S1P modulator FTY720 limits matrix expansion in acute anti-thy1 mesangioproliferative glomerulonephritis

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the first protocol (injury experiment), administration of FTY720 was started 5 days before disease induction. Glomerular-inducible nitric oxide (NO) synthesis and mesangial cell injury were analyzed 24 h after antibody injection. In the second protocol (matrix expansion experiment), FTY720 treatment was begun 24 h after disease induction. Six days later, on day 7 after antibody administration, effects of S1P modulation on proteinuria, glomerular matrix expansion, collagen III accumulation, and expression of TGF-β1, fibronectin, and plasminogen activator inhibitor type-1 (PAI-1) were determined.

METHODS

Materials

Unless otherwise indicated, materials, chemicals, or culture media were purchased from Sigma (Taufkirchen, Germany).

Animals

Male Wistar rats (180–250 g) were obtained from Charles River (Sulzfeld, Germany) and fed a normal protein diet (22.5% protein, Altromin, Lage, Germany) for at least 3 days before the start of the experiment to allow equilibration. Body weight was determined at the beginning and end of each experiment. Animals were housed at a constant room temperature with a 12:12-h dark-light cycle. Food and water intakes were monitored. Animal care and treatment were in conformity with the guidelines of the American Physiological Society and approved by local authorities.

Induction of Acute Anti-thy1 Glomerulonephritis

Acute anti-thy1 glomerulonephritis was induced by tail vein injection of the monoclonal antibody OX-7 (1 mg/kg body wt in PBS) as previously described (15). The antibody OX-7 binds to a thy1-like antigen on the surface of mesangial cells and causes a complement- and NO-dependent mesangial cell lysis within 24 h (11). Control animals were injected with equal volumes of PBS only. OX-7 antibody was produced from a hybridoma cell line as previously described (14). The antibodies were diluted in PBS (pH 7.4) and stored at −70°C until use.

FTY720 Treatment

FTY720 was kindly provided by Novartis Pharma GmbH (Basel, Switzerland). The drug was given with the food in a dose of 1.5 mg/kg body wt for the first 24 h. Afterwards, the FTY720 dose was reduced to 0.3 mg/kg body wt until the end of the experiments. The doses were chosen on the basis of previous reports showing effective reduction in blood lymphocyte count in rat disease models (3). The drug-containing food was produced in our laboratory by using the flour of the standard rat chow (22.5% protein, A1311, Altromin) as previously described (17). Briefly FTY720 was mixed into the dry food flour in appropriate amounts, water was added to form pellets, and the air-dried pellets were subsequently given to the animals.

Experimental Groups and Design

The actions of FTY720 were separately analyzed in the injury phase (injury protocol, day 1 after antibody injection) and the subsequent matrix expansion phase of acute anti-thy1 glomerulonephritis (matrix expansion protocol, day 7 after antibody injection). In the injury experiment, glomerular cell number and inducible NO production were analyzed as indicators of mesangial cell injury. In the matrix expansion protocol, glomerular matrix score and expression of TGF-β1, fibronectin, and PAI-1 protein were measured as indicators of glomerular matrix expansion. FTY720 was given starting 5 days before and continuing until 24 h after injection of anti-thy1-antibody in the injury protocol and from day 1 until day 7 after antibody administration in the matrix expansion protocol.

Injury Protocol

Effects of FTY720 on the injury phase of acute anti-thy1 glomerulonephritis (day 1 after antibody injection). Five days before antibody injection, Wistar rats were assigned to the following groups: 1) PBS-injected controls (n = 6, control); 2) anti-thy1 antibody-injected animals, no treatment (GN; n = 14); and 3) anti-thy1 antibody-injected rats plus FTY720 (GN+FTY720; n = 14).

One day after antibody injection, the histological degree of mesangial cell lysis and the release of basal and lipopolysaccharide (LPS)-stimulated nitrite of cultured glomeruli were analyzed. At this point, mesangial cell lysis is complete and inducible glomerular NO production is markedly increased (15).

Matrix Expansion Protocol

Effects of FTY720 on the matrix expansion phase of anti-thy1 glomerulonephritis (day 7 after antibody injection). One day after antibody injection, when the mesangial cell lysis had occurred and fibrotic response had started (15), Wistar rats were assigned to the following groups: 1) PBS-injected controls (n = 6, control); 2) anti-thy1 antibody-injected animals, no treatment (GN; n = 14); and 3) anti-thy1 antibody-injected rats plus FTY720 (GN+FTY720; n = 12).

