7500 real-time PCR system and 7500 system SDS software (Applied Biosystems; Foster city, CA). The following primers were used: S1P1 forward 5’-GTT TAG ACC ACG AGT CCT GCC-3’ and reverse 5’-AGC TTT TCC TTT GGA GAG-3’; S1P2 forward 5’-GGC GTA CCA AGT GCT CAG C-3’ and reverse 5’-CCT TGG TGT AAT TGT AGT GAA GCA-3’; S1P3 forward 5’-GGA GCC CCT AGA CGG GAG T-3’ and reverse 5’-CCG ACT GCC GGA AGA GTG T-3’; S1P4 forward 5’-GCT GGA ACT CAC-3’ and reverse 5’-GAT GAA GCT-3’.
TTT ATA GAC CAG G-3' and reverse 5'-AGA AAG CGT GCC ATA GGC AG-3'; S1P₁, forward 5'-GAG TCG CGG TTA CAG GAG ACT T-3' and reverse 5'-GCG TGC TGT GTC CTG CC-3'; and glyceraldehyde-3-phosphate dehydrogenase, forward 5'/H11032 GAG ACT T-3 and reverse 5'/H11032 AGA AAG CGT GCC-3'. PCR cycling conditions consisted of 95°C for 15 min followed by 45 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 40 s. Real-time PCR analysis of S1P receptors was expressed as the relative amount of the indicated mRNA normalized by that of glyceraldehyde-3-phosphate dehydrogenase.

Statistical analysis. Results are expressed as means ± SD. Groups of data were compared by the Mann-Whitney U-test. Differences were considered to be statistically significant at \( P < 0.05 \).

RESULTS

Treatment with FTY720 suppresses the development of colitis induced by adoptive transfer of naïve CD4⁺CD45RB<sup>high</sup> T cells. We first tested whether FTY720 suppressed the development of colitis originally induced by the adoptive transfer of splenic CD4⁺CD45RB<sup>high</sup> T cells from normal BALB/c mice into CB-17 SCID mice (Fig. 2A). Mice were administered daily with FTY720 (0.3 mg/kg) or DW orally starting 1 day before transfer. The control DW-treated mice manifested progressive weight loss from 2 wk after transfer (Fig. 2B) and clinical symptoms of colitis (Fig. 2C) such as diarrhea with increased mucus in the stool, anorectal prolapse, and hunched posture by 3–4 wk. In contrast, FTY720-treated mice appeared healthy with a gradual increase of body weight and without clinical symptoms during the whole period of observation (Fig. 2B and C). In total, the assessment of colitis by clinical scores showed a clear difference between the control DW-treated mice and FTY720-treated mice (Fig. 2D). Histological examination showed a marked elongation of the villi with a massive infiltration of mononuclear cells in the LP of the colon from control DW-treated mice (Fig. 2E). In contrast, the elongation of the villi was mostly abrogated and only a few mononuclear cells were observed in the LP of the colon from FTY720-treated mice (Fig. 2E). This difference was also confirmed by the histological scoring of multiple colon sections, which was 0.4 ± 0.8 in FTY720-treated mice versus 4.2 ± 0.8 in control DW-treated mice (P < 0.05; Fig. 2F).

A further quantitative evaluation of CD4⁺ T cell infiltration was made by isolating CD4⁺ T cells from each tissue or the peripheral blood. Only a few CD4⁺ T cells were recovered from the colonic tissue of FTY720-treated mice compared with control DW-treated mice (Fig. 3). The number of CD4⁺ cells recovered from the colon of control DW-treated mice (23.6 ± 18.4 × 10<sup>5</sup> cells) far exceeded the number of originally injected cells (3 × 10<sup>5</sup> cells), indicating extensive T cell migration and/or proliferation in the inflamed colon, which was mostly abrogated in FTY720-treated mice. Similarly, the numbers of CD4⁺ cells in the spleen, MLN, and peripheral blood from FTY720-treated mice was significantly decreased compared with those from control DW-treated mice (Fig. 3). In contrast, the number of CD4⁺ cells in the PLN from FTY720-treated mice was comparable with that in DW-treated mice (Fig. 3).

