FTY720, a Novel Immunosuppressant, Induces Sequestration of Circulating Mature Lymphocytes by Acceleration of Lymphocyte Homing in Rats. II. FTY720 Prolongs Skin Allograft Survival by Decreasing T Cell Infiltration into Grafts But Not Cytokine Production In Vivo

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*J Immunol* 1998; 160:5493-5499; 
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FTY720, a Novel Immunosuppressant, Induces Sequestration of Circulating Mature Lymphocytes by Acceleration of Lymphocyte Homing in Rats. II. FTY720 Prolongs Skin Allograft Survival by Decreasing T Cell Infiltration into Grafts But Not Cytokine Production In Vivo

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FTY720, a novel immunosuppressant, prolonged the survival of WKAH skin allografts transplanted into MHC-incompatible F344 rats. In this allograft model, the median survival time of the control group was 7 days, whereas those of the groups given FTY720 orally at 0.1 mg/kg and cyclosporin A (CsA) at 10 mg/kg were 10.5 and 11 days, respectively. In contrast, FTY720 (0.1 mg/kg) combined with CsA (10 mg/kg) synergistically prolonged allograft survival with a median survival time exceeding 70 days. To elucidate the mechanisms of this remarkable synergistic effect, mRNA expressions of IL-2 and IFN-γ and that of CD3 (Δ-chain), which reflects T cell infiltration, in allografts were temporally analyzed using a semiquantitative PCR method. In WKAH skin allografts, mRNA levels of IL-2, IFN-γ, and CD3 were increased as compared with isograft controls, peaking on days 4 to 5. CsA (10 mg/kg) significantly inhibited elevations of IL-2 and IFN-γ mRNA, while slightly inhibiting that of CD3 mRNA in allografts. On the contrary, FTY720 (0.1 mg/kg) markedly inhibited elevation of CD3 mRNA, while slightly inhibiting those of IL-2 and IFN-γ mRNA. FTY720 (0.1 mg/kg) combined with CsA (10 mg/kg) almost completely suppressed the intragraft expressions of mRNA for IL-2, IFN-γ, and CD3. Immunohistochemical staining and flow cytometric analysis also confirmed that FTY720 decreased T cell infiltration into allografts. From these results, the synergistic effect of FTY720 combined with CsA on prolongation of allograft survival is presumably based on the respective inhibitions of T cell infiltration and cytokine production in grafts. The Journal of Immunology, 1998, 160: 5493–5499.

2-Amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720), a synthetic immunosuppressant, showed more potent immunosuppressive activity than CsA and FK506 in skin and cardiac allograft models (1–4). However, unlike CsA and FK506, FTY720 up to 1000 nM affected neither lymphocyte proliferation nor IL-2 production in rat allogeneic MLC in vitro (5). FTY720 combined with CsA showed a remarkable synergistic effect in prolonging skin, cardiac, and renal allograft survival in rats and dogs (3–7). As described in our preceding report, FTY720 led to disappearance of lymphocytes, especially T cells, from peripheral blood and thoracic duct lymph within 3 to 24 h after a single oral administration to rats (5). On the contrary, there were marked increases in the numbers of lymphocytes in peripheral lymph nodes, mesenteric lymph nodes, and Peyer’s patches. These observations were attributed to the acceleration of lymphocyte-trafficking to lymphoid tissues mediated by lymphocyte-homing receptors, including CD62L, CD49d, and CD11a (5). FTY720-induced acceleration of lymphocyte homing and subsequent sequestration of lymphocytes within lymphoid tissues appears to be related to the immunosuppressive activity of FTY720, since the integration and control of systemic immune responses depend on regulated lymphocyte homing (8).

An allograft is thought to be rejected by intragraft immune responses, including infiltration of T cells into the graft and the activation of these T cells by Th1-associated cytokines (9–14). The aim of the current study was to determine the effects of FTY720 and CsA on intragraft immune responses, and thereby to elucidate the mechanism underlying the remarkable synergistic effect produced by the combination of FTY720 and CsA. Using a semiquantitative PCR method, we temporally analyzed mRNA levels of IL-2 and IFN-γ and that of CD3, which reflects T cell mass, in WKAH skin allografts transplanted to MHC-incompatible F344 rats treated with FTY720, CsA, and both. The infiltration of CD3+ T cells into allografts was also analyzed by flow cytometry and immunohistochemical staining with anti-rat CD3 mAb.