Seven days after disease induction, histological glomerular matrix accumulation, and protein production of TGF-β, fibronectin and PAI-1 of cultured glomeruli were determined. Furthermore, glomerular collagen III-staining intensity was analyzed histologically. To document that TGF-β expression reflected actual matrix accumulation, a histological glomerular matrix score was used. In addition, renal expression of the matrix protein fibronectin was measured as an indicator for matrix protein production. The protease inhibitor PAI-1 was used as sensitive marker of the matrix degrading system. In acute anti-thy1 glomerulonephritis, the fibrotic response peaks 7 days after antibody injection (16). For analysis of cell infiltration, renal tissue was immunohistologically stained for CD3-positive (lymphocytes) and ED1-positive (macrophages) and ED3-positive (activated macrophages) cells.

Urine Collection and Measurement of Proteinuria, Systolic Blood Pressure, and Heart Rate

In the matrix expansion protocol, 24-h urine was collected from each rat the day before death, using metabolic cages. Urinary protein concentration was measured by a pyrogallol red method using a microplate technique (17). Proteinuria is expressed as milligrams of protein per 24 hours. Systolic blood pressure and heart rate were measured before death in conscious animals by tail cuff method as previously described (17).

Euthanasia

At the end of each experiment, animals were anesthetized with ketanest (50 mg/kg body wt, Pharmacia GmbH, Erlangen, Germany)/xylazine (10 mg/kg body wt, Bayer Vital GmbH, Leverkusen, Germany). Following a midline abdominal incision, 5–10 ml of heparinized blood were drawn from the abdominal aorta and the kidneys were subsequently perfused with 30 ml of ice-cold PBS. For histological examination, a cortical segment from one kidney was fixed in 10% neutral buffered formalin.

Blood Analysis

Total and differential white blood cell counts were determined in an automated cell counter (XE 2100, Sysmex GmbH, Norderstedt, Germany) using standard fluorescent flow cytometry technology (17).
Glomerular Isolation and Culture

Glomeruli from individual rats were isolated by a graded sieving technique (160-, 125-, and 71-µm mesh metal sieves) as described previously (14). Glomeruli were suspended in DMEM supplemented with 0.1 U/ml insulin, 100 U/ml penicillin, and 100 µg/ml streptomycin at a density of 2,000 per ml.

Basal and LPS-Stimulated Glomerular NO Production

In the injury protocol, glomeruli were cultured at a density of 2,000 per ml at 37°C/5% CO₂ for 48 h (basal glomerular NO production). Additional samples were cultured in the presence of 10 µg LPS from Escherichia coli (serotype 0127:B8) to stimulate inducible NO production (stimulated glomerular NO production). Nitrite served as indicator of NO production and was determined by the Griess reaction in glomerular culture supernatants (15). Briefly, 50 µl of sample were mixed with 100 µl Griess reagent (0.05% N-[1-naphthyl] ethylenediamine dihydrochloride, 0.5% sulfanilamide in 45% glacial acetic acid) in 96-well plates. After 10-min incubation in the dark, absorbance was read at 570 nm in an automated plate reader (MRX II, Dynex Technologies). Two samples from each rat with and without LPS stimulation were analyzed. Standard samples were prepared with sodium nitrite.

Glomerular Production of TGF-β1, Fibronectin, and PAI-1

In the matrix expansion protocol, glomeruli were cultured at a density of 2,000 per ml at 37°C/5% CO₂. After 48-h incubation, supernatants were harvested and stored at −20°C until analysis. In previous experiments, we showed that the TGF-β1, fibronectin, and PAI-1 production by glomeruli ex vivo is constant over 48 h and correlates closely to the actual glomerular matrix accumulation in vivo (13). TGF-β1 content of culture supernatant was measured after acid activation using a commercially available ELISA kit (TGF-β1 DuoSet, R&D Systems, Wiesbaden, Germany) according to the manufacturer’s instructions. Fibronectin and PAI-1 levels were measured with inhibitory ELISA as previously described (20). Three samples from each rat were analyzed.

