Anti–Transforming Growth Factor Antibody at Low but Not High Doses Limits Cyclosporine-Mediated Nephrotoxicity Without Altering Rat Cardiac Allograft Survival

Potential of Therapeutic Applications

Ashwani K. Khanna, PhD; Matthew S. Plummer, BS; Gail Hilton, BS; Galen M. Pieper, PhD; Steven Ledbetter, PhD

Background—Long-term treatment of cardiac transplant recipients with cyclosporine results in a progressive decline in kidney function in a large number of patients. This complication is one of the most important prognostic parameters that determine the outcome of cardiac transplantation. Transforming growth factor-β (TGF-β) is one of the most potent mediators of the fibrogenic effects of cyclosporine.

Methods and Results—With the use of an experimental rodent model, heterotopic heart transplantation was performed, creating histocompatibility-disparate allografts. Because TGF-β in part mediates both the immunosuppressive and nephrotoxic effects of cyclosporine, recipients were treated with cyclosporine with and without anti–TGF-β antibody to determine whether anti–TGF-β antibody could reduce the nephrotoxic effects of cyclosporine. Intrarenal expression of TGF-β, collagen, fibronectin, matrix metalloproteinase-2, and tissue inhibitor of metalloproteinase-2 was studied with the use of reverse transcription–polymerase chain reaction. Intrarenal expression of TGF-β protein was studied by immunohistochemistry and with the use of ELISA to quantify circulating levels of TGF-β protein in plasma. Cyclosporine-induced graft survival (immunosuppressive effect) was abrogated with a higher concentration (2.5 mg/kg) of anti–TGF-β antibody, whereas a lower concentration (1 mg/kg) inhibited both cyclosporine-induced expression of fibrogenic molecules and renal toxicity.

Conclusions—These results provide credence to the pivotal role of TGF-β in immunosuppression-associated renal toxicity in recipients of cardiac transplantation. Furthermore, these findings support a potentially significant therapeutic use of optimal concentration of anti–TGF-β antibody to ameliorate cyclosporine-associated nephrotoxicity in cardiac transplant recipients. (Circulation. 2004;110:3822-3829.)

Key Words: transplantation ■ coronary disease ■ kidney ■ transforming growth factors

The occurrence of chronic renal failure after cardiac transplantation is a major risk factor, and the 5-year risk is ≈7%.1 A number of clinical studies2–9 suggest that one of the common clinical complications after heart transplantation is chronic cyclosporine-induced nephrotoxicity, which generally leads to progressive renal failure.10 Van Gelder et al11 demonstrated that a significant rise in serum creatinine was found in cases compared with controls as early as 3 months after transplantation. Cyclosporine remains the central immunosuppressive agent for cardiac allograft recipients, even though its use leads to a greater than one-third decrease in creatinine clearance by 6 months after transplantation with progression to end-stage renal failure, requiring hemodialysis in 6.5% of cardiac transplant recipients. End-stage kidney disease may develop in 1% to 3% of cyclosporine-treated heart transplant recipients, and most patients show a decreased glomerular filtration rate.12 Recently, English et al13 compared the effect of tacrolimus and cyclosporine on nephrotoxic effects in pediatric cardiac transplant recipients and observed similar nephotoxicity of both immunosuppressants. These findings demonstrate the need for a better strategy utilizing alternative or adjunctive agents that would allow decreased dosing as a means to achieve reduced nephrotoxicity of cyclosporine and other immunosuppressive agents while maintaining equivalent rates of graft survival.

