FTY720, a New Immunosuppressant, Promotes Long-Term Graft Survival and Inhibits the Progression of Graft Coronary Artery Disease in a Murine Model of Cardiac Transplantation

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Background—Effective immunosuppression is a critical determinant of organ and patient survival in cardiac transplantation. The present study was designed to determine the potency of FTY720, a new synthesized immunosuppressant, and examine its clinical potential as an immunosuppressant.

Methods and Results—Hearts of DBA/2 mice were transplanted heterotopically in C57BL/6 mice. Recipients were treated with oral FTY720 in doses of 0.3, 1, 3, or 10 mg·kg\(^{-1}\)·d\(^{-1}\) or with 40 mg·kg\(^{-1}\)·d\(^{-1}\) of cyclosporin A (CsA) as a comparative treatment. The median graft survival time (MST) was significantly prolonged by treatment with FTY720 10 mg·kg\(^{-1}\)·d\(^{-1}\). MST was not prolonged by FTY720 1 mg·kg\(^{-1}\)·d\(^{-1}\) or CsA. However, FTY720 1 mg·kg\(^{-1}\)·d\(^{-1}\) combined with CsA 40 mg·kg\(^{-1}\)·d\(^{-1}\) resulted in a significant prolongation of MST. Histopathological studies performed 5 days after transplantation demonstrated remarkable suppression of inflammatory response by treatment with FTY720 10 mg·kg\(^{-1}\)·d\(^{-1}\). Interleukin (IL)-2 and interferon (IFN)-\(\gamma\) production was not suppressed; however, cytotoxic T lymphocyte activity was strongly suppressed in vitro. In addition, IL-2–stimulated T-cell proliferation and class I and class II MHC antigen expression on IFN-\(\gamma\)–stimulated macrophages were strongly inhibited by FTY720. Histopathological studies 60 days after transplantation (DBA/2-B10.D2) demonstrated a beneficial effect on graft atherosclerosis.

Conclusions—FTY720 promoted long-term cardiac graft survival and strongly inhibited the progression of graft atherosclerosis. These observations suggest that FTY720 has a promising clinical potential in cardiac transplantation. (Circulation. 1999;100:1322-1329.)

Key Words: FTY720 ■ immunology ■ transplantation ■ rejection ■ grafting ■ hormones

Since its introduction into clinical use, cyclosporin A (CsA) has greatly increased the success of cardiac transplantation.\(^1\)\(^-\)\(^4\) However, further improvements in immunosuppressive therapy are much needed, because rejection, infection, and drug toxicity remain the most common causes of morbidity and mortality in the cardiac transplant population.\(^5\)\(^-\)\(^6\) Furthermore, despite successful immunosuppressive treatment, graft atherosclerosis has emerged as a leading cause of late graft failure.\(^2\)\(^-\)\(^7\)\(^-\)\(^8\) Therefore, considerable effort is being devoted to the development of new, more effective, and less toxic immunosuppressant agents. FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diolhydrochloride) is a new synthetic immunosuppressant isolated from \textit{Isaria sinclairii}. Its exact mechanism of action remains to be clarified.\(^9\)\(^-\)\(^11\)

The aims of this study were to measure the immunosuppressant potency of FTY720 in a murine model of cardiac transplantation and to examine its mechanism of action as well as its therapeutic potential. CsA was used as a reference immunosuppressant to estimate the severity of rejection in an acute and chronic rejection model.\(^12\)

Methods

Mice and Cardiac Transplantation
Male DBA/2 (H-2\(^b\)), C57BL/6 (H-2\(^b\)), C3H/He (H-2\(^k\)), and B10.D2 (H-2\(^b\)) mice 7 to 9 weeks old were obtained from the Shizuoku Agricultural Cooperation Association (Shizuoku, Japan). DBA/2 mice served as transplant donors, and heterotopic cardiac transplantation was performed as previously described.\(^12\)\(^-\)\(^13\) Time of rejection was defined as the day of cessation of cardiac activity examined by daily abdominal palpation.