Light and Immunohistochemistry Microscopy

All microscopic examinations were performed in a blinded fashion. Three-micrometer sections of paraffin-embedded tissue were stained with periodic acid-Schiff (PAS) and analyzed using a computer-based morphometric system. Glomerular sections were examined on a Leica DM LB2 light microscope (Leica Microsystems, Wetzlar, Germany) connected to a PL-A662 video camera and the Axiovision 2.05 image analysis system (both Carl Zeiss Vision, München, Germany) using a 10 × 10 orthographic grid overlaid on digital images. In the injury protocol, the number of cell nuclei was counted in 15 glomeruli at 800× magnification (both Carl Zeiss Vision, München, Germany) using a computer-based morphometric system and integrates both the area and the intensity of collagen III was analyzed using a computer-based morphometric system and integrates both the area and the intensity of collagen III staining. Glomerular sections were examined using a microscope-based morphometric system and integrates both the area and the intensity of collagen III staining.

Effects of FTY720 on Body Weight, Blood Pressure, and Heart Rate

Animals’ mean body weights at the end of the experiment were not significantly different in the injury protocol (control 255 ± 3 g, GN 247 ± 9 g, GN+FTY720 257 ± 2 g, P = not significant [NS]) and in the matrix expansion protocol (control 244 ± 2 g, GN 254 ± 12 g, GN+FTY720 250 ± 12 g, P = NS). Mean food and water intakes did not significantly differ between the groups.

Animals’ heart rates at the end of each experiment, i.e., after 6 days of FTY720 administration, were similar between the groups of the injury protocol (control 425 ± 24 beats/min, GN 395 ± 12 beats/min, GN+FTY720 410 ± 5 beats/min, P = NS) and the matrix expansion protocol (control 409 ± 38 beats/min, GN 386 ± 8 beats/min, GN+FTY720 408 ± 7 beats/min, P = NS).

Effects of FTY720 on White Blood Cell Count

Induction of acute anti-thy1 glomerulonephritis, i.e., injection of anti-thy1 antibody in itself, already brought about a significant 35% reduction in total blood leukocyte count in the injury phase and 25% in the matrix expansion phase, respectively (Fig. 1, A and C). Subsequent differential cell analysis revealed that this effect was due to a decrease in blood lymphocyte count (injury protocol: −41%, and matrix expansion protocol: −31%; Fig. 1, B and D). This finding is explained by the direct anti-thy1-induced lysis of thy1-positive lymphocytes (=CD90-positive cells) and occurs in parallel to the mesangial cell damage in the kidney.

Compared with the values of the untreated anti-thy1-injected animals, administration of FTY720 resulted in a marked and highly significant further reduction in total leukocyte count by 78% and lymphocyte count by 89% in the injury protocol, and by 77 and 88% in the matrix expansion protocol, respectively (Fig. 1, A–D). The numbers of blood monocytes, granulocytes, basophils, and eosinophils were not affected by SIP modulation, documenting the lymphocyte selectivity of FTY720 actions on blood leukocytes circulation. The reductions of both total leukocyte and lymphocyte numbers were more pronounced in the injury than in the matrix expansion experiment (Fig. 1, A–D).
Effects of FTY720 on the Injury Phase of Acute Anti-thy1 Glomerulonephritis

Compared with the normal control animals, injection of anti-thy1 antibody resulted in a significantly reduced glomerular cell number (control 62.6 ± 0.7 cell nuclei/glomerular cross section vs. GN 45.5 ± 0.9 cell nuclei/glomerular cross section; Fig. 2, A–D) as well as basal (control 0.7 ± 0.2 nmol/ml vs. GN 4.2 ± 1.0 nmol/ml, P < 0.01; Fig. 2E) and LPS-stimulated glomerular NO production (control 3 ± 1 nmol/ml vs. GN 36 ± 4 nmol/ml, P < 0.001; Fig. 2F), indicating the level of iNOS expression. Compared with the nephritic animals, the FTY720 administration for 6 days before and during the injury phase showed no significant action on disease activity (glomerular cell number: 45.1 ± 0.8 cell nuclei/glomerular cross section, basal and LPS-stimulated glomerular NO production: 6.8 ± 1.2 and 41 ± 5 nmol/ml, all P = NS vs. GN; Fig. 2, A–F).

