Rapamycin Reverses Chronic Graft Vascular Disease in a Novel Cardiac Allograft Model

Robert S. Poston, MD; Margaret Billingham, MD; E. Grant Hoyt; Jeffrey Pollard, BS; Randi Shorthouse, BS; Randall E. Morris, MD; Robert C. Robbins, MD

Background—Chronic graft vascular disease (CGVD) in cardiac allografts has been defined as a slowly evolving vasculopathy unresponsive to conventional immunosuppression. We compared 4 rodent models of CGVD to evaluate the reproducibility of CGVD in heart allografts. Rapamycin (Rapa) and cyclosporine (CSA) were then used to treat CGVD.

Methods and Results—Hearts were harvested and placed heterotopically into allogenic recipients. CGVD scores of PVG allografts from ACI recipients treated with CSA on days 1 through 10 were significantly elevated on day 90 (n=16) compared with other models (immunosuppression used): (1) Lewis to F344 recipients (CSA), (2) Brown Norway to Lewis (FK506), and (3) DA to Wistar-Firth (methylprednisolone, azathioprine, CSA). Although delayed (day 60 to 90) CSA treatment had no effect (n=6), delayed Rapa (3 mg·kg⁻¹·d⁻¹ IP) reversed CGVD in PVG grafts (0.22±0.19 on day 90, n=6). ACI isografts showed no evidence of CGVD (n=6) at day 90. Immunohistochemistry of PVG grafts revealed perivascular infiltrates consisting of CD4⁺ T cells and limited numbers of macrophages persisting up to day 90. Flow cytometry demonstrated increased levels of anti-donor antibody at day 90, which was significantly inhibited by Rapa treatment.

Conclusions—PVG grafts developed a significant increase in CGVD without evidence of ongoing myocardial rejection. This CGVD appeared to be mediated by both cellular and humoral mechanisms, given CD4⁺ perivascular infiltrates and increased levels of anti-donor antibody. The anti-CGVD effectiveness of Rapa during a period in which there was little myocardial cellular infiltrate supports a novel mechanism of effect such as smooth muscle or B-cell inhibition.

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Key Words: transplantation ■ immunology ■ coronary disease ■ antibodies ■ immunohistochemistry

Chronic graft vascular disease (CGVD), a slowly evolving process thought to be secondary to chronic rejection, has been defined as the development of progressive coronary artery narrowing despite adequate immunosuppression. Many animal models have been described that report the histological findings of concentric neointimal hyperplasia of coronary arteries. However, no model that closely simulates the above definition has been established. The development of a reliable animal model is crucial for advancing our understanding of the pathogenesis and treatment of CGVD. The demonstration that rapamycin is a potent immunosuppressive that also inhibits neointimal hyperplasia in nontransplant models has further heightened the need for a well-quantified transplant model.

There are 3 reports of CGVD with coronary narrowing developing in heterotopic cardiac grafts in the rat within 90 days after transplantation. The purpose of this study was to reproduce and quantitatively compare these reports with the results seen in the PVG to ACI model. The model found to consistently give the highest level of CGVD while closely simulating the human condition would be used to evaluate the effectiveness of rapamycin at blocking this process.

Methods

Drugs

Cyclosporine (CSA; 100 mg/mL, Sandoz Pharmaceuticals) was diluted in olive oil and administered by gavage (ie, PO) or was dissolved in 100% ethanol and administered intramuscularly (IM). FK506 (gift of Fujisawa USA) was supplied as pure drug powder, dissolved in 100% ethanol, and administered via intraperitoneal (IP) injection until the animal was euthanized. Methylprednisolone (The Upjohn Co) was supplied as a sodium succinate powder, dissolved in deionized water, and administered PO. Azathioprine (Burroughs Wellcome Co) was supplied as a lyophilized sodium salt, dissolved in deionized water, and administered PO until euthanasia. Rapamycin (gift of S.N. Sehgal, Wyeth-Ayerst, Hanover, NJ) was supplied in pure form and stored at 4°C. It was suspended in 0.2% carboxymethyl cellulose and administered IP.
Animals
Adult male (8 to 10 weeks old, 230 to 270 g) Lewis (RT1⁺), Fischer (RT1⁺), Brown Norway (BN, RT1⁻), DA (RT1⁺), Wistar-Firth (WF, RT1⁻), PVG (RT1⁺), and ACI (RT1⁺) rats were obtained from Harlan Sprague-Dawley, Indianapolis, Ind. All animals were maintained in the animal care facilities at Stanford University Medical Center at 21±2°C with a time-regulated light period and were provided water and dry food ad libitum. Periodic serological analysis of room sentinel animals showed that all rats were free of acute viral infection. All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals, prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