Pharmaceuticals and Chemicals
FTY720 was provided as a pure powder by Yoshitomi Pharmaceutical Industries, Ltd. It was dissolved in water and stored at 4°C. Oral

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1322
Csa (Sigma) was commercially purchased. FTY720 was administered in doses of 0.3, 1, or 10 mg/kg·d⁻¹, and Csa in doses of 40 or 120 mg/kg·d⁻¹. As the combination treatment, FTY720 1 or 3 mg/kg·d⁻¹ was combined with 40 mg·kg⁻¹·d⁻¹ of Csa. Both drugs were administered orally, once daily, beginning on the day of transplantation. RPMI 1640 medium and FCS (Gibco), recombinant human interleukin-2 (rHL-2) (Amersham), mouse anti-mouse MHC class I antibody (Chemicon International), and rat anti-mouse MHC class II antibody (Southern Biotechnology) were commercially purchased. Recombinant mouse interferon-γ (rMIF-γ) (Shionogi Pharmaceutical Industries) and superparamagnetic microbeads conjugated with monoclonal anti-mouse Thy 1.2 antibodies (Daiichi Kagaku Pharmaceutical Industries) were graciously provided.

Histopathological Examination

Five days after transplantation, C57BL/6 recipient mice were killed, and allotrlogs were harvested and stained with hematoxylin-eosin and Masson’s trichrome. Histopathological examination was performed by 3 observers blinded to all background data. The intensity of mononuclear cell infiltration, myocardial necrosis, and coronary vasculitis were independently graded for severity on a scale of 0 (none present), 1 (minimal grade), 2 (low grade), 3 (moderate grade), and 4 (high grade), and scores were averaged. To evaluate the efficacy of treatment with FTY720 on graft atherosclerosis, B10.D2 recipient mice were killed 60 days after transplantation. Total vessel area (TV), neointimal area (NIA), medial area (MA), and luminal area (LA) were measured with a computerized morphometric system.

Cytotoxic T Lymphocyte Assay (51Cr Release Assay) In Vitro

Cytotoxic T lymphocyte (CTL) assay was performed as described previously.13 Spleen cells from C57BL/6 mice (recipient strain) and DBA/2 mice (donor strain) were used as responder and stimulator cells, respectively. Responder cells (5.6×10⁶) were cocultured with 4.0×10⁶ stimulator cells treated with mitomycin C (final concentration 25 μg/mL) in 24-well plates in 2 mL of sensitization medium consisting of RPMI 1640–10 (RPMI 1640 supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin sulfate, 5×10⁻⁵ mol/L 2-mercaptoethanol, and 10% heat-inactivated FCS) containing 1×10⁻³ mol/L sodium pyruvate. FTY720 (10⁻⁷, 10⁻⁸, and 10⁻⁹ mol/L in final concentration) dissolved in water or the vehicle only (control) was added to the cultures at their initiation. After 1-way mixed lymphocyte reaction (MLR) for 5 days, CTL activity was assessed by a 51Cr release assay at an effector-to-target ratio of 100:1, 33:1, or 10:1. Radioactivity was measured with an automatic gamma scintillation counter. Percentage specific cytotoxicity was calculated as % specific cytotoxicity=(cpm experiment-cpm spontaneous release)/(cpm experiment-cpm spontaneous release)×100.

CTL Activity (51Cr Release Assay) In Vivo

Spleen cells of DBA/2 mice were harvested, suspended in RPMI 1640-10 containing 10 μg/mL of LPS, and cultured for 12 hours. Cells were resuspended in RPMI, and C57BL/6 mice were alloimmunized by intravenous injection of 1×10⁹ spleen cells in a volume of 200 μL of RPMI as described previously.13 C57BL/6 mice received FTY720 at 1, 3, or 10 mg·kg⁻¹·d⁻¹ or the vehicle daily. To boost CTL activity, on day 5, they received an intraperitoneal injection of the same amount of spleen cells stimulated as described above. C57BL/6 mice were killed on day 8, spleen cells were harvested, and CTL activity against spleen cells of DBA/2 or C3H/He mice was assessed by 51Cr release assay.