We next examined cytokine production by LP CD4⁺ T cells from control PBS-treated mice and FTY720-treated mice. As shown in Fig. 4, LP CD4⁺ T cells from FTY720-treated mice produced significantly less TNF-α, IFN-γ, and IL-2 compared with those from control DW-treated mice upon in vitro stimulation. In contrast, the production of IL-4, IL-5, or IL-10 was not significantly affected. These results suggested that FTY720 prevented the development of colitis primarily by promoting the sequestration of naïve CD4⁺ T cells and/or inhibiting the egress of colitogenic CD4⁺ T cells in the MLN, followed by inhibiting the development of pathogenic Th1 cells producing TNF-α, IFN-γ, and IL-2.

We further evaluated whether FTY720 affected cell differentiation of the transferred CD4⁺CD45RB<sup>high</sup> T cells in vivo. As shown in Fig. 5, almost all CD4⁺ T cells in any tissue and...
the blood from DW- or FTY720-treated SCID mice had a phenotype of TEM cells, which express CD62L^low CD44^high, in contrast to the originally transferred CD4^+ CD45RB^high CD62L^− CD44^low T cells (data not shown). The results indicated that cell activation/differentiation from naïve T cells to TEM cells in this model was not impaired by FTY720 treatment.

**Treatment with FTY720 suppresses the development of colitogenic CD4^+ TEM cell-mediated colitis.** Although we found that FTY720 suppressed the development of chronic colitis induced by the adoptive transfer of naïve CD4^+ CD45RB^high CD62L^− CD44^low T cells into SCID mice (Figs. 2–5), it was still unclear how FTY720 controlled the migration property of colitogenic memory T cells in memory T cell-mediated chronic colitis. Because it was possible that the effect of FTY720 in the CD4^+ CD45RB^high TEM cell transfer model was owing to the promotion of the sequestration of naïve CD4^+ T cells and/or to the inhibition of the egress of colitogenic CD4^+ memory T cells in the MLN, as previously demonstrated by others (3), we used our recently established colitogenic CD4^+ TEM cell-mediated chronic colitis model (29) under the condition without impact of naïve T cells. CB-17 SCID mice were injected intraperitoneally with LP CD4^+ TEM (CD44^high CD62L^−; as shown in the inset in Fig. 6A) cells obtained from colitic SCID mice originally induced by CD4^+ CD45RB^high T cells and were treated with DW or FTY720 (0.3 mg/kg) daily starting 1 day before transfer over a period of 4 wk (Fig. 6A). As shown in Fig. 6B, control DW-administered mice manifested progressive weight loss from 2–4 wk after the transfer. These mice had diarrhea with increased mucus in the stool, anorectal prolapse, and hunched posture by 4 wk. In contrast, FTY720-treated mice appeared healthy without any clinical signs during the whole period of observation (Fig. 6B). At 4 wk after the transfer, the colon from control DW-treated mice, but not from FTY720-treated mice, was enlarged and had a greatly thickened wall (Fig. 6C). In total, the assessment of clinical scores showed a clear difference between control DW-treated mice and FTY720-treated mice (Fig. 6D). Histological examination showed a marked inflammation in the LP of the colon from control DW-treated mice (Fig. 6E). In contrast, it was mostly abrogated in the LP of the colon from FTY720-treated mice (Fig. 6, E and F).