Heart Transplantation
Both donor and recipient rats were anesthetized with methoxyflurane (inhalational) and sodium pentobarbital (50 mg/kg IP). Hearts were harvested and placed heterotopically into the abdomens of allogenic recipients by the methods described by Ono and Lindsay. After reperfusion and successful return of cardiac contractions, recipients were weighed daily, and graft function was monitored by abdominal palpation and scored in a range from 0 (no contractions) to 4 (vigorous contractions). Hearts were considered acutely rejected when palpation scores were <1. Subacute rejection was diagnosed by the presence of palpation scores ≥2 with histological confirmation of massive inflammatory infiltrates.

Immunosuppression
Postoperative treatment was administered exactly as reported in each publication and varied for each model as follows: (1) Lewis hearts to F344 recipients with CSA 2.5 mg·kg⁻¹·d⁻¹ PO (n=6) or IM (n=6) or 5.0 mg·kg⁻¹·d⁻¹ IM (n=6) for 10 days postoperatively, (2) BN to Lewis with 1 mg·kg⁻¹·d⁻¹ FK506 IP (n=6) until euthanization, (3) DA to WF with methylprednisolone 0.5 mg·kg⁻¹·d⁻¹ PO, azathioprine 2 mg·kg⁻¹·d⁻¹ PO, and CSA 5 mg·kg⁻¹·d⁻¹ PO (n=8) until euthanization, (4) PVG to ACI with CSA 10 mg·kg⁻¹·d⁻¹ PO (n=24) for 10 days, and (5) ACI to ACI recipients (isograft control). After preliminary results had established that the PVG to ACI model displayed the highest level of CGVD with minimal evidence of acute rejection, this model was used to assess the anti-CGVD effects of rapamycin. After an initial 10-day course of CSA to promote long-term PVG allograft survival, ACI recipients were retreated between postoperative days (POD) 60 and 90 with 1 of 3 regimens: CSA vehicle (olive oil) alone, CSA 10 mg·kg⁻¹·d⁻¹ PO, or rapamycin 3 mg·kg⁻¹·d⁻¹ IP, and euthanized on day 90 for graft analysis.

Evaluation of CGVD
Grafts were removed for histological analysis of CGVD on days 30, 60, and 90. After harvest, grafts were sectioned perpendicular to the long axis of the heart and fixed in buffered formalin for 24 hours. Thin hematoxylin and eosin (H-E)–stained sections of paraffin-embedded samples were examined by a pathologist (M.B.) blinded as to experimental group and were assigned a CGVD score. This score was the mean score for all the individual vessels in a section and therefore represented the fact that normal and occluded vessels were often found in the same sections (ie, displayed large SDs). Individual vessels were subjected to a 5-point grading scale from 0 to 4 (0 for no involvement, 1 for partial intimal involvement, 2 for concentric intimal thickening, 3 for more severe concentric involvement up to 50% luminal narrowing, and 4 for >50% up to complete occlusion) (Figure 1).