Production of IL-2 and IFN-γ in MLR

One-way MLR was performed in 24-well plates as described previously.14 FTY720 (10⁻⁸, 10⁻⁹, and 10⁻¹⁰ mol/L in final concentration) dissolved in water or in the control vehicle only was added to the culture medium at the initiation of culture. The supernatants were harvested at 24 hours after initiation and stored at −70°C until ELISA. Murine IL-2 and IFN-γ in the supernatants were quantified by use of ELISA systems purchased from Endogen.

IL-2 and IFN-γ Gene Expression In Vivo

Five days after transplantation, the allotrlogs were harvested and total RNA was isolated as described previously.15 The RNA concentration was measured spectrophotometrically. Total RNA (3 μg) was subjected to first-strand cDNA synthesis, diluted 1:10, and the 1 μL of aliquot was amplified by PCR as described previously.15 Sense primer (A) and antisense primer (B) cDNA sequences of IL-2, IFN-γ, and GAPDH were as follows: IL-2 (A), 5'-AACCGCCACCCACTTCAAA-3'; IL-2 (B), 5'-TTGGATGATGCTTTGACA-3'; IFN-γ (A), 5'-AAGCGTACAC-
IL-2 and IFN-γ gene expression was analyzed by 32 cycles of amplification and GAPDH by 22 cycles.

**T-Cell Proliferation Assay**

(5-Bromo-2′-Deoxyuridine–ELISA)

The cell proliferation ELISA system was purchased from Boehringer Mannheim. Spleen cells from C57BL/6 mice were suspended in RPMI 1640-10 medium passed through nylon wool columns. Subsequently, superparamagnetic microbeads conjugated with monoclonal anti-mouse Thy 1,2 antibodies were added, and magnetic cell sorting was performed to obtain T cells. 5-Bromo-2′-deoxyuridine–ELISA was performed according to the manufacturer’s instructions. Absorbance was measured at 450 nm and 690 nm with an ELISA reader.

**rmIFN-γ–Stimulated MHC Antigen Expression on Murine Peritoneal Macrophages**

Nonstimulated murine peritoneal macrophages were collected as described previously.17 Cells numbering $5 \times 10^6$ in 200 μL of culture medium containing 20 ng/mL of rmIFN-γ were cultured with final concentrations of FTY720 of $10^{-6}$, $10^{-5}$, and $10^{-7}$ mol/L. Cell ELISA was performed as described previously.17,18 In brief, after the nonspecific binding sites were blocked with 10% normal rabbit serum, a first antibody was added to each well. After 1 hour of culture, plates were washed with PBS, a second antibody was added, and the culture was continued for 45 minutes. After washing, streptavidin–alkaline phosphatase (diluted 1:100 in PBS) was added. After 30 minutes of culture, each well was washed, phosphate substrate solution was added, and absorbance was measured at 405 nm with an ELISA reader. For the class I MHC expression assay, a mouse anti-mouse class I MHC antibody was used as the first antibody and biotinylated rabbit anti-mouse IgG as the second antibody; for the class II MHC expression assay, a rat anti-mouse class II MHC antibody was used as the first antibody and biotinylated rabbit anti-rat IgG as the second antibody.

**Statistical Analysis**

Differences between 2 nonparametric groups in the allograft survival experiments and scores for histopathological findings were examined by the Mann-Whitney U test. Percentage cytolysis in CTL assay, levels of IL-2 and IFN-γ in culture supernatants, T-cell proliferation in T-cell proliferation assay, and MHC antigen expression assay on rmIFN-γ–stimulated macrophages were compared by 1-way ANOVA, followed by Fisher’s protected least significant difference. A value of $P < 0.05$ was considered significant.