Furthermore, a few CD4^+ T cells were recovered from the colonic tissue of FTY720-treated mice compared with control DW-treated mice (Fig. 7). The numbers of CD4^+ cells recovered from the colon of control DW-treated mice (91.4 ± 63.7 × 10^5 cells) far exceeded the number of originally injected memory cells (5 × 10^5 cells), indicating extensive T cell migration and/or proliferation in the inflamed colon, which was mostly abrogated in FTY720-treated mice. Similarly, the numbers of CD4^+ cells in the spleen and peripheral blood from FTY720-treated mice was significantly decreased compared with control DW-treated mice (Fig. 7). Somewhat at odds, the numbers of CD4^+ cells in the MLN from FTY720-treated mice was comparable with that from DW-treated mice (Fig. 7). Unlike the CD4^+ CD45RB^high T cell transferred model, transferred colitogenic LP CD4^+ TEM cells could not be detected in the PLN.

**Fig. 3.** Mononuclear cells from the spleen, PB, peripheral lymph node (PLN), MLN, and lamina propria (LP; LPMC) were isolated from the colon at 5 wk after transfer. Cells were stained with FITC-anti-CD3 and PE-anti-CD4, and the total number of CD4^+ cells was determined by flow cytometry. Data are means ± SE of 7 mice/group. *P < 0.01. Data are indicated as the mean ± SD of seven mice in each group.

**Fig. 4.** Cytokine production by LP CD4^+ T cells. LP CD4^+ T cells were isolated from PBS- or FTY720-treated mice 5 wk after CD4^+ CD45RB^high T cell transfer or normal BALB/c mice (12 wk old) and stimulated with anti-CD3 and anti-CD28 monoclonal antibodies for 48 h. Interferon (IFN)-γ, interleukin (IL)-2, IL-4, IL-10, and tumor necrosis factor (TNF)-α concentrations in culture supernatants were measured by a specific ELISA (IL-10) or a mouse T helper (Th1/Th2 cytokine bead array kit (IL-2, IL-4, IL-5, TNF-α, and IFN-γ). Data are means ± SD of 6 mice/group. *P < 0.05.
the characteristics of TEM cells, including the MLN, indicating blood from DW-treated or FTY720-treated SCID mice retained PCR analysis. As depicted in Fig. 10, colitogenic LP TEM cells that FTY720 did not affect the TEM-to-TCM conversion in this CD4 and the blood of DW-treated or FTY720-treated mice transferred with Fig. 9, however, almost all CD4 S1P receptors in sorted normal splenic CD4 receptors (5), we finally evaluated the expression pattern of Because it has been reported FTY720-phosphate acts via S1P model.

We further determined whether FTY720 affected the cell differentiation of transferred CD4+CD62L+CD44high TEM cells in vivo, because we initially speculated that memory T cells residing in lymph nodes preferentially express CD62L, which is termed as central memory T (TCM) cells. As shown in Fig. 9, however, almost all CD4+ T cells in any organ and the blood from DW-treated or FTY720-treated SCID mice retained the characteristics of TEM cells, including the MLN, indicating that FTY720 did not affect the TEM-to-TCM conversion in this model.

Colitogenic CD4+ TEM cells express receptors for S1P. Because it has been reported FTY720-phosphate acts via S1P receptors (5), we finally evaluated the expression pattern of S1P receptors in sorted normal splenic CD4+, CD4+CD45RBhigh, and colitic LP CD4+ TEM cells using quantitative PCR analysis. As depicted in Fig. 10, colitogenic LP TEM cells contained mRNA encoding S1P1, S1P2, and S1P4 receptors, albeit to a lesser extent compared with naive CD4+CD45RBhigh T cells, with only a minimal representation of S1P3 and S1P5 receptors.

Fig. 5. Expression of CD62L and CD44 on CD4+ T cells in various organs and the blood of DW-treated or FTY720-treated mice transferred with CD4+CD45RB+TEM cells. Freshly isolated cells from DW-treated or FTY720-treated mice transferred with CD4+CD45RB+ TEM cells at 5 wk after transfer were stained with FITC-labeled anti-CD4 and PE-labeled anti-CD62L or PE-labeled anti-CD44 monoclonal antibodies. Samples were analyzed by flow cytometry. Lymphocytes were identified by characteristic forward angle and side-scatter profiles. LPL, LP lymphocytes. Data are displayed as a dotted plot (4-decade log scale), and quadrant markers were positioned to include >98% of control Ig-stained cells in the bottom left. Percentages in each quadrant are indicated. Results shown are representative of 3 mice/group.