Immunohistochemistry
PVG grafts were procured at 30 and 60 days and, after treatment with delayed CSA or rapamycin, at 90 days (n=3 for each group) for sectioning and immunohistochemical analysis. Sections were embedded in OCT compound (Miles), snap-frozen in liquid N₂, and stored at −80°C. After samples were brought to −20°C, 6-μm thin sections were placed onto poly-L-lysine–precoated slides (Sigma-Diagnostics) and stained for CD4, CD8, macrophage, and major histocompatibility complex (MHC) class I and class II antibodies by the avidin-biotin–complex method outlined in the Histostain SP immunohistochemistry kit (Zymed Laboratories). Briefly, sections were air-dried at room temperature, fixed in −20°C acetone, rehydrated in 1% BSA-PBS, and incubated with 1 of the following primary antibodies (obtained from Serotec) for 45 minutes: W3/25 (MHC class I), and 46 (MHC class II). This was followed by incubation with a biotinylated goat anti-mouse IgG (Zymed Laboratories) for 15 minutes. The avidin-biotin complex was applied, and diamobenzidine tetrahydrochloride was used as the chromogen. The substitution of 1% BSA-PBS for the primary antibody served as the negative (reagent) control. Rat cervical lymph nodes and cardiac allografts at day 3 in untreated recipients served as the positive control. Sections were scored on a 3-point scale (0, +, and ++ ) for CD4, CD8, macrophage, B cell, and MHC class I and II staining intensity by a pathologist (M.B.) blinded to experimental group. Immediately adjacent myocardial sections were taken for H&E staining to facilitate identification of vessels with CGVD (score >2). By comparing immunohistochemistry with H&E sections, the relative risk of vasculopathy (score ≥2) for any given vessel staining positive.
 aliquots in plastic tubes at a concentration of 10^6 cells/mL. Cells released were washed 3 times in PBS solution and divided into

- Compression of thymus tissue between glass slides. The thymocytes were then incubated with test sera for 30 minutes at 4°C and washed

- Serum. Lymphocytes were harvested from PVG rats by gentle

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- Cells from all

- Groups were washed twice with fluorescence-activated cell sorter

- Laboratories) at 4°C for 30 minutes. Autofluorescence was ac-

- Mouse anti-rat IgG alloantibodies were

- Were then incubated with test sera for 30 minutes at 4°C and washed

- Cells were harvested from PVG rats by gentle compression of thymus tissue between glass slides. The thymocytes released were washed 3 times in PBS solution and divided into aliquots in plastic tubes at a concentration of 10^6 cells/mL. Cells then incubated with test sera for 30 minutes at 4°C and washed with PBS through a calf-serum cushion. IgG alloantibodies were stained by incubation of the cells with PBS solution containing fluorescein isothiocyanate–conjugated goat antibodies specific for the Fc portion of rat IgG (dilution 1:100) (Jackson Immunoresearch Laboratories) at 4°C for 30 minutes. Autofluorescence was accounted for by analyzing cells without added stains. Cells from all groups were washed twice with fluorescence-activated cell sorter (FACS) buffer and then analyzed on FACSscan (Becton-Dickinson). Cells (8 x 10^5) were acquired by list mode and gated by forward light scatter versus side light scatter, which excluded dead cells and debris. Fluorescence data were collected by use of logarithmic amplification and expressed as median channel fluorescence intensity (rather than mean) because of the nonsymmetrical nature of the data.

- **Statistical Analysis**

  CGVD scores for each group were calculated from the average score (0 to 4) for all vessels in the worst scoring section of an individual

  graft (sections taken from superior, middle, and inferior portions of each graft). Parametric data (mean ischemic times, mean of median channel fluorescence) were compared between multiple groups by ANOVA with post hoc Dunnett’s test. Nonparametric data (weighted mean CGVD scores, mean survival times) were compared by use of the Mann-Whitney U test. The Fischer exact test was used for comparison of the incidence of acute and subacute rejection between groups and for determining the relative risk of a perivascular CD4 infiltrate resulting in significant vasculopathy (score ≥2) for any given vessel. Significance was assigned to values of $P<0.05$. Data were expressed in all cases as mean±SD. All calculations were made with the statistical program InStat for MacIntosh, version 2.0 (GraphPad Software).