Effects of FTY720 on the Matrix Expansion Phase of Acute Anti-thy1 Glomerulonephritis

Seven days after injection of anti-thy1 antibody, disease was characterized by a significant increase in proteinuria (GN 46 ± 8 mg/24 h vs. control 6 ± 2 mg/24 h, P < 0.001; Fig. 3), histological matrix accumulation (GN 78.1 ± 1.3% vs. control 18.6 ± 2.9%, P < 0.001; Fig. 4) and glomerular production of TGF-β1 (GN 1,190 ± 129 pg/ml vs. control 117 ± 20 pg/ml), fibronectin (GN 23,167 ± 1,051 ng/ml vs. control 1,425 ± 260 ng/ml), and PAI-1 (GN 880 ± 41 ng/ml vs. control 499 ± 28 ng/ml; P < 0.001 for all parameters; Fig. 5, A–C). Glomerular collagen III staining intensity increased from 0.4 ± 0.1 to 3.9 ± 0.4% (P < 0.001; Fig. 6). Treatment with FTY720 significantly decreased proteinuria (GN+FTY720 29 ± 3 mg/24 h, P < 0.05 vs. GN), histological matrix accumulation (GN+FTY720 68.9 ± 2.5, P < 0.01 vs. GN), and glomerular production of TGF-β1 (GN+FTY720 725 ± 84, P < 0.01 vs. GN), fibronectin (GN+FTY720 19,691 ± 784, P < 0.05 vs. GN), and PAI-1 (GN+FTY720 19,774 ± 17.5, P < 0.05 vs. GN) as well as glomerular collagen-staining intensity (GN+FTY720 1.4 ± 0.1%, P < 0.001 vs. GN; Figs. 3–6).

Seven days after induction of anti-thy1 glomerulonephritis, matrix accumulation was paralleled by a 3.9-fold increase in CD3-positive cells (GN 1.3 ± 0.1 cells/glomerular section; Fig. 7A) and an 18-fold increase in glomerular infiltration with ED1-positive cells, respectively (GN 3.8 ± 0.2 cells/glomerular section; Fig. 7B). Indicating its relative lymphocyte specificity, treatment with FTY720 markedly limited glomerular lymphocyte infiltration by 65% (GN+FTY720 0.4 ± 0.1 CD3-positive cells/glomerular section, P < 0.001 vs. GN),
whereas glomerular macrophage number was moderately decreased by 22% (GN+FTY720 3.0 ± 0.3 ED1-positive cells/glomerular section, \( P < 0.05 \) vs. GN; Fig. 7). The number of ED3-positive macrophages increased following induction of anti-thy1 glomerulonephritis (control 0.27 ± 0.06 vs. GN 0.60 ± 0.09 cells/glomerular section) and decreased with FTY720 treatment (0.20 ± 0.12 cells/glomerular section, \( P = 0.08 \)).

In their entirety, the results show that FTY720 selectively decreases blood lymphocyte count and subsequently reduces glomerular lymphocyte infiltration in acute anti-thy1 glomerulonephritis. Subsequently, S1P modulation significantly prevented the increases in proteinuria, glomerular matrix accumulation, and TGF-\( \beta \) overexpression of the matrix expansion phase, while having no marked influence on the degree of mesangial cell injury and inducible NO production of the injury phase of acute anti-thy1 glomerulonephritis.

**DISCUSSION**

Administration of the S1P modulator FTY720 has recently been shown to slow the progressive course of experimental chronic anti-thy1-induced glomerulosclerosis toward renal fibrosis and insufficiency (17). In this previous experiment, FTY720 treatment began 7 days after injection of anti-thy1 antibody, i.e., when glomerular matrix expansion already occurred. S1P modulation substantially prevented tubulointerstitial overexpression of the key fibrosis mediator TGF-\( \beta \), and...
subsequent matrix accumulation. The functional relevance of this beneficial effect was indicated by lower blood creatinine levels as well as by higher blood erythrocyte counts. After the relatively late start of treatment, glomerular TGF-β overexpression and matrix expansion were only moderately and not significantly decreased through FTY720, which supports the assumption that the progression phase of anti-thy1 renal disease had predominately been targeted. In the treatment group, blood lymphocyte number was selectively reduced by more than 90%, again documenting the main action of FTY720. Blood lymphopenia subsequently prevented infiltration of the fibrosing kidneys with lymphocytes, while the number of infiltrating macrophages was not significantly altered. Taken together, these recent results in chronic anti-thy1 glomerulosclerosis provided strong evidence for the concept that lymphocytes actively participate in the progression of chronic, not primarily immune-driven renal diseases.