DISCUSSION

In the present study, we demonstrated that FTY720 suppressed the development of colitis induced by the adoptive transfer of colitogenic LP TEM cells into SCID mice under the condition without the impact of naïve T cell migration. In addition, we also found that FTY720 ameliorates the development of colitis induced by the adoptive transfer of naïve...
REGULATION OF CD4+ TEM CELLS BY FTY720

CD4+CD45RBhigh T cells into SCID mice, in which all processes responsible for the development of colitis, such as the initiation of naïve T cells by antigen-bearing dendritic cells and the elicitation to effector or TEM cells in secondary lymphoid organs, are involved. Because we demonstrated that colitogenic LP TEM cells as well as normal splenic CD4+CD45RBhigh T cells expressed several S1P receptors that are targets for FTY720-phosphate, the present study provides the suggestion that FTY720 can directly control memory CD4+ T cell-mediated immune diseases in addition to the impact of naïve T cells.

Because it has been largely thought that the effect of FTY720 treatment on autoimmune diseases in the previous preventive protocols is owing to the sequestration of naïve T cells rather than pathogenic memory T cells, it was unclear...
whether FTY720 directly affects the migration property of memory T cells in autoimmune established stages. To overcome this issue, we conducted the adoptive transfer of only colitogenic LP CD4⁺ TEM cells into SCID mice in the present study. As there were no naïve T cells in this transfer system because almost all isolated LP CD4⁺ T cells from colitic mice had a characteristic of CD44highCD62L⁻ CD45RBlow TEM cells, we could directly evaluate the effect of FTY720 on colitogenic LP CD4⁺ TEM cells. Surprisingly, we found that FTY720 suppressed the development of colitogenic memory TEM cell-mediated colitis, indicating that FTY720 affects the migration of colitogenic LP CD4⁺ TEM cells. Consistent with this, we demonstrated that colitogenic LP TEM cells as well as normal splenic CD4⁺ CD45RBhigh T cells expressed several S1P receptors (S1P₁, S1P₂, and S1P₃). However, we found that the numbers of CD4⁺ T cells in the MLN were also significantly decreased in FTY720-treated mice transferred with colitogenic LP TEM cells compared with DW-treated mice. Although the result might indicate that FTY720 could not sequester colitogenic CD4⁺ TEM cells in FTY720-treated mice, it is more likely that colitogenic CD4⁺ TEM cells in PBS-treated mice had a greater chance to repeat the migration into the MLN and the egress from the MLN to sites of inflammation and could expand their number, which exceeded the original transferred cell number (3 × 10⁵ cells). However, it is possible that the finding that there were decreased numbers of CD4⁺ T cells in the various compartments may be a result of the lack of the colitis in FTY720-treated mice and not necessarily a direct effect of FTY720 on egress or sequestration, because we could not detect any differences of cell numbers in the MLN between TEM cell-transferred DW-treated mice and FTY720-treated mice. Nonetheless, we found that FTY720 caused a significant loss of CD4⁺ TEM cells from the blood (lymphopenia) and spleen, reducing numbers by ~100 to 1,000-fold compared with DW-treated mice, indicating that FTY720 induced a smaller chance to recirculate into the MLN repeatedly, followed by no development of colitis. Further studies will be needed to address this issue using another short-term in vivo transfer system.