- **Results**

  All groups

  Total ischemic times (from cardioplegic arrest of the donor heart to return of contractions in the recipient’s abdomen) ranged from 17 to 26 minutes, with no statistically significant differences in the mean ischemic times between groups ($P=NS$, ANOVA) (Tables 1 and 2). No recipient death occurred after any of the protocols. But whereas recipients of other groups gained weight over the observation period, weight loss was seen in WF recipients receiving triple therapy (azathioprine, methylprednisolone, CSA) and ACI recipients in the delayed rapamycin group ($-0.35±0.09$ and $-0.43±0.12$ g/d, respectively).

- **Histology in Strong-Responder CGVD Models**

  In the models using strong-responder recipients (ie, Fischer, Lewis, WF), acute rejection (especially acute vascular rejec-

### TABLE 1. Models of CGVD

<table>
<thead>
<tr>
<th>Model</th>
<th>Treatment</th>
<th>POD</th>
<th>Ischemic Times, min</th>
<th>n</th>
<th>CGVD Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVG→ACI</td>
<td>CSA 10 mg·kg⁻¹·d⁻¹·P0×10 d</td>
<td>30</td>
<td>22.6±1.9</td>
<td>6</td>
<td>0.96±1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>22.2±1.6</td>
<td>8</td>
<td>0.88±1.12</td>
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<tr>
<td></td>
<td></td>
<td>90</td>
<td>21.7±2.3</td>
<td>12</td>
<td>1.39±0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
<td>22.1±1.9</td>
<td>5</td>
<td>0.96±1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>22.2±1.6</td>
<td>8</td>
<td>0.88±1.12</td>
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<td>12</td>
<td>1.39±0.68</td>
</tr>
</tbody>
</table>

*MP indicates methylprednisolone.

†Rapamycin 3 mg

‡P<0.05 vs delayed CSA and positive control.

### TABLE 2. Effect of Delayed (Day 60–90) Immunosuppression

<table>
<thead>
<tr>
<th>Group</th>
<th>Recipient Treatment</th>
<th>Harvest Date</th>
<th>n</th>
<th>CGVD Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACI isograft</td>
<td>None</td>
<td>90</td>
<td>6</td>
<td>0.10±0.09</td>
</tr>
<tr>
<td>PVG allograft</td>
<td>Continuous CSA day 1–90 (negative control)</td>
<td>90</td>
<td>6</td>
<td>0.23±0.38</td>
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<tr>
<td>PVG allograft</td>
<td>Initial CSA (day 1–10)</td>
<td>90</td>
<td>12</td>
<td>1.39±0.68</td>
</tr>
<tr>
<td></td>
<td>+ vehicle day 60–90 (positive control)</td>
<td>90</td>
<td>6</td>
<td>1.15±0.64</td>
</tr>
<tr>
<td></td>
<td>+ CSA* day 60–90</td>
<td>90</td>
<td>6</td>
<td>1.15±0.64</td>
</tr>
<tr>
<td></td>
<td>+ Rapamycin 3 mg·kg⁻¹·d⁻¹·P0</td>
<td>90</td>
<td>6</td>
<td>0.22±0.19†</td>
</tr>
</tbody>
</table>

*CSA 10 mg·kg⁻¹·d⁻¹·P0.

†Rapamycin 3 mg·kg⁻¹·d⁻¹·P0.
tion) appeared to be a major confounding factor. Compared with other groups, Lewis grafts in Fischer recipients demonstrated a significantly higher incidence ($P<0.05$, Fischer exact test) of acute and subacute rejection (ie, weakened beat with inflammatory infiltrates and fibrosis). In our hands, all Lewis grafts from the low-dose CSA group (2.5 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$ IM or PO $\times$ 10 days) demonstrated such significant inflammatory infiltrates and vascular rejection that our pathologist (M.B.) could not assign a CGVD score to any of the sections. Histology revealed extensive vasculopathy in a majority of vessels, but only in association with extensive myocyte necrosis and inflammatory infiltrate (Figure 1A). Grafts harvested on POD 60 from recipients receiving higher immunosuppression (CSA 5 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$ IM $\times$ 10 days) showed a reversal of this vascular disease (CGVD score, 0.21±0.74, n=6) (Figure 1B). Similarly, despite the use of protocols identical to that reported,8,9 WF to DA and Lewis to BN models showed only minimal inflammation and vasculopathy with CGVD scores of 0.31±0.17 (n=8) and 0.49±0.41 (n=6), respectively (Table 1). Only a single graft in either group demonstrated examples of vessels scoring $\geq$2. Of note, this particular graft had functional and histological evidence of subacute rejection.