**Results**

**Effect of FTY720 and CsA on Cardiac Allograft Survival**

Over the course of the study, no significant differences in mean body weight were observed among the various treatment and control groups, except for the 6 mice treated with CsA $120 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, which suffered rapid loss of weight and died within 12 days after transplantation. The saline-treated C57BL/6 recipient mice rejected all DBA/2 cardiac allografts within 12 days, with a median graft survival time (MST) of 10 days. CsA $40 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ increased graft survival only slightly, with an MST of 14.0 days. Likewise, treatment with FTY720 at 1 mg · kg⁻¹ · d⁻¹ had little effect on graft survival, with an MST of 13.5 days. In contrast, FTY720 at a dose of 10 mg · kg⁻¹ · d⁻¹ caused a marked prolongation of graft survival, with an MST >50 days...
Furthermore, the combination therapy of FTY720 1 mg kg\(^{-1}\) d\(^{-1}\) and CsA 40 mg kg\(^{-1}\) d\(^{-1}\), resulting in an MST >35.5 days, was particularly noteworthy (Table 1).

**Histopathological Examination**
The grafts of 5 mice treated with FTY720 at 10 mg kg\(^{-1}\) d\(^{-1}\) were compared with those of 5 saline-treated control mice and those of 5 mice treated with CsA 40 mg kg\(^{-1}\) d\(^{-1}\). In comparison with the saline-treated mice, treatment with CsA 40 mg kg\(^{-1}\) d\(^{-1}\) had no significant effect on mononuclear cell infiltration or necrosis. In contrast, FTY720 treatment was associated with a striking inhibition of these inflammatory manifestations. Although CsA was not effective in preventing coronary vasculitis, FTY720 had evident beneficial effects (Figures 1 and 2).

**Effect of FTY720 on the Generation of Antigen-Specific CTL**
C57BL/6 cells treated with the control vehicle showed an increase in CTL response to DBA/2 target cells after MLR for 5 days. In contrast, cells treated with FTY720 showed a decrease in CTL activity at a concentration of 10\(^{-9}\) mol/L and complete CTL activity inhibition at 10\(^{-7}\) mol/L. These C57BL/6 cells showed little cytolytic activity when the target cells were from a third strain. Likewise, nonsensitized C57BL/6 spleen cells showed little cytolytic activity against DBA/2 target cells. FTY720 at 3 mg kg\(^{-1}\) d\(^{-1}\) demonstrated significantly suppressed CTL activity, and complete inhibition was observed with 10 mg kg\(^{-1}\) d\(^{-1}\) (Figure 3).

**Effect of FTY720 on IL-2 and IFN-\(\gamma\) Production**
In the MLR assay, the addition of FTY720 in concentrations of 10\(^{-6}\), 10\(^{-7}\), and 10\(^{-8}\) mol/L to the culture caused only a mild concentration-dependent suppression of IL-2 production. The effect on IFN-\(\gamma\) production was comparable to that

![Figure 3](http://circ.ahajournals.org/)
**Figure 3.** Inhibition of CTL activity by treatment with FTY720. Graphs represent cytolytic activities at effector-to-target ratios of 10, 33, and 100. Values are mean±SEM of triplicate cultures. \(*P<0.05\) vs control. A, Marked inhibition of CTL activity at concentrations of 10\(^{-9}\) mol/L and complete suppression at 10\(^{-7}\) mol/L. B, Spleen cells of C57BL/6 cocultured with DBA/2 cells displayed no significant CTL activity against third party (C3H/He) cells. C, Marked inhibition of CTL activity in a dose of 3 mg kg\(^{-1}\) d\(^{-1}\) and complete suppression by 10 mg kg\(^{-1}\) d\(^{-1}\). D, Spleen cells of C57BL/6 alloimmunized with DBA/2 spleen cells exerted only slight CTL activity against third-party (C3H/He) cells.

![Figure 4](http://circ.ahajournals.org/)
**Figure 4.** IL-2 and IFN-\(\gamma\) production in allogenic MLR incubated in presence of FTY720 or control vehicle. Values are mean±SEM of 3 wells. \(*P<0.05\) vs control, \(\uparrow P<0.01\) vs control. A, Concentration-dependent suppression of IL-2 production by FTY720 during MLR. Whereas CTL assay showed suppression of CTL activity by FTY720 at concentrations as low as 10\(^{-9}\) mol/L, IL-2 production was weakly suppressed by concentrations as high as 10\(^{-5}\) mol/L. B, As in case of IL-2, FTY720 suppressed IFN-\(\gamma\) production weakly after 24 hours of MLR.
observed on IL-2 production (Figure 4). Conversely, both IL-2 and IFN-γ gene expression was strongly suppressed by treatment with 10 mg · kg⁻¹ · d⁻¹ of FTY720. This is consistent with the histopathological finding of a distinct suppression of both mononuclear cell infiltration and inflammatory response by FTY720 treatment in the acute phase (Figure 5).