The present study in acute anti-thy1 glomerulosclerosis expands and completes previous findings in chronic progressive glomerulosclerosis in several important ways. 1) S1P modulation by FTY720 significantly abrogated the degree of the matrix expansion on day 7, when started just 1 day after antibody injection and before the glomerular matrix began its build-up. This was indicated by several relevant disease measures such as lower proteinuria, glomerular histological matrix protein accumulation, and protein expression of TGF-β1, fibronectin, and PAI-1. 2) FTY720 acutely and dramatically reduces blood lymphocyte number as anticipated. With this pharmacological manoeuvre, we were able to prevent recruitment of lymphocytes into the diseased glomeruli by ~75%, while infiltration with macrophages was only moderately affected. 3) In contrast to the matrix expansion phase, induction of disease, i.e., antibody-induced mesangial cell injury, was not significantly altered by S1P modulation, as indicated by the degree of mesangial cell loss and glomer-
ular inducible NO synthesis. In the following paragraphs, we will discuss the relevance of these findings.

Like acute anti-thy1 glomerulonephritis, lupus nephritis in MRL-lpr/lpr mice represents an immune-induced kidney disease characterized by renal overproduction of TGF-β and expansion of extracellular matrix protein. In MRL-lpr/lpr mice, Naoe et al. (23) previously reported that early intervention with FTY720 significantly prolonged survival when MRL-lpr/lpr mice received an oral daily dose of 1 mg/kg FTY720 for 14 days, beginning at 16 wk of age. Subsequent studies by Minota et al. (12) revealed that S1P modulation in MRL-lpr/lpr mice reduced production of anti-double-strand DNA antibodies and number of B-cells as well as normalized T-cell proliferation and IL-2 production. This resulted in reduced immune complex deposition and action in the lupus kidneys. Thus, with regard to the mechanism involved, FTY720 was protective in chronic lupus nephritis by reducing the activity of the immune system and subsequently lowered immune renal tissue damage. In contrast to murine lupus nephritis, the immune injury to the mesangium in acute anti-thy1 glomerulonephritis is brief, transient, and mainly restricted to the first 24 h–48 h after antibody injection. The following glomerular matrix expansion is not further exposed to primary immune attacks. However, a secondary white cell infiltration ensues that potentially modulates glomerular matrix expansion. In this sense, the antifibrotic benefits during the anti-thy1 matrix expansion phase go beyond those detected in the studies in lupus nephritis. They indicate that FTY720 can also directly interfere with the cascade leading to glomerular matrix accumulation, without affecting the degree of the immunological injury.

This beneficial effect of FTY720 on the matrix expansion phase of acute anti-thy1 glomerulonephritis is in line with a recent study by Shimizu et al. (6), which elegantly analyzed the role of various lymphocyte subsets in a comparable rat model of mesangioproliferative glomerulonephritis. Shimizu et al. employed subset-specific lymphocyte lysing anti-CD8 monoclonal antibodies, which deplete both CD8+ T-lymphocytes and natural killer (NK) cells, and they also used anti-CD5 mAb, which diminish both CD4-positive and CD8-positive T-lymphocytes, respectively. Administration of neutralizing anti-CD5 mAb significantly suppressed glomerular infiltration with CD4-positive T-lymphocytes and went along with lower proteinuria and mesangial matrix expansion. In contrast, anti-CD8 mAb treatment completely prevented recruitment of NK-cells into the diseased glomeruli, but no protective effect on mesangial injury could be evaluated by glomerular matrix expansion score, total number of cells in glomeruli, α-smooth muscle actin-staining score, and type I collagen-staining score and proteinuria. Together, the experiments conducted by Shimizu et al. and our group jointly point toward the concept that lymphocytes play an active role in the matrix expansion phase of anti-thy1-induced glomerulonephritis. At this stage of the research, we can only speculate on what cellular and molecular mechanisms may be operating. First, it appears to be safe to expect that these pathways are different from those functions which lymphocytes classically exert in specific im-

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**Fig. 5. Effects of FTY720 on glomerular protein expression of TGF-β (A), fibronectin (B), and PAI-1 (C) 7 days after induction of acute anti-thy1 GN (matrix expansion protocol). FTY720 treatment was begun 24 h after disease induction and continued until day 7. Glomeruli were harvested from individual animals and cultured at a density of 2,000 ml for 48 h. **P < 0.01 and *P < 0.05 vs. GN.**
munity, autoimmunity, and transplant rejection. It is quite likely that indirect immune-modulating actions are involved. In this sense, there is evidence that infiltrating activated lymphocytes themselves release profibrotic mediators such as TGF-β and ANG II and thereby directly amplify matrix expansion by the resident glomerular cells (10, 19). In addition, lymphocytes are important in the glomerular recruitment and activity of monocytes/macrophages that, in turn, may promote glomerular matrix protein accumulation (19, 21, 27). In the present study, we observed a 22% reduction in the glomerular macrophage infiltration (ED1-positive cells) 7 days after antibody injection.