Materials and Methods

Animals

Inbred strains of male F344 and WKAH rats were purchased from Charles River Japan (Atsugi, Kanagawa, Japan) and Japan SLC (Hamamatsu, Shizuoka, Japan), respectively. All rats were used at 4 to 6 wk of age.

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Received for publication October 17, 1997. Accepted for publication January 30, 1998.

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2 Abbreviations used in this paper: FTY720, 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride; CsA, cyclosporin A; FK506, tacrolimus; HPRT, hypoxanthine-guanine phosphoribosyltransferase.
Agents

FTY720 was synthesized according to previously described methods (1), dissolved in distilled water, and given orally. CsA (oral solution of Sandimmune, Sandoz, Basel, Switzerland) was dissolved in pure olive oil (Sigma Chemicals, St. Louis, MO) and administered orally. Control animals received the vehicle only.

Rat allogeneic MLC

Allogeneic MLC was performed using nylon nonadherent spleen cells from F344 rats as responder cells and mitomycin C–treated spleen cells from WKAH rats as stimulator cells (5, 15). Responder cells at a concentration of 5 × 10^7 cells/well were cocultured with equal numbers of stimulator cells in RPMI 1640 medium containing 5 μM 2-ME and 10% FCS (Boehringer Mannheim, Mannheim, Germany).

After a 48-h culture at 37°C in 5% CO_2, the cells were recovered and used for RNA isolation.

Rat skin allograft

Full thickness skin grafts (square pieces 2.0 × 2.0 cm) were transplanted to the lateral thorax of 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. The rat strain combination for MHC-incompatible transplantation was WKAH (RT1^k) to F344 (RT1^b) (3, 5). Either FTY720 or CsA was orally administered daily to the transplanted animals at the aforementioned doses.

mRNA analysis using RT-PCR

The skin grafts removed from the transplanted recipients or alloantigen-stimulated lymphocytes were homogenized with RNA isolation reagent (Nippon Gene, Tokyo, Japan). Total RNA was isolated according to the manufacturer’s protocol and then quantitated spectrophotometrically. Total RNA from the graft (1.2 μg) or from the lymphocytes (0.6 μg) was reverse transcribed in a 60-μl volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl_2, 200 mM each dNTP (dATP, dGTP, dTTP, and dCTP), 0.625 U M-MLV reverse transcriptase (Promega), and 28 (CD3), or 24 (HPRT) μM of each primer pair, and 200 μM of dNTP (dATP, dGTP, dTTP, and dCTP). Primer sequences were as follows (GenBank accession numbers): IL-2, 351 (5′-GAGGCTGACGACAGCTGCT-3′); IFN-γ, 351 (5′-AGCCGAGAAGCGTAAAGCT-3′); CD3, 351 (5′-GGTGAAAGTGGCTTGGCT-3′); and HPRT, 351 (5′-GGATGGAACTGGCTCTGCT-3′). cDNA was synthesized at 37°C for 10 min and 42°C for 30 min.

Five microliters of cDNA was amplified in a 25-μl volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl_2, 200 mM each dNTP (dATP, dGTP, dTTP, and dCTP), 200 nM appropriate primer pair, and 1.5 μM of each dNTP, for 35 cycles of 94°C for 15 s, 65°C for 1 min, and 72°C for 1 min. After an initial denaturation step, the cDNA amplification mixture was subjected to amplification cycles, each cycle consisting of denaturation (94°C for 15 s), annealing (65°C (IFN-γ), 60°C (CD3), and 608 bp, respectively. After an initial denaturation step, the cDNA amplification mixture was subjected to amplification cycles, each cycle consisting of denaturation (94°C for 15 s), annealing (65°C (IFN-γ, CD3, and HPRT) or 72°C (IL-2 for 15 s) and extension (72°C for 15 s) using a thermal cycler (Gene Amp PCR System 9600, Perkin-Elmer Cetus, Foster, CA).