**rhIL-2–Stimulated T-Cell Proliferation Assay**

rhIL-2 stimulation of murine splenic T cells induced significant T-cell proliferation, which was markedly suppressed by FTY720. This inhibitory effect on T-cell proliferation was already noticeable at the lowest concentration of 10⁻⁹ mol/L, and nearly complete inhibition of T-cell proliferation was observed at a concentration of 10⁻⁷ mol/L (Figure 6). During culture, no significant decrease in the number of T cells was observed (data not shown).

**rmIFN-γ–Stimulated MHC Antigen Expression on Murine Peritoneal Macrophages**

rmIFN-γ stimulation of murine peritoneal macrophages induced significant upregulation of the MHC antigen expression, which was strongly inhibited by FTY720. This inhibitory effect was already noticeable at the lowest concentration of 10⁻⁹ mol/L, and nearly complete inhibition was observed at a concentration of 10⁻⁷ mol/L (Figure 7).

**Effect of FTY720 on Graft Atherosclerosis**

Allografts transplanted to B10.D2 mice were harvested 60 days after transplantation (Table 2). Percent LA, % NIA, and % MA were calculated as NIA/TVA × 100%, LA/TVA × 100%, and MA/TVA × 100%, respectively. Saline-treated control mice (n=7) displayed advanced graft atherosclerosis. In contrast, treatment with FTY720 10 mg · kg⁻¹ · d⁻¹ (n=5) was associated with a striking attenuation of the progression of atherosclerosis (P<0.01 versus control). Treatment with CsA at 40 mg · kg⁻¹ · d⁻¹ (n=5) was associated with minimal effects. It is particularly noteworthy that the progression of atherosclerosis was remarkably attenuated (Figures 8 and 9) by combination treatment with FTY720 at 3 mg · kg⁻¹ · d⁻¹ and CsA at 40 mg · kg⁻¹ · d⁻¹ (n=5).

**Discussion**

This study examined the effects of FTY720 on cardiac allograft survival and on histopathological changes in an acute and chronic cardiac allograft rejection model. CsA, the comparison drug, was used in high doses because of its specific pharmacokinetics in mice.29,30 Corrected for body

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**Figure 5.** Representative analysis of IL-2, IFN-γ, and GAPDH mRNA by semiquantitative PCR.

**Figure 6.** Recombinant human IL-2–stimulated T-cell proliferation assay. Values are mean±SEM in triplicate cultures. Partial and complete inhibitory effects by FTY720 on T-cell proliferation in concentrations of 10⁻⁹ and 10⁻⁷ mol/L, respectively. In contrast, FTY720 had no significant effect on nonstimulated T cells. *P<0.05 vs control.

**Figure 7.** Class I and class II MHC antigen expression on rmIFN-γ–stimulated murine peritoneal macrophages (cell ELISA). Values are mean±SEM. FTY720 10⁻⁹ mol/L markedly suppressed both class I and class II MHC antigen expression and completely suppressed it at a concentration of 10⁻⁷ mol/L. †P<0.05 vs control, ‡P<0.01 vs control.
point to a mechanism of action of FTY720 different from that observed with CsA.CTL activity was strongly suppressed in vitro. These results demonstrate typical graft atherosclerosis in this chronic cardiac rejection model. All grafts surviving to 60 days were harvested for histopathological studies.