The present study would provide another impact in terms of a characteristic of memory T cell trafficking for the maintenance of autoimmune diseases. Although some investigators have suggested that LP T cells do not migrate out of the gut (12), our results indicated that LP CD4⁺ TEM cells are needed to constitutively recirculate into the MLN and are restimulated by antigen-bearing dendritic cells in the MLN to maintain colitogenic CD4⁺ TEM cells for sustaining chronic colitis. An earlier study (32) has demonstrated that the thoracic duct lymph (TDL), which empties into the blood, contains many lymphocytes. In a landmark experiment, Gowans and Knight (7) transferred radiolabeled thoracic duct lymphocytes intravenously into naïve syngeneic recipients and detected donor memory lymphocytes in their TDL again, providing the first formal demonstration that memory lymphocytes recirculate continuously between blood and lymph. Consistent with this notion, we also found that LP CD4⁺ T cells obtained from FTY720-treated SCID mice transferred with colitogenic CD4⁺ TEM cells in FTY720-treated mice produced less amounts of Th1 cytokines compared with DW-treated SCID mice, indicating that the recirculation and restimulaton of Th1 cells in lymph nodes, in this case, the MLN, are essential for the maintenance of Th1-mediated autoimmune disorders.

In nohuman primates treated with FTY720 for over 100 days, however, ~10% of peripheral blood CD4⁺ T cells were refractory to depletion by FTY720 (23). It is likely that these cells represent long-lived TEM cells. Because TEM cells lack the lymph node homing receptors CCR7 and CD62L (28) and express high levels of inflammatory chemokine receptors (28), and they therefore could preferentially reside in nonlymphoid tissues (16) in contrast to naïve and TCM cells. Thus it is possible that TEM cells might not be trapped by FTY720 treatment in the lymph node but be controlled by the unidentified mechanism of action of FTY720 in our model. Conversely, Henning and colleagues (9) recently reported that FTY720 rescues the homing defect in both CCR7⁻/⁻ mice and plt mice, which lack expression of CCL19 and CCL21-ser, both ligands for CCR7 on high endothelial cells (9), suggesting that FTY720 enables TEM cells to sequester into lymph nodes in a CCR7/CD62L-independent mechanism for lymphocyte homing through endothelial venules, which is strongly augmented in the presence of FTY720. Further studies will be needed to address this issue.

The ultimate goal of any treatment for autoimmune diseases, including IBDs, is antigen-specific suppression of pathology. Autoaggressive antigen-specific lymphocytes need to be eliminated or controlled to prevent tissue damage and halt the progression of clinical disease. Although it is so far poorly understood regarding the self-antigens and commensal bacteria flora responsible for the pathogenesis of IBDs, colitogenic memory T cells rather than naïve T cells should be the targets for the treatment of IBDs, even if the primary, initiating self-antigens/commensal bacteria flora are unknown and inflammation is progressive (19, 25). Although Mizushima and colleagues (18) recently demonstrated that FTY720 treatment of established colitis in IL-10⁻/⁻ mice ameliorated colitis, it was still unclear whether FTY720 affected the migration of naïve T cells or colitogenic memory T cells. In light of this, the present SCID colitis model induced by the adoptive transfer of colitogenic CD4⁺ TEM cells is a useful model, because we can assess a character of memory T cells without the impact of naïve T cells or colitogenic memory T cells. In light of this, the present SCID colitis model induced by the adoptive transfer of colitogenic CD4⁺ TEM cells is a useful model, because we can assess a character of memory T cells without the impact of naïve T cells. Because we demonstrated that FTY720 could control and suppress colitogenic CD4⁺ TEM cell-mediated colitis, FTY720 might be beneficial for the treatment of ongoing or established IBDs as well as their prevention.

In conclusion, we demonstrated here that FTY720 was useful to suppress memory T cell-mediated chronic colitis without the impact of naïve T cell activation using memory CD4⁺ TEM cell adoptive transfer experiments. Although we have to pass many critical checkpoints, this study indicates that FTY720 offers a hope for the treatment of human IBDs.

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