The number of amplification cycles was 30 (IL-2), 26 (IFN-γ), 28 (CD3), or 24 (HPRT). An aliquot (10 μl) of the PCR product was electrophoresed on 2% agarose gel, and amplified DNA fragments were stained with SYBR Green I (Molecular Probes, Eugene, OR). Fluorescence intensity of the specific band was visualized and measured using a fluorescence image analyzer (Forte Imager 575; Molecular Dynamics, Sunnyvale, CA). The specificity of PCR products was verified by restriction analysis, using two restriction enzymes indicative of the expected amplified sequence in a previous report (16).

Measurement of numbers of CD3+ T cells in peripheral blood and skin allografts

Peripheral blood was periodically collected from a tail vein of allografted rats. The skin graft removed from transplanted recipients was chopped into small fragments, then incubated for 1 h at 37°C on a rocker in 4 ml of RPMI 1640 containing 10% FCS, 2 mg/ml collagenase (Calzyme, San Luis Obispo, CA), and 20 μg/ml DNase I (Boehringer Mannheim). The digested skin graft was passed through a stainless steel mesh, and skin parenchymal cells were sedimented over Ficoll buffer, density 1.090 g/cm^3 (Immuno-Biologic Laboratories, Fujioaka, Japan), at 1700 × g for 20 min. The cells were stained with FITC-conjugated anti-rat CD3 mAb (clone: G4.18, Pharmingen, La Jolla, CA) and streptavidin-Cy-Chrome (PharMingen). Numbers of CD3+ T cells were determined by flow cytometry with EPICS XL-MCL (Coulter, Miami, FL).

FTY720 decreases T cell infiltration into allografts in rats

RESULTS

Semiquantitative assessment of the measurement of IL-2, IFN-γ, and CD3 mRNA levels in allografts by the PCR method

For semiquantitative assessment of the measurement of IL-2, IFN-γ, and CD3 mRNA levels in allografts by the PCR method in our present study, a twofold dilution of cDNA solution prepared from allografts separated 5 days after transplantation (day 5) was amplified with IL-2, IFN-γ, CD3-, or HPRT-specific primers. The fluorescence intensity of the PCR product diminished as the cDNA level in the reaction mixture decreased (Fig. 1). Thus, the analysis by the PCR method was semiquantitative in this study.

Temporal analysis of IL-2, IFN-γ, and CD3 mRNA levels in rat skin allografts

Levels of IL-2, IFN-γ, and CD3 mRNA in allografts on days 2, 3, 4, 5, and 6 were analyzed by the PCR method (Fig. 2). Isografts were analyzed in parallel to control for the nonspecific inflammatory response associated with the transplantation procedure. Control skin tissue (before transplant) contained detectable levels of IL-2, IFN-γ, and CD3 mRNA. The mRNA background levels of the isografts were only slightly enhanced. In contrast, IL-2, IFN-γ, and CD3 mRNA levels of allografts were markedly enhanced, peaking on day 4 (IL-2 and IFN-γ) and day 5 (CD3).

Effects of FTY720, CsA, and both on survival of rat skin allografts (WKAH) transplanted to MHC-incompatible rats (F344)

Figure 3 shows the effects of FTY720, CsA, and both on survival of rat skin allografts (WKAH) transplanted to MHC-incompatible rats (F344). In this allograft model, the median allograft survival was 7 days in vehicle-treated recipients (control group). The respective median graft survivals for FTY720-treated recipients at

Immunohistochemical staining of skin allograft sections

Skin allografts were removed, embedded in tissue compound, and frozen in liquid N_2. Cryostat sections (6 μm thickness) were prepared, fixed with ice-cold acetone, and stained by immunoperoxidase methods. Briefly, sections were incubated with 5 mg/ml of biotinylated anti-rat CD3 mAb (clone: G4.18) for 1 h, and then with an avidin-biotinylated peroxidase complex (ABC kit, Vectastain, Vector, Burlingame, CA) for 1 h. Ab binding was visualized using a peroxidase substrate containing 0.05% 3,3′-diaminobenzidine and 0.01% hydrogen peroxide in 0.1 M Tris-HCl buffer (pH 7.3).