The exact mechanism of action of FTY720 remains to be clarified but is suspected to be different from that of CsA, which is known to inhibit IL-2 production from helper T cells. IL-2 is involved both in the proliferation of mature T cells and in the generation of CTL.22 In this study, FTY720 strongly inhibited production in MLR, whereas CTL generation was completely suppressed in vitro. In our in vitro experiments, however, the number of murine T cell cultured in 10−7 mol/L of FTY720 did not decrease (data not shown), and MLR assay showed only mild suppression of IL-2 and IFN-γ production. Furthermore, the recovery of the peripheral lymphocytes counts in vivo occurs within only 14 days.9 These findings suggest that the immunosuppressive properties of FTY720 in therapeutic doses cannot be explained by apoptotic lymphocyte death.

It was recently reported that oral and intravenous treatment of rats with FTY720 causes a rapid redistribution of peripheral lymphocytes to the lymphatic system, and this phenomenon was suggested to be the mechanism of action of this compound.11 Although the determinants of this lymphocyte homing process remain to be clarified, it may be one of the likely mechanisms of action of this drug. Our study also identified the existence of other properties of FTY720. We had hypothesized that one of the mechanisms of action of FTY720 might be its interference with IL-2 response, because 10−7 mol/L of FTY720 caused only mild suppression of IL-2 production in MLR, whereas CTL generation was completely suppressed in vitro. In this study, FTY720 strongly inhibited rhIL-2-stimulated T-cell proliferation in a concentration as low as 10−9 mol/L and nearly completely suppressed it at a concentration of 10−7 mol/L. These results are consistent with our hypothesis formulated earlier.

Graft coronary artery disease is a major problem in clinical cardiac transplantation, because ≈50% of patients who survive 5 years show significant atherosclerosis on routine angiography.23,24 It has been postulated that the atherosclerotic lesions emerge in response to immune-mediated damage due to previous acute rejection or to the persistence of such immune mechanisms into the chronic posttransplant phase.25,26 Several studies have addressed the prevention of graft coronary artery disease without satisfactory answer. The efficacy of CsA in the prevention of atherosclerosis in allogenic transplants has not been reliable.27,28 The DBA/2-B10.D2 transplantation technique was used in our experiments because of the need for nonimmunosuppressed controls to test the efficacy of FTY720 on graft atherosclerosis. DBA/2 and B10.D2 mice share major histocompatibility antigens but differ in minor antigens, and it has been reported that nonimmunosuppressed recipients surviving >60 days develop typical graft atherosclerosis in this model.12 The daily administration of 40 mg·kg−1·d−1 CsA PO for 60 days had no apparent effect on graft atherosclerosis in comparison with control mice. In contrast, FTY720 10 mg·kg−1·d−1 was shown to be effective in the prevention of graft atherosclerosis.

The histopathological studies showed that treatment with the highest dose of FTY720 was associated with strong suppression of acute rejection in comparison with control mice or CsA-treated mice. Coronary vasculitis, characterized by perivascular cuffing and intraluminal accumulation of mononuclear leukocytes, was also attenuated by FTY720 treatment. These findings point to a strong immunosuppressive potency of FTY720.

The exact mechanism of action of FTY720 remains to be clarified but is suspected to be different from that of CsA, which is known to inhibit IL-2 production from helper T cells. IL-2 is involved both in the proliferation of mature T cells and in the generation of CTL.22 In this study, FTY720 caused only mild suppression of IL-2 production; however, CTL activity was strongly suppressed in vitro. These results point to a mechanism of action of FTY720 different from that of CsA.

Oral administration of FTY720 to normal rats induces a remarkable decrease in the number of peripheral lymphocytes.9 It had been hypothesized that FTY720 induces apoptotic lymphocyte death and that its immunosuppressive effect is attributable to the induction of lymphocytic apoptosis.9 Our in vitro experiments, however, the number of murine T cell cultured in 10−7 mol/L of FTY720 did not decrease (data not shown), and MLR assay showed only mild suppression of IL-2 and IFN-γ production. Furthermore, the recovery of the peripheral lymphocytes counts in vivo occurs within only 14 days.9 These findings suggest that the immunosuppressive properties of FTY720 in therapeutic doses cannot be explained by apoptotic lymphocyte death.