Histology in the PVG to ACI Model

Although PVG and ACI rats are mismatched across major MHC antigens, ACI rats have been shown to be “weak responders” to allogenic stimuli.12 As a result, indefinite recipient immunosuppression was not necessary to avoid acute or subacute rejection. After a short course of CSA (10 mg/kg PO $\times$ 10 days), acute rejection was seen in only 3 allografts, with the remaining grafts (41 of 44, 93.2%) demonstrating long-term survival (palpation scores $\geq$3 on day 60). Histological analysis of these grafts on POD 90 demonstrated the full range of vasculopathy (Figure 2), with higher mean CGVD scores than the other groups (Table 1). Between PODs 60 and 90, daily CSA administration did not significantly affect CGVD scores compared with the vehicle control group (1.15±0.64, n=5 versus 1.39±0.68, n=12, respectively). Conversely, rapamycin treatment resulted in a significant reversal in CGVD (0.22±0.19, n=6, $P<0.05$ versus the other 2 groups, Mann-Whitney $U$ test) (Table 2). Daily CSA immunosuppression from POD 0 until euthanasia on POD 90 prevented graft inflammation and subsequent vasculopathy (CGVD score, 0.23±0.38, n=6).

Immunohistochemistry in PVG to ACI

Immunohistochemical examination revealed almost no CD4 or CD8 cells or macrophages, with low baseline expression of MHC class I and II antigens in native PVG hearts at baseline. On POD 30 after transplantation into ACI recipients, the expression of all 5 antigens was dramatically increased. Although CD8 and MHC class I and II levels returned to baseline on day 90, most of the PVG allografts showed a decreased but persistent CD4 and macrophage infiltrate, primarily in a perivascular distribution (Figure 3). The presence of a CD4-positive perivascular infiltrate around any given artery, as seen around 48% of the graft vessels (37 of 76 total vessels, n=17), was significantly associated with a CGVD score $\geq$2 ($P<0.02$, Fischer’s exact test) and provided for a relative risk of vasculopathy of 2.11 (95% CI, 1.3 to 3.8). However, although delayed rapamycin (POD 60 to 90) reversed CGVD, it appeared to have no impact on these low-level, persistent perivascular infiltrates (11 of 24 vessels, 46%, n=6).

Donor-Specific IgG Titters in PVG to ACI

Serum from naive ACI rats showed no preformed anti-RT1$^c$ antibodies in excess of nonspecific binding (median channel fluorescence [MCF], 57.5±4.3; 95% CI, 52.2 to 62.8). In
addition, isograft recipients and allograft recipients treated with continuous (day 1 to 90) CSA (negative control) also showed no increase in titers (58.8 ± 5.2; 95% CI, 52.1 to 63.9 and 59.9 ± 7.2; 95% CI, 51.3 to 66.2, respectively, n=5 each). However, anti-RT1<sup>c</sup> antibody titers were found to be significantly elevated allograft recipients treated with a short course of CSA (positive control) 30 days after allograft transplantation (MCF, 80.9 ± 10.8; 95% CI, 67.5 to 94.3, n=5, P<0.05 versus naive ACI at day 1, ANOVA with post hoc Dunnett’s test). These anti-donor antibody titers decreased but remained persistently elevated at days 60 and 90 (Figure 4A). Delayed rapamycin (day 60 to 90) resulted in a strong inhibition of the anti-donor antibody response seen on day 90 compared with the vehicle control group (MCF, 56.1 ± 4.7; 95% CI, 50.3 to 61.9, n=5 versus 64.4 ± 8.5; 95% CI, 60.0 to 68.7, n=17, P<0.05, ANOVA with post hoc Dunnett’s test). Conversely, delayed CSA treatment during this time period did not affect the anti-donor antibody response (MCF, 62.0 ± 9.9; 95% CI, 49.7 to 74.3, n=5), as shown in the graph and by the representative flow cytometry plots (Figure 4B).