We performed studies in an established rat cardiac transplantation model to define the causal mechanism behind cyclosporine-mediated nephrotoxicity.14 The results demon-
strated a significant increase in intrarenal expression of transforming growth factor-β (TGF-β) and profibrogenic genes in cardiac transplant recipients treated with cyclosporine for 120 days compared with isograft controls. Earlier, in a nontransplantation experimental model, we demonstrated that TGF-β mediates at least some of the immunosuppression of cyclosporine and is nephrotoxic itself.\textsuperscript{5,6} In this study we explored the effects of anti–TGF-β antibody on immunosuppression (graft survival) and nephrotoxicity after long-term treatment with cyclosporine in a rat heart transplantation model. This experimental model employs completely mismatched strains, and the allograft survival requires treatment with cyclosporine or other immunosuppressant therapy. Recipients were treated with cyclosporine alone or along with different concentrations of either anti–TGF-β antibody or control antibody to study their effects on the intrarenal expression of TGF-β, collagen, fibronectin, matrix metalloproteinase-2 (MMP-2), and tissue inhibitor of metalloproteinase-2 (TIMP-2). We also studied intrarenal expression of TGF-β protein and circulating levels of plasma TGF-β protein. Samples of renal tissue and plasma from rats treated with cyclosporine alone or with either anti–TGF-β antibody or control antibody therapy were compared with similar samples from animals receiving isografts with no immunosuppression therapy. We also compared the renal histopathological changes in these groups.

**Methods**

**Experimental Groups, Preparation, and Transplantation**

This study was divided into 6 groups (n=6 in each) including the following: untreated control; cyclosporine; cyclosporine plus 2.5 mg/kg control antibody; cyclosporine plus 2.5 mg/kg anti–TGF-β antibody; cyclosporine plus 1.0 mg/kg anti–TGF-β antibody; and isograft controls. Heterotopic heart transplantsations were performed on the entire study population with the use of rat strains Wistar Furth isograft controls. Heterotopic heart transplantations were performed with cyclosporine or other immunosuppressant therapy. We also compared the renal histopathological changes in these groups.

**Immunosuppression Protocols**

Immunosuppression was accomplished with the use of cyclosporine in the appropriate groups. Cyclosporine was used at a dosage of 2.5 mg/kg IM for the duration of the experiment with and without anti–TGF-β or control antibody. Treatment of anti–TGF-β antibody or control antibody was administered by intraperitoneal injection every third day until the time of euthanasia or organ rejection.

1D11 is a murine monoclonal antibody (IgG1) that specifically neutralizes the biological activity of TGF-β1, TGF-β2, and TGF-β3. The antibody recognizes active and not latent TGF-β. The control antibody (13C4) is also a murine monoclonal antibody (IgG1) that specifically binds shigella toxin. Both antibodies were produced and purified at Genzyme Corporation. Antibodies were determined to be free of detectable endotoxin.

**Measurement of TGF-β Protein in Rat Plasma**

TGF-β protein was measured as previously described.\textsuperscript{18} Plasma from rats was separated and stored at −70°C. TGF-β1 protein was assayed on acid-activated samples by a sandwich ELISA with the use of a commercial TGF-β1-specific kit (Promega).

**Histopathology and Immunohistochemistry for TGF-β1 Protein Expression in Kidney Tissues**

One kidney from each animal was fixed in formalin and paraffin imbedded. We used hematoxylin-eosin and Masson’s trichrome staining to assess histological changes. TGF-β1 was identified with the use of immunohistochemistry.\textsuperscript{14} Formalin-fixed, paraffin-embedded tissues were sectioned at 5 μm, deparaffinized in xylene, and rehydrated in graded ethanol to PBS. After endogenous peroxidase activity was blocked with methanol/peroxide (18:1 vol/vol) for 30 minutes, nonspecific binding was blocked for 1 hour with 1.5% avidin/biotin diluted in PBS supplemented with 10% normal horse serum and 3% BSA. The tissue sections were then incubated overnight at 4°C with 1D11 monoclonal antibody (50 mg/mL) in the aforementioned PBS. After multiple washings with PBS, the slides were incubated for 1 hour with 1:1000 diluted biotin-labeled anti-mouse IgG horse anti-serum at room temperature, then washed again extensively in PBS and then in the ABC solution for 30 minutes. The slides were then developed for 10 minutes in diaminobenzidine and rinsed with water for 10 minutes. The slides were then counter-stained with hematoxylin followed by dehydration in graded ethanol and xylene. The slides were mounted with Permount for evaluation. Samples from each group were graded for histopathological changes and immunohistochemistry staining. The intensity of immunostaining was graded from 0 (no staining) to +4 (maximum staining).