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doses of 0.1 mg/kg and 1 mg/kg were 10.5 and 21 days, respectively, while those of CsA-treated recipients at doses of 10 mg/kg and 30 mg/kg were 11 and 21 days, respectively. Thus, 0.1 mg/kg and 10 mg/kg were regarded as subtherapeutic doses for FTY720 and CsA, respectively. Combination treatment with FTY720 (0.1 mg/kg) and CsA (10 mg/kg) resulted in a remarkable prolongation, with median survival exceeding 70 days.

Figure 4 shows the time course change in the number of CD3\(^+\) T cells in the peripheral blood of allograft F344 rats. FTY720 (0.1 mg/kg) alone or combined with CsA (10 mg/kg) markedly decreased numbers of peripheral blood T cells during the administration period when compared with vehicle (control) or CsA (10 mg/kg) alone.

Effects of FTY720, CsA, and both on IL-2, IFN-\(\gamma\), and CD3 mRNA levels in rat skin allografts

Figure 5 shows IL-2, IFN-\(\gamma\), and CD3 mRNA levels in allografts of the recipients treated with FTY720 (0.1 mg/kg), CsA (10 mg/kg), and both agents on days 4, 5, 6, and 7. The mRNA levels in allografts of vehicle-treated recipients (control) on day 7 were not determined, as preparing the total RNA from rejected allografts was impossible. In the recipients treated with FTY720 (0.1 mg/kg), IL-2 and IFN-\(\gamma\) mRNA levels were enhanced in allografts and peaked on day 5, although the peak level was lower than that of vehicle-treated recipients. FTY720 (0.1 mg/kg) markedly suppressed the elevation of CD3 mRNA in the allograft until day 6, but failed to maintain this suppressive effect on day 7. On the contrary, CsA (10 mg/kg) markedly inhibited the elevations of IL-2, IFN-\(\gamma\), and CD3 mRNA in allografts, while having little effect on the CD3 mRNA level. In recipients treated with both FTY720 (0.1 mg/kg) and CsA (10 mg/kg), the elevations of IL-2, IFN-\(\gamma\), and CD3 mRNA in allografts were markedly suppressed, and the levels were similar to those in isografts. This suppressive effect was maintained at least until day 14 (data not shown).

Figures 6 and 7 show the effects of subtherapeutic and therapeutic doses of FTY720 or CsA on IL-2, IFN-\(\gamma\), and CD3 mRNA
levels in skin allografts 5 days after transplantation. A therapeutic dose of FTY720 (1 mg/kg) markedly suppressed the elevation of CD3 mRNA, while having little effect on the elevations of IL-2 and IFN-γ mRNA levels in the allograft. A subtherapeutic dose of FTY720 (0.1 mg/kg) had a similar effect. A subtherapeutic dose of CsA (10 mg/kg) did not, whereas a therapeutic dose of CsA (30 mg/kg) did, markedly inhibit the elevation of CD3 mRNA in allografts. Both doses of CsA markedly inhibited the elevation of IL-2 and IFN-γ mRNA.

FTY720 did not affect mRNA expressions of IL-2 and IFN-γ in rat allogeneic MLR

CsA was reported to inhibit mRNA expression of Th1-associated cytokines, including IL-2 and IFN-γ, by Ag- or mitogen-stimulated Th cells (20, 21). The effects of FTY720 on alloantigen-induced mRNA expressions of IL-2 and IFN-γ were examined in comparison with those of CsA in allogeneic MLR using splenic T cells of F344 rats as responder cells and mitomycin C-pretreated WKAH rat spleen cells as stimulator cells (Fig. 8). CsA at 100 nM completely inhibited IL-2 and IFN-γ mRNA expressions in allograft-stimulated T cells. Unlike CsA, FTY720 up to 1000 nM affected neither IL-2 nor IFN-γ mRNA expression.