**Discussion**

CGVD is a process that limits the success of solid-organ transplantation. After the first year after cardiac transplantation, intimal thickening has been demonstrated by intravascular coronary ultrasound in up to 75% of patients, and CGVD becomes the leading cause of death in these patients. The effect of currently used immunosuppressants on the incidence of CGVD is controversial. Both clinical and experimental studies have suggested that effective immunosuppression with cyclosporine, the main agent in use in transplant recipients today, has minimal impact on the eventual development of graft vasculopathy. Conversely, second-generation immunosuppressives, such as rapamycin, have been shown to inhibit smooth muscle cell growth and neointimal hyperplasia, indicating a new CGVD prevention strategy.

Little has been established regarding either the pathophysiology of human CGVD or the criteria for an animal model of this process. Because clinical data suggest a multifactorial pathogenesis, we feel that an appropriate model should demonstrate vasculopathy in cardiac allografts in which ongoing T-cell–mediated alloimmunity in the form of acute or subacute rejection is not the sole or major pathogenesis. In other words, the development of vasculopathy should be relatively resistant to standard T-cell–directed immunosuppressants such as CSA. The anti-CGVD potential of novel agents like rapamycin can be analyzed only after the development of such a clinically relevant model.

The most commonly reported model of CGVD in the literature involves cardiac transplantation from Lewis to Fischer strain rats. In our hands, transplantation across this minor histocompatibility mismatch (RT3 and possibly others) with postoperative doses of cyclosporine as reported resulted in an unacceptable incidence of acute Lewis allograft rejection. Although the surviving allografts demonstrated the classic lesions of neointimal hyperplasia (ie, chaotic organization of spindle-shaped cells inside the internal elastic laminae of multiple vessels), it was associated with a weakened cardiac beat and massive inflammatory infiltrates and fibrosis suggesting a more indolent form of acute rejection, or subacute rejection. The prevention of these lesions by high-dose CSA (5 mg·kg<sup>−1</sup>·d<sup>−1</sup>) confirmed acute rejection as the main underlying pathogenesis. Although these findings agree with those of Handa et al, we did not conclude that optimal CSA dosing plays a role in preventing human CGVD. Instead, vasculopathy in this model is probably a reflection of the amount of acute rejection suppression. This strict dependence on acute rejection reveals the limitations of this model for drawing conclusions about the human condition. Similarly, immunosuppression pharmacodynamics in high-
responder Lewis and WF recipients appeared to be the sole factor responsible for the development of CGVD in major MHC-mismatched BN and DA grafts, which limited the clinical relevance of these models as well. One of 2 outcomes was seen in the surviving grafts: impressive coronary occlusive lesions in the setting of subacute rejection or normal coronary arteries with minimal to no inflammation. Although acute rejection episodes are a risk factor for the future development of chronic rejection, the high rates of graft attrition from CGVD have not been significantly affected by the increasing success of clinicians in suppressing early acute rejection, as seen with these models.