**Detection of mRNA by Reverse Transcription and Polymerase Chain Reaction in Kidney Tissues**

Total RNA was isolated from a small piece of kidney tissue from rats with the use of SV Total RNA Isolation System (Promega), and quality of RNA was verified by 260/280-nm ratio. Then 1 μg of RNA was reverse transcribed to cDNA with the use of Superscript First Strand Synthesis System for reverse transcription–polymerase chain reaction (RT-PCR) (Life Technologies). The amplification by PCR was performed with the use of 1 μL of cDNA and 2 μL each of 2.5 mmol/L coding and noncoding oligonucleotide primers and Platinum PCR Supermix (Life Technologies). The primer sequences were as follows: TGF-β1: coding 5′-ACC CCT GAG CTG TCT TAC TGT TTG-3′ and noncoding 5′-ACG TGG AGT TTA TCT TGT-3′; β-actin: coding 5′-GAT CTG CCA CAC CCT CTT CTA-3′ and noncoding 5′-GCA GGA TGT CGT GAG GGA GAG-3′; collagen I: coding 5′-ACC AGG TAG TCC TAA CGG AGT-3′ and noncoding 5′-CAC ATG TGT GTC TTT TCT-3′; fibronectin: coding 5′-ATG ATG AGG TGC ACG TGT CTG-3′ and noncoding 5′-TGAT GGC TCA TTA TCT TGC-3′; collagen I: coding 5′-ACC AGG TAG TCC TAA CGG AGT-3′ and noncoding 5′-GAG TAG TGG TCA AAG TTT-3′; fibronectin: coding 5′-ATG ATG AGG TGC ACG TGT CTG-3′ and noncoding 5′-TGAT GGC TCA TTA TCT TGC-3′; β-actin: coding 5′-GAT CTG CCA CAC CCT CTT CTA-3′ and noncoding 5′-GCA GGA TGT CGT GAG GGA GAG-3′; and noncoding 5′-ACC AGG TAG TCC TAA CGG AGT-3′ and noncoding 5′-GAG TAG TGG TCA AAG TTT-3′.
each primer pair to determine the cycle number for optimal amplification. The PCR products were resolved in 1% agarose gel electrophoresis. Ethidium bromide–stained specific bands were visualized under UV light and photographed. The densitometric analysis of the specific bands was made with the use of Alpha-Imager (Alpha Innotech Corp), and data are represented as the ratio of the specific gene to β-actin.

Data Analysis
Data are expressed as mean±SEM. Differences between appropriate groups were then analyzed by ANOVA.

Results
Effect of Anti–TGF-β Antibody on Immunosuppressive Effects of Cyclosporine
The rat heterotopic heart transplantation model employing the completely mismatched strain combination Wistar Furth (WF) (RTI') into Lewis (LEW) (RTI), which was used in these studies, requires chronic immunosuppression to prevent rejection. We administered a TGF-β–neutralizing antibody or an isotype-matched control antibody to determine whether TGF-β expression affects allograft survival in cyclosporine-treated animals within the first 30 days.

Grafts from untreated animals were rejected on average 11 days after transplantation (n=6; 11±1.5 days). In contrast, grafts from cyclosporine-treated animals survived for an average of 117 days (n=6; 117±9 days; P<0.001). To better understand the role of TGF-β in the pathogenesis of chronic rejection, heart transplant recipients were dosed with 1.0 or 2.5 mg/kg of anti–TGF-β antibody 3 times weekly beginning at 3 days after transplantation. Control antibody was administered at an identical concentration and dosing schedule. No differences in survival were observed between the groups with untreated allografts and cyclosporine plus 2.5 mg/kg anti–TGF-β antibody (Figure 1). Animals in the cyclosporine plus 2.5 mg/kg control antibody group did not experience rejection but were euthanized on similar days as animals treated with cyclosporine plus anti–TGF-β antibody to compare histological changes (not shown).