Indirect evidence that lymphocyte depletion was the main mechanism of the beneficial actions of FTY720 on matrix expansion in anti-thy1 glomerulonephritis has recently been provided by in vitro studies by Huwiler et al. (26). In cultured mesangial cells, this research group found that phosphorylated FTY720 is profibrotic in that it induces CTGF through an intracellular crossactivation of the TGF-β signaling cascade.

Fig. 6. Effects of FTY720 on glomerular collagen III expression 7 days after induction of acute anti-thy1 GN (matrix expansion protocol). Shown are characteristic photographs of glomerular collagen expression of a normal control (A), GN (B), and GN with FTY720-treated animal (C) and histomorphometric analysis of glomerular collagen III staining intensity (D). FTY720 treatment was begun 24 h after disease induction and continued until day 7. Collagen III analyses were performed with a standard APAAP technique and is expressed as % per glomerular area. ***P < 0.01 vs. GN.

Fig. 7. Effects of FTY720 on glomerular cell infiltration 7 days after induction of acute anti-thy1 GN (matrix expansion protocol). Shown are CD3-positive cells (A) representing lymphocytes and glomerular ED1-positive cells (B) indicating macrophages. Analyses were performed with a standard APAAP technique and results are expressed as number of positive cells per glomerular cross section. ***P < 0.01 vs. GN and *P < 0.05 vs. GN.
This feature was shared by phosphorylated S1P. Although we cannot exclude that this action may occur in vivo as well, the overall antifibrotic outcome in the matrix expansion experiments with FTY720 suggests that the greater functional importance lies in lymphocyte depletion achieved by S1P modulation rather than in direct action on resident renal cells. In an observation in support of this interpretation, no acute renal histological alterations were detected upon 7-day oral administration of FTY720 to rats and no reduction in renal or hepatic blood flow and function was seen in mice treated at doses of 1 mg FTY720·kg\(^{-1}\)·day\(^{-1}\) (24). In addition, histological examination of the kidneys of normal rats treated with FTY720 at rather high doses of 5 mg·kg\(^{-1}\)·day\(^{-1}\) for 3 wk did not reveal signs of either glomerulosclerosis or tubulointerstitial fibrosis or atrophy (24). Beyond mesangial cells, glomerular endothelial cells may have been affected by FTY720.

Since S1P receptor modulation has shown beneficial actions in a well-established experimental model of human mesangio-proliferative glomerulonephritis, it is well worth speculating on its future applicability in human glomerular disease. So far, FTY720 has shown very promising results as a single therapy in both patients with relapsing multiple sclerosis. In a recently published proof-of-concept study, FTY720 reduced markedly the number of lesions detected on MRI and indices of clinical disease activity (7). In human kidney transplantation, trials have been stopped since FTY720 in combination therapy with steroids and cyclosporine failed to show superior efficacy to mycophenolate mofetil (25). In addition, FTY720, together with cyclosporine and steroids, was linked to relevant side effects such as macula oedema, bradycardia, and liver enzyme elevation (personal comment by K. Budde). The bradycardia from FTY720 is transient and most probably due to an interaction of FTY720-P with S1P3 receptor on atrial sinus node myocyte, which in turn leads to a negative chronotropic response (22). Other than FTY720, novel, more specific S1P receptor modulators, such as SEW2871, are in development. These might be more effective and potentially less prone to side effects. Thus S1P receptor modulation as single therapy may well become a therapeutic option for human mesangio-proliferative renal disease.

In conclusion, the present study shows that FTY720-induced lymphopenia extinguated the glomerular fibrotic response and proteinuria 7 days after induction of acute anti-thy1 glomerulonephritis. These findings expand and complete previous studies showing antifibrotic effects of FTY720 in chronic progressive anti-thy1 glomerulosclerosis. They indicate that lymphocytes actively contribute to immune-modulated matrix expansion in acute anti-thy1 glomerulonephritis.

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REFERENCES

results from the first phase 2A study in de novo renal transplantation. 

26. Xin C, Ren S, Eberhardt W, Pfeilschifter J, Huwiler A. The immuno-
modulator FTY720 and its phosphorylated derivative activate the Smad 
signalling cascade and upregulate connective tissue growth factor and 
collagen type IV expression in renal mesangial cells. Br J Pharmacol 147: 

27. Zatz R, Noronha IL, Fujihara CK. Experimental and clinical rationale 
for use of MMF in nontransplant progressive nephropathies. Am J Physiol 