The PVG to ACI model, by using low-responder recipients that require only a short initial course of CSA to allow long-term allograft survival, appears to circumvent the strict influence of acute rejection. This protocol allows for prolonged exposure of PVG allografts to longer-term injuries, including more indolent cell-mediated and humoral immune rejection. Figure 4. A, Anti-PVG antibody titers (IgG) in ACI allograft recipients treated with initial 10-day course of CSA and no other postoperative therapy (positive control) were significantly elevated vs serum from naive ACI rats, isograft recipients, and allograft recipients treated with continuous, daily CSA (negative control) at all time points of evaluation (P<0.05, n=5 at each point, ANOVA with post hoc Dunnett’s test). Between days 60 and 90, an additional course of immunosuppression using CSA (10 mg · kg⁻¹ · d⁻¹ PO, n=5) had no effect, whereas use of rapamycin (Rapa) (3 mg · kg⁻¹ · d⁻¹ IP, n=5) significantly decreased anti-donor antibody titers (*P<0.05 vs POD 90 Control). B, Representative flow cytometry plots demonstrating a shift in histogram obtained from serum of delayed CSA treatment group compared with plot from naive rat serum indicating presence of anti-donor antibodies. Conversely, rapamycin eliminated response with titers similar to those seen in naive rats.
moral immune responses, unhindered by the requirement of ongoing immunosuppression after day 10. Between days 60 and 90, the PVG allografts showed increasing CGVD scores in the setting of decreasing graft inflammation, which was neither prevented nor reversed by the daily administration of CSA during this period. Interestingly, this "point of no return" was also seen in a study by Forbes et al. in which allografts retransplanted back into isogenic recipients failed to reverse CGVD changes after day 60, although they had shown improvement in vasculopathy when retransplanted at earlier times. The persistence of both low-level CD4+ perivascular infiltrates in the PVG grafts and anti-RT1<sup>e</sup> antibodies in the ACI recipients implicates both humoral and cellular immunity, consistent with a multifactorial pathogenesis. Conversely, the prevention of CGVD after daily CSA treatment (days 1 to 90) illustrates that even in this model, CGVD and acute rejection are not mutually exclusive. Therefore, the effects of rapamycin were assessed between days 60 and 90 after an initial short course of CSA to avoid the confounding actions of the acute rejection response.

Compared with an equipotent dose of CSA, daily rapamycin between days 60 and 90 led to a significant reduction in CGVD. Delayed rapamycin treatment did not appear to affect the already decreasing level of overall graft inflammation or to alter the persistent CD4+ perivascular infiltrates. These data support a mechanism of action for rapamycin that is not explained solely by a reduction in T-cell–mediated immunity. Rapamycin is thought to inhibit the final common pathway of CGVD pathophysiology, smooth muscle cell growth. In addition to direct inhibitory effects on smooth muscle cells, rapamycin fails to prevent inducible nitric oxide synthetase upregulation as seen with standard immunosuppressants. Such an effect is hypothesized to result in a high local production of nitric oxide, a potent smooth muscle cell growth inhibitor.

Finally, anti-HLA (ie, RT1 in the rat) antibodies have been shown to correlate clinically and experimentally with the classic findings of CGVD. The small but statistically significant inhibition of anti-RT1<sup>e</sup> antibody formation in rapamycin-treated ACI recipients compared with vehicle controls implies an additional point of action for this drug. Although it is difficult to know the true clinical significance given the small shift in MCF seen, the data of Russell et al. suggest that only very low levels of anti-HLA antibodies were required by passive transfer to antibody-deficient recipients to restore the ability to produce classic CGVD lesions. In addition, antibodies to non-HLA graft endothelial cell antigens that are exposed to the recipient immune system after injury (eg, vimentin) have also been found to correlate to the development of CGVD. For technical reasons, we assessed only anti-HLA antibodies in these studies. More work needs to be done investigating the molecular and cellular mechanisms of rapamycin in this CGVD model. We present a rodent model of CGVD that more closely simulates the human condition in that PVG allografts in ACI recipients display a progressive increase in CGVD scores without evidence of ongoing myocardial rejection. The effectiveness of delayed rapamycin administration during a period in which there was little myocardial cellular infiltrate supports the idea that this drug affects CGVD by novel mechanisms, such as smooth muscle or B-cell inhibition. Further investigations using this model should provide insight into the pathophysiology of human CGVD and the effectiveness of other novel methods of treating this currently intractable condition.

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References


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