In sharp contrast, the animals that were injected with 1.0 mg/kg of antibody did not experience rejection in the same manner as animals that received cyclosporine plus 2.5 mg/kg of anti–TGF-β antibody, and the mean survival time was 99±8 days. This was highly significant compared with the higher-dose antibody group (P<0.01). The data strongly support our hypothesis that the immunosuppressive effects of cyclosporine are mediated by TGF-β because anti–TGF-β at a concentration of 2.5 mg/kg totally abolished the efficacy of cyclosporine. We also hypothesized that rats injected with a lower dose of anti–TGF-β antibody could have less severe renal pathological changes because the fibrogenic properties of TGF-β would be neutralized by anti–TGF-β antibody, and the current data support this. We have analyzed the mRNA expression of different profibrotic genes in renal tissues and the histopathological analysis of renal tissues from recipients treated with cyclosporine, cyclosporine plus control antibody, and cyclosporine plus anti–TGF-β antibody.

Effect of Anti–TGF-β Antibody on Nephrotoxic Effects of Cyclosporine
These experiments were performed to study the role of TGF-β in nephrotoxic effects of cyclosporine. Along with cyclosporine, recipients were also treated with TGF-β or control antibody (2.5 or 1.0 mg/kg for each) 3 times per week. Groups included isografts, untreated allografts, cyclosporine-treated allografts, cyclosporine plus control antibody (2.5 mg/kg), cyclosporine plus anti–TGF-β antibody (2.5 mg/kg), cyclosporine plus control antibody (1.0 mg/kg), cyclosporine plus anti–TGF-β antibody (1.0 mg/kg), and group F isotype controls. Anti–TGF-β antibody at 2.5 mg/kg but not 1.0 mg/kg abrogated the immunosuppressive effects of cyclosporine. Cyclosporine plus control antibody–treated recipients did not experience rejection but were euthanized for comparison with anti–TGF-β antibody–treated recipients.

Renal Function
Renal function was measured by quantifying creatinine and blood urea nitrogen (BUN) levels in plasma of rats obtained after euthanasia from all experimental animals studied. Quantification was performed with the use of kits from Sigma. BUN levels were elevated in the cyclosporine-treated animals versus isografts (18.6±0.85 versus 12.6±0.4 mg/dL; P<0.0001), and decreased levels were observed in cyclosporine plus anti–TGF-β antibody–treated animals (13.8±0.63 mg/dL; P<0.03) but not with cyclosporine plus control antibody–treated animals (17.8±0.45 mg/dL). A significant increase in serum creatinine (0.75±0.03 versus 0.43±0.2 mg/dL; P<0.0001) was observed in cyclosporine-treated recipients compared with isografts. Similar to BUN levels, animals treated with cyclosporine plus anti–TGF-β antibody (0.49±0.06 mg/dL; P<0.003) but not control antibody (0.68±0.08 mg/dL) exhibited decreased creatinine levels.

Intrarenal Expression of TGF-β, Collagen, and Fibronectin mRNA
The results in Figure 2, expressed as gene/β-actin ratio (n=6 each), demonstrate the effect of long-term treatment of cyclosporine with and without anti–TGF-β or control anti-
long-term treatment of cyclosporine resulted in increased intrarenal expression of collagen mRNA (P<0.0001) compared with isografts. A statistically significant decrease of intrarenal expression of TGF-β, collagen, and fibronectin was seen in cyclosporine plus anti-TGF-β antibody–treated compared with cyclosporine-treated animals, whereas no difference with cyclosporine plus control (C) antibody–treated recipients was seen. TGF-β: *P<0.02, **P<0.003. Collagen: #P<0.0001, ##P<0.0008. Fibronectin: #P<0.0001, ###P<0.0002.