We would like to analyze the data provided to understand the effects of FTY720 on graft coronary artery disease. The chart provided shows the percentage of total vessel area affected by atherosclerosis in different treatment groups. The control group shows the highest percentage of vessel area affected, followed by the CsA 40 mg/kg/day group, and the FTY720 10 mg/kg/day group shows the least percentage. This indicates that FTY720 is effective in preventing graft coronary artery disease.

### TABLE 2. Survival of DBA/2 Mice Allografts Transplanted in B10.D2 Mice

<table>
<thead>
<tr>
<th>Treatment (n)</th>
<th>Graft Survival Time, d</th>
<th>MST, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (19)</td>
<td>6, 8, 9, 10, 10, 10, 10, 11, 11, 13, 13, 14, &gt;60, &gt;60, &gt;60, &gt;60, &gt;60, &gt;60, &gt;60, &gt;60, &gt;60</td>
<td>13</td>
</tr>
<tr>
<td>CsA 40 mg·kg−1·d−1 (9)</td>
<td>15, 25, 34, 36, &gt;60, &gt;60, &gt;60, &gt;60, &gt;60</td>
<td>&gt;60</td>
</tr>
<tr>
<td>FTY720 10 mg·kg−1·d−1 (7)</td>
<td>10, 17, &gt;60, &gt;60, &gt;60, &gt;60, &gt;60</td>
<td>&gt;60</td>
</tr>
<tr>
<td>CsA 40 mg·kg−1·d−1 + FTY720 3 mg·kg−1·d−1 (6)</td>
<td>12, &gt;60, &gt;60, &gt;60, &gt;60, &gt;60</td>
<td>&gt;60</td>
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DBA/2 and B10.D2 mice share major histocompatibility antigen but differ in minor antigen, and nonimmunosuppressed recipients that survived >60 days demonstrate typical graft atherosclerosis in this chronic cardiac rejection model. All grafts surviving to 60 days were harvested for histopathological studies.
associated with significant suppression of neointimal proliferation and preservation of % LA. Furthermore, combination treatment with 3 mg \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1} \) of FTY720 and 40 mg \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1} \) of CsA also prevented graft atherosclerosis.

IFN-\( \gamma \) is suspected to play a central role in the initiation of a cytokine and growth factor cascade leading to the development of graft atherosclerosis.\(^{29-31} \) IFN-\( \gamma \) regulates the proliferation and function of activated T lymphocytes and plays a pivotal role in organ rejection by activating macrophages.\(^{32-34} \) It also promotes inflammation in transplanted coronary arteries\(^{31,32,35} \) and upregulates adhesion molecules, including vascular cell adhesion molecule-1 or intercellular adhesion molecule-1, in the medial smooth muscle and endothelial cells.\(^{36-38} \) Cardiac allografts to IFN-\( \gamma \)-deficient mice have been reported to show a lesser degree of graft atherosclerosis, and progression of atherosclerosis was attenuated by anti-IFN-\( \gamma \) antibody treatment.\(^{39,40} \) In our study, FTY720 caused only mild suppression of IFN-\( \gamma \) production in MLR assay; however, it did limit coronary arterial narrowing at 60 days after transplantation. FTY720 strongly suppressed the MHC antigen expression in rmlIFN-\( \gamma \)-stimulated macrophages. These results indicate that FTY720 inhibits the activation of IFN-\( \gamma \)-stimulated macrophages, which may lead to the prevention of both acute rejection and development of graft atherosclerosis.

Human cardiac transplantation is in need of less toxic immunosuppressants that are effective in both acute and chronic allograft rejection. Our results strongly suggest that FTY720 is effective in preventing both early and late graft coronary artery disease. Our study also demonstrated the effectiveness of the drug as an immunosuppressant and at least partially clarified its mechanism of action. In addition, combination treatment with subtherapeutic doses of CsA and FTY720 was effective. Combination therapy with CsA reduces the production of IL-2 and interferes with the response of IL-2 and IFN-\( \gamma \), leading to a higher long-term graft acceptance. It is our hopeful expectation that the use of FTY720 in human cardiac transplantation will be associated with favorable results.

**Acknowledgments**

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