Effect of FTY720 on CD3⁺ T cell infiltration of rat skin allografts

Rat skin allografts were digested by collagenase on day 5, and then the number of CD3⁺ T cells in allografts were analyzed by flow cytometry.

FIGURE 5. Effects of FTY720 (0.1 mg/kg), CsA (10 mg/kg), and both on IL-2, IFN-γ, and CD3 mRNA levels in rat skin allografts (WKAH donor to F344 recipient) on days 4, 5, 6, and 7 after transplantation. FTY720 (0.1 mg/kg) and CsA (10 mg/kg) were given orally on consecutive days following transplantation. Levels of IL-2, IFN-γ, and CD3 mRNA in rat skin allografts of the recipients treated with FTY720, CsA, or both were analyzed using the PCR method. As a control, those of vehicle-treated recipients were also analyzed. All PCR reactions for each time point were performed using aliquots from the same cDNA preparation. The analysis of allografts was repeated using a second set of animals with essentially the same results. Each PCR reaction was conducted at least twice with essentially the same results.

FIGURE 6. Effects of subtherapeutic and therapeutic doses of FTY720 (0.1 mg/kg and 1 mg/kg) or CsA (10 mg/kg and 30 mg/kg) on IL-2, IFN-γ, and CD3 mRNA levels in rat skin allografts (WKAH donor to F344 recipient) 5 days after transplantation. The agents were given orally on consecutive days following transplantation. Levels of IL-2, IFN-γ, and CD3 mRNA in rat skin allografts from recipients treated with FTY720 or CsA were analyzed by the PCR method. As a control, those of vehicle-treated recipients were also analyzed. All PCR reactions for an individual point were performed using aliquots from the same cDNA preparation. Each lane represents an individual animal. Each PCR reaction was conducted at least twice with essentially the same results.

FIGURE 7. Effects of subtherapeutic and therapeutic doses of FTY720 (0.1 mg/kg and 1 mg/kg) or CsA (10 mg/kg and 30 mg/kg) on IL-2, IFN-γ, and CD3 mRNA levels in rat skin allografts (WKAH donor to F344 recipient) 5 days after transplantation. The fluorescence intensity of the specific band described in Figure 6 was measured with a fluorescence image analyzer. Each column represents the mean ± SE of three animals.

FIGURE 8. Effects of FTY720 and CsA on IL-2, IFN-γ, and CD3 mRNA levels in allo-MLC between F344 responder and WKAH stimulator. R, F344 lymphocytes; R+S, F344 lymphocytes cocultured with mitomycin C-treated WKAH lymphocytes. Nylon-nonadherent splenic lymphocytes from F344 rats were cocultured with mitomycin C-treated splenic lymphocytes from WKAH rats. After a 48-h culture, lymphocytic IL-2, IFN-γ, and CD3 mRNA levels were analyzed by the PCR method. Each lane represents an individual culture. All PCR reactions for a point were performed using aliquots from the same cDNA preparation.
cytometry with anti-rat CD3 mAb. As shown in Figure 9, FTY720 (0.1 mg/kg) markedly decreased CD3⁺ T cells in allografts. FTY720 (0.1 mg/kg) and CsA (10 mg/kg) were given orally on consecutive days following transplantation. Rat skin allografts were digested by collagenase on day 5; then numbers of CD3⁺ T cells in the allografts were analyzed by flow cytometry with anti-rat CD3 mAb. As a control, those of vehicle-treated recipients were also analyzed. Each column represents the mean ± SE of three animals.

As shown in Figure 9, FTY720 (0.1 mg/kg) markedly decreased CD3⁺ T cells in allografts. FTY720 (0.1 mg/kg) combined with CsA (10 mg/kg) had essentially the same effect. In addition, T cell infiltration into allografts on day 5 was assessed by immunohistochemical staining with anti-rat CD3 mAb. As shown, A (×200) and B (×400) of Figure 10, CD3⁺ T cell infiltration was seen in the dermis of allografted skin in the control recipient. In contrast, CD3⁺ T cell infiltration was hardly seen in the dermis of allografted skin in FTY720-treated recipients (Fig. 10C, ×200).