**Intrarenal Expression of MMP-2 and TIMP-2**

The results shown in Figure 3 demonstrate that long-term treatment with cyclosporine resulted in increased intrarenal expression of collagen mRNA (P<0.0001) compared with isografts. There was no difference (P=0.12) in TGF-β mRNA expression between isografts and recipients treated with cyclosporine plus anti–TGF-β antibody (1.0 mg/kg), whereas a significant difference (P<0.0003) was observed between cyclosporine-treated and cyclosporine plus anti–TGF-β antibody (2.5 mg/kg)–treated recipients. No difference was observed between cyclosporine–treated and cyclosporine plus control antibody–treated recipients. For collagen, long-term treatment of cyclosporine resulted in increased intrarenal expression of collagen mRNA (P<0.0001) compared with isografts. There was a significant difference (P<0.0008) observed between cyclosporine-treated and cyclosporine plus anti–TGF-β antibody–treated recipients (Figure 2). No difference was observed between cyclosporine-treated and cyclosporine plus control antibody–treated recipients. Similar results were obtained with fibronectin mRNA (Figure 2); long-term treatment of cyclosporine resulted in increased intrarenal expression of fibronectin mRNA (P<0.0001) compared with isografts. A significant difference (P<0.0002) was observed between cyclosporine-treated and cyclosporine plus anti–TGF-β antibody–treated recipients. No difference was observed between cyclosporine–treated and cyclosporine plus control antibody–treated recipients.

**Figure 2. TGF-β, collagen, and fibronectin mRNA expression.** Results are expressed as ratio of TGF-β, collagen, and fibronectin with housekeeping gene β-actin. A statistically significant increased expression in cyclosporine (CsA)-treated recipients was seen compared with isografts. A statistically significant decrease of intrarenal expression of TGF-β, collagen, and fibronectin mRNA was seen in cyclosporine plus anti–TGF-β antibody–treated compared with cyclosporine-treated animals, whereas no difference with cyclosporine plus control (C) antibody–treated recipients was seen. TGF-β: *P<0.02, **P<0.003. Collagen: #P<0.0001, ##P<0.0008. Fibronectin: #P<0.0001, ###P<0.0002.

**Figure 3. MMP-2 and TIMP-2 mRNA expression.** Results are expressed as ratio of MMP-2 and TIMP-2 with housekeeping gene β-actin. A statistically significant increased expression in cyclosporine (CsA)-treated recipients was seen compared with isografts. A statistically significant decrease of intrarenal expression of MMP-2 and TIMP-2 mRNA was seen in cyclosporine plus anti–TGF-β antibody–treated compared with cyclosporine–treated animals, whereas no difference with cyclosporine plus control (C) antibody–treated recipients was seen. MMP-2: *P<0.0002, **P<0.0004. A decrease of intrarenal expression of TIMP-2 mRNA was seen in cyclosporine plus anti–TGF-β antibody–treated compared with cyclosporine–treated animals, which did not reach significant levels, whereas no difference with cyclosporine plus control antibody–treated recipients can be seen. TIMP-2: *P<0.03, ##P<0.18.

**Figure 4. Circulating levels of TGF-β protein.** With the use of ELISA kits, circulating levels of TGF-β protein in plasma samples were quantified. A statistically significant increased expression in cyclosporine (CsA)-treated recipients was seen compared with isografts. A statistically significant decrease of TGF-β levels was seen in cyclosporine plus anti–TGF-β antibody–treated compared with cyclosporine–treated animals, whereas no difference with cyclosporine plus control (C) antibody–treated recipients was seen. *P<0.0001, **P<0.0001.
Effect of Anti–TGF-β Antibody on Circulating Levels of TGF-β Protein
The results shown in Figure 4 demonstrate that compared with isografts, long-term treatment with cyclosporine resulted in a significant ($P<0.0001$) increase in circulating levels of TGF-β protein. There was no difference in TGF-β levels among isografts and cyclosporine plus control antibody–treated recipients; however, a statistically significant difference ($P<0.003$) was observed between cyclosporine-treated recipients and cyclosporine plus anti–TGF-β antibody–treated recipients. TGF-β protein expression in cyclosporine-treated recipients was not different in control antibody plus cyclosporine–treated recipients compared with isografts, long-term treatment with cyclosporine resulted in histopathological changes similar to those in patients receiving long-term treatment with cyclosporine. Histopathological analysis of periodic acid–Schiff (PAS)– and hematoxylin-eosin–stained slides from renal tissues of cardiac transplant recipient rats treated with (1) cyclosporine alone, (2) cyclosporine plus control antibody, and (3) cyclosporine plus anti–TGF-β antibody (1.0 mg/kg) was performed. Tubular vacuolization and vascular changes, including increased PAS staining of vascular structures, were found in cyclosporine–treated and cyclosporine plus control antibody–treated animals. Of particular note, PAS staining was also diminished in anti–TGF-β–treated recipients.

