Stimulation of soluble guanylyl cyclase inhibits mesangial cell proliferation and matrix accumulation in experimental glomerulonephritis

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EXCESSIVE MESANGIAL CELL (MC) proliferation and matrix accumulation are characteristic for the most common glomerulonephritis in humans, mesangial proliferative glomerulonephritis (e.g., IgA-nephropathy), but they also occur in other types of renal disease such as lupus and membranoproliferative glomerulonephritis, as well as to some extent in diabetic nephropathy. Despite the increasing knowledge about MC pathophysiology, no specific antiproliferative treatment in these human diseases has been established so far, a fact that may be due to the lack of orally applicable compounds without severe side effects. Anti-Thy1 nephritis in the rat is a well-established animal model for mesangial proliferative glomerulonephritis. After intravenous injection of the anti-Thy1 antibody, specific binding to MC mediates complement-induced mesangiolysis, which is followed by a marked proliferative reaction of the mesangium starting on day 2, peaking on day 4/5, and ceasing afterward. Diseased glomeruli transiently develop hypercellularity, proteinuria, and a marked increase in extracellular matrix proteins, all of which are typical hallmarks of mesangial proliferative glomerulonephritis in humans.

Guanylyl cyclase is a ubiquitously distributed cytoplasmic enzyme that exists in two isoenzyme forms, a membrand-bound form stimulated by various peptides and the intracellular soluble guanylyl cyclase (sGC). The intracellular sGC is a heme-containing heterodimer, consisting of an α (73–88 kDa) and a β (70 kDa)-subunit (19). The α1β1 isoenzyme is thought to be the major isoform. Previous studies have shown that sGC is expressed in various renal cell types in vivo, such as glomerular arterioles, granular cells, fibroblasts, podocytes, and MC (22, 35). Nitric oxide (NO) is a major sGC stimulator in vivo (1), even though the effects of NO are mediated by various physiological pathways (21). Previous studies demonstrated inhibition of MC proliferation (28) and matrix expansion (36) by NO treatment in vitro. Inhibition of NO production caused glomerulosclerosis, hypertension, and matrix expansion in healthy rats in vivo (4, 26) as well as aggravated anti-glomerular basement membrane nephritis in rats (9). In contrast, a NO-generating β-blocker prevented renal injury in the remnant kidney model (34).

Nevertheless, it is still unknown whether these beneficial NO-dependent effects in renal disease are due to stimulation of sGC or the result of other activated pathways. Recently, a novel group of pyrazolopyridine derivatives was developed (32), which demonstrated a highly specific, NO-independent stimulation of sGC in a cell-free environment as well as in endothelial cells and platelets (31). In vivo studies demonstrated potent vasodilatation and a subsequent dose-dependent decrease in blood pressure in spontaneously hypertensive and normal rats, as well as inhibition of platelet aggregation (10, 33). In a canine model of congestive heart failure, treatment with the specific sGC stimulator BAY 41–2272 improved cardiac output (5). In contrast, nothing is known about the regulation and potential therapeutic role of sGC stimulation in inflammatory kidney disease.

In this study, we investigated the regulation and therapeutic potential of direct stimulation of sGC in experimental mesangial proliferative glomerulonephritis in the rat in vivo.

METHODS

Animal model and experimental design. Experimental mesangial proliferative glomerulonephritis was induced in seven Sprague-Dawley rats per group (150–200 g; Charles River, Sulzfeld, Germany) by a single injection of 0.3 ml/100 g body wt of the mouse monoclonal anti-Thy1 antibody OX-7. All animals were fed standard rat chow.
by D. Kerjaschki, University of Vienna, Vienna, Austria) (18); PL-1, IV; Southern Biotechnology Associates, Birmingham, AL) (12), or a polyclonal antibody to collagen IV (goat anti-human/bovine collagen libidum.

DIRECT SGC STIMULATION INHIBITS MC PROLIFERATION

daily doses from applications were given within 18 h as loading doses, followed by an amount of the solvent solution via oral gavage. The first three experiments were performed in rats per group either received BAY 41–2272 therapy or solvent solution analogous to the other experiments but without consecutive induction of anti-Thy1 nephritis. Glomeruli and tissues were harvested for further analysis as described above.

To verify changes in sGC protein expression in isolated glomeruli during anti-Thy1 nephritis, a fourth experiment was performed, where anti-Thy1 nephritis was induced in six rats as described above. Kidneys of three rats each on days 2 and 6 were removed and used for isolation of glomeruli and consecutive Western blot analysis. Isolated glomeruli of three normal, healthy rats served as controls.

Tissue processing and immunohistochemical staining. Renal biopsies were fixed in methyl Carnoy’s solution or 3% paraformaldehyde, embedded in paraffin, and cut into 5-μm sections for indirect immunoperoxidase staining as described elsewhere (7, 12).

To perform immunoperoxidase staining, tissue sections were incubated with the following primary antibodies as indicated: a murine IgG monoclonal antibody (mAb) against the proliferating cell nuclear antigen (PCNA; 19A2; Coulter Immunology, Hialeah, FL) (12); ED-1, a murine IgG1 mAb to a cytoplasmic antigen present in monocytes, macrophages, and dendritic cells (Serotec, Oxford, UK) (12); OX-7, a murine IgG1 mAb specific for MC (Serotec) (12); a polyclonal antibody to collagen IV (goat anti-human/bovine collagen IV; Southern Biotechnology Associates, Birmingham, AL) (12), or a monoclonal antibody against fibroconnectin (GIBCO, Invitrogen, Karlsruhe, Germany) (27); JG-12, a monoclonal antibody against the AGE-receptor for staining of glomerular capillaries (kindly provided by D. Kerjaschki, University of Vienna, Vienna, Austria) (18); PL-1, a murine monoclonal antibody against rat platelets (kindly provided by W. W. Baker, Groningen, The Netherlands) (2); a monoclonal antibody against the β1-subunit of sGC (Alexis Biochemicals, Grunenberg, Germany) (35).

Negative controls for immunostaining included either deleting the primary antibody or substitution of the primary antibody with equivalent concentrations of an irrelevant murine mAb or preimmune rabbit IgG. All tissue sections were incubated with primary antibodies overnight at 4°C. Afterward, specific biotinylated secondary antibodies (all by Zymed, San Francisco, CA) were applied, followed by peroxidase-conjugated avidin