Discussion

Patterns of cytokine expression in grafts of various transplantation models have been reported previously, and elevation of cytokine expressions in such grafts are believed to contribute to their rejection (9–14, 22, 23). These reports suggested that alloreactive T cells in allografts were activated by Th1-associated cytokines (IL-2 and IFN-γ) but not Th2-associated cytokines (IL-4 and IL-10), and that Th2-associated cytokines were essential for xenograft rejection. In addition to the elevation of cytokine expressions, T cell infiltration into the graft is thought to be an important component of graft rejection. Increased T cell infiltration of grafts has, in fact, been described in various graft models (10, 23). The expression of adhesion molecules related to T cell trafficking is enhanced in grafts, and mAbs of these molecules inhibit graft rejection (11, 24, 25). In our present MHC-incompatible allograft model, mRNA expressions of Th1-associated cytokines (IL-2 and IFN-γ) in the allografts were markedly enhanced (Fig. 2), whereas rat CD3 mAb. As shown, A (×200) and B (×400) of Figure 10, CD3⁺ T cell infiltration was seen in the dermis of allografted skin in the control recipient. In contrast, CD3⁺ T cell infiltration was hardly seen in the dermis of allografted skin in FTY720-treated recipients (Fig. 10C, ×200).

FIGURE 9. Effects of FTY720 and CsA on numbers of CD3⁺ T cells in rat skin allografts. FTY720 (0.1 mg/kg) and CsA (10 mg/kg) were given orally on consecutive days following transplantation. Rat skin allografts were digested by collagenase on day 5; then numbers of CD3⁺ T cells in the allografts were analyzed by flow cytometry with anti-rat CD3 mAb. As a control, those of vehicle-treated recipients were also analyzed. Each column represents the mean ± SE of three animals.

FIGURE 10. Immunohistochemical staining of CD3⁺ T cells in sections of rat skin allografts 5 days after transplantation. Photomicrographs represent dermis of allografted skin in control (A, ×200, and B, ×400) and FTY720-treated recipients (C, ×200) on day 5. Arrowheads indicate CD3⁺ T cells stained with anti-rat CD3 mAb (G 4.18). FTY720 at 0.1 mg/kg was given orally on consecutive days following transplantation. Sections were stained with anti-rat CD3 mAb employing immunoperoxidase methods.
that of a Th2-associated cytokine (IL-4) was undetectable (data not shown). In parallel, CD3 mRNA levels as a marker of T cell infiltration in the allografts were markedly enhanced (Fig. 2). T cell infiltration was also confirmed by immunohistochemical staining and flow cytometry with anti-CD3 mAb (Figs. 9 and 10). Therefore, infiltration of T cells into allografts and the activation of these T cells by Th1-associated cytokines presumably contributed to the allograft rejection observed in our present model.

E. C. Butcher and L. J. Picker suggested, in their review, that integration and control of systemic immune responses depended on regulated lymphocyte homing (8). As described in the preceding companion paper, FTY720 accelerated lymphocyte-homing, leading to the subsequent disappearance of T cells from peripheral blood and thoracic duct lymph in rats (5). The current study assessed the effects of FTY720, which modulates lymphocyte homing, on intragraft immune responses. FTY720 significantly reduced the number of peripheral blood T cells in skin-allografted rats (Fig. 4). On the other hand, FTY720 markedly decreased T cell infiltration into allografts while, in contrast to CsA, having little effect on IL-2 and IFN-γ mRNA expressions in allografts (Figs. 5 to 10). In addition, FTY720 had no effect on IL-2 and IFN-γ mRNA expressions by alloantigen-stimulated rat T cells in vitro (Fig. 8). These findings suggest that FTY720 prolongs allograft survival by decreasing the T cell infiltration into grafts but not cytokine productions. It is probable that the decreasing effect of FTY720 on T cell infiltration is due to reduction in the number of circulating T cells by acceleration of lymphocyte homing to lymph nodes and Peyer’s patches (5).

Cytokine mRNA level and T cell mass were not parallel in allografts (Figs. 2 and 5). Levels of IL-2 and IFN-γ mRNA peaked on day 4 and decreased on days 5 to 6, while that of CD3 mRNA peaked on day 5 and decreased on day 6 in control allografts (Fig. 2). In FTY720-treated recipients, fewer T cells infiltrated into allografts, and were presumably activated by alloantigen. Levels of IL-2 and IFN-γ mRNA peaked on day 5 and decreased on days 6 to 7, while that of CD3 mRNA was increased on day 7 in the allografts (Fig. 5). It is possible that this discrepancy between cytokine mRNA level and T cell mass in allografts is due to the change of ability for cytokine transcription by infiltrated T cells. This ability in control and FTY720-treated recipients presumably peaked on days 4 and 5, respectively. On the other hand, FTY720 markedly decreased T cell infiltration into allografts, while having much less effect on the levels of IL-2 and IFN-γ mRNA on day 5 (Fig. 7). The ability for cytokine transcriptions by infiltrated T cells in FTY720-treated recipients may be higher than that in control recipients on day 5.

In the current study, we determined the effects of FTY720 and CsA at high and low doses, to characterize these agents in detail (Figs. 6 and 7). The low dose of CsA (10 mg/kg) markedly inhibited the elevations of IL-2 and IFN-γ mRNA in allografts, while having little effect on that of CD3 mRNA (Figs. 5, 6, and 7). The effect of subtherapeutic CsA (10 mg/kg) on prolongation of the allograft survival is presumably due to insufficient decrease in T cell infiltration into the allografts (Figs. 5, 6, and 7). In contrast, the high dose of CsA (30 mg/kg) successfully inhibited T cell infiltration into the allografts (Figs. 6 and 7). Although the data are not shown, the high dose of CsA (30 mg/kg), in contrast to the low dose (10 mg/kg), markedly reduced peripheral blood T cells in skin-allografted rats. CsA (30 mg/kg) appears to reduce circulating T cells by inhibiting intrathymic differentiation (26–28), and thereby this drug may decrease T cell infiltration into allografts. In contrast, FTY720 appears to reduce circulating T cells by acceleration of lymphocyte homing (5). Thus, mechanism of the effect of CsA on the inhibiting T cell infiltration into allografts is thought to be different from that of FTY720. Both high and low doses of FTY720 equivalently inhibited T cell infiltration (Figs. 6 and 7). However, the high dose is much more effective in prolonging allografts (Fig. 3). These findings indicate that additional factors besides decrease in T cell infiltration contribute to immunosuppression at the high dose of FTY720. We have recently confirmed that repeated oral administration of FTY720 decreased the alloreactivity of lymph node T cells in skin-allografted rats dose dependently (our unpublished observations). Further analyses are currently being performed to clarify the detailed mechanism of this effect.

In our present allograft model, the immunosuppressive efficacy of FTY720 (0.1 mg/kg) was essentially the same as that of CsA (10 mg/kg), while the effects of FTY720 (0.1 mg/kg) on sequential expressions of IL-2, IFN-γ, and CD3 mRNA in allografts were entirely different from those of CsA (10 mg/kg). FTY720 more effectively inhibited T cell infiltration into allografts, while CsA was more effective in reducing IL-2 and IFN-γ expressions (Figs. 5, 6, and 7). Although FTY720 markedly decreased T cell infiltration into allografts, a small number of T cells were infiltrated. FTY720 is presumably unable to inhibit cytokine production by these infiltrated T cells. It is likely that this cytokine production is successfully inhibited in the case of combination treatment with FTY720 and CsA. From these observations, the synergistic effect of FTY720 combined with CsA on prolongation of allograft survival is presumed to be based on the respective inhibitions of T cell infiltration and cytokine production in grafts.

In this study, we initially characterized intragraft immune responses during FTY720 and CsA treatment, and proposed a mechanism underlying the remarkable synergistic effect produced by simultaneous treatment with both agents. 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.

References