Figure 5. Intrarenal expression of TGF-β protein. Representative slides of immunohistochemical staining for TGF-β in renal tissues from isografts, cyclosporine (CsA), cyclosporine plus anti–TGF-β–, and cyclosporine plus control antibody–treated animals are shown. Results demonstrate a significantly higher staining of intrarenal TGF-β protein expression in cyclosporine–treated recipients, which was not different in control antibody plus cyclosporine–treated recipients. TGF-β protein staining in animals treated with cyclosporine plus anti–TGF-β antibody (1.0 mg/kg) was abolished but was slightly higher than that in isografts.

Discussion
Our earlier studies focused on the potential role of TGF-β in the pathogenesis of cyclosporine-induced nephrotoxicity. In this study we extend our previous investigation to examine the effect of a TGF-β antibody in a cardiac transplantation model. We show for the first time that it is possible to separate the immunosuppressive and nephrotoxic effects of cyclosporine, which we attribute to upregulated TGF-β, in organ transplantation. TGF-β is a potent immunosuppressant, and it was not surprising that a neutralizing antibody could reduce the therapeutic efficacy of cyclosporine. However, it was surprising that the fibrogenic properties of cyclosporine were abrogated on 2.5-fold lower dosing without compromise to the immunosuppressive activity. Importantly, these data demonstrate that optimal dosing with a TGF-β antagonist can discriminate the nephrotoxic and immunosuppressive activities of cyclosporine, and this discovery may have clinical utility. Clearly, further dose-response studies are necessary to better understand the dose-efficacy relationship.
nephrotoxicity by a TGF-β-related mechanism include spironolactone, losartan, vitamin E, pirfenidone, and angiotensin receptor blockade. A number of studies have implicated TGF-β as a key mediator of cyclosporine-induced nephrotoxicity, but definitive evidence has only recently been reported. Our laboratory and those of others have examined the effects of administering TGF-β-neutralizing antibodies to nontransplantation models of cyclosporine nephrotoxicity. These studies have shown that blocking TGF-β can normalize the expression of collagen types III and I and substantially reduce overall tissue fibrosis when examined histologically. The effects of TGF-β-neutralizing antibodies were not limited to reduction in fibrosis but also showed protection of tubular epithelial cells and reduction in markers of tissue hypoxia. Finally, these studies also demonstrated reduction in the expression of TGF-β isoforms, which is likely to interrupt the positive feedback loop that is known to regulate TGF-β production.

In this study, however, we examined the effect of administering a TGF-β-neutralizing antibody in a rat cardiac transplantation model. A direct effect of TGF-β inhibition on immunosuppression-induced nephrotoxicity in an organ transplantation model has not been attempted previously. The results from the present study demonstrate that treating recipients of cardiac transplants with cyclosporine plus 2.5 mg/kg of anti-TGF-β antibody reduces the immunosuppressive effects of cyclosporine. In contrast, a lower dose of antibody combined with cyclosporine resulted in prolonged survival and decreased expression of fibrogenic genes, including TGF-β mRNA. These data provide evidence that TGF-β is a direct mediator of cyclosporine-induced nephrotoxic effects in recipients of cardiac transplantation.

These studies are significant because numerous clinical studies have confirmed that long-term treatment of cardiac transplantation patients with cyclosporine is associated with severe nephrotoxicity. Van Gelder et al noted that end-stage renal disease occurred in 8% of heart transplant recipients treated with cyclosporine. Goldstein et al observed that 6.5% of patients developed end-stage renal failure. Tinawi et al documented a sequential increase in serum creatinine levels in cardiac transplant recipients. Griffiths et al also observed severe renal histopathological changes in heart transplant recipients treated with cyclosporine, with tubular atrophy being the most prominently observed feature; earlier, Falkenhain et al demonstrated that the renal damage increased in relation to the time of exposure and dose of cyclosporine.

On the basis of the documented role of MMP-2 and TIMP-2 in renal damage, we compared the intrarenal expression of MMP-2 and TIMP-2 mRNA in kidney tissues of rat heart transplant recipients treated with cyclosporine versus cyclosporine plus anti-TGF-β antibody and isograft controls. Our results demonstrated that the expression of MMP-2 was significantly increased in renal tissues from rats treated with cyclosporine compared with recipients treated with cyclosporine plus anti-TGF-β antibody and isograft controls. These results demonstrate that the increased expression of MMP-2 is associated with the nephrotoxic effects of cyclosporine in a rat cardiac transplantation model. Interestingly, TGF-β, like collagen and fibronectin, is known to induce the expression of MMP-2. This increased expression of MMP-2 was also observed in a nontransplantation rat model studying the mechanism of the nephrotoxic effects of cyclosporine, which correlated with increased expression of TGF-β. Anti-TGF-β antibody treatment in cyclosporine-treated rats significantly reduced collagen TGF-β, collagen, and fibronectin mRNA levels. These results support the findings of this study. The results of this study also demonstrate that the expression of TIMP-2 was significantly higher in cyclosporine-treated recipients than in cyclosporine plus anti-TGF-β antibody–treated recipients and isograft controls. Increased expression of MMP-2 and TIMP-2 has been noted in other clinical and experimental models of tissue fibrosis; the critical component is a persistent increase in both molecules, signaling increased fibrosis, and the ratio of TIMP-2 and MMP-2 may be a better indicator of the precise role of these tissue repair molecules in nephrotoxicity/fibrosis.
Additionally, our results uniquely demonstrate that intrarenal expression of TGF-β protein was increased in cyclosporine-treated recipients compared with isografts. The treatment of the recipients with cyclosporine plus anti–TGF-β antibody, but not control antibody, inhibited intrarenal TGF-β protein expression. Furthermore, renal histological changes similar to those observed during clinical cyclosporine nephrotoxicity were observed in cyclosporine-treated recipients and were significantly reversed by anti–TGF-β antibody treatment but not with control antibody.

The results from this study demonstrate the feasibility of anti–TGF-β antibody as a therapeutic strategy to limit the nephrotoxic effects of long-term treatment with cyclosporine in organ transplantation. However, similar to these experiments, a detailed study with dose effects of antibody needs to be performed. Furthermore, by in vitro, in vivo, and clinical studies, we have demonstrated induction of TGF-β with other immunosuppressive agents such as tacrolimus and sirolimus. 25-29 Therefore, it is attractive to hypothesize that anti–TGF-β antibody may provide similar benefits to counteract adverse nephrotoxic effects observed with long-term use of these drugs as well.

In summary, these new data demonstrate that long-term treatment of rat heart transplant recipients with cyclosporine results not only in histological change and renal function change but also in significantly increased expression of profibrogenic genes; improvement in renal function; and histological alteration with concomitant use of anti–TGF-β antibody with cyclosporine. These results provide strong evidence for the promising therapeutic application of anti–TGF-β antibody to prevent nephrotoxicity. This may have clinical relevance in organ transplantation and other diseases, including diabetic nephropathy.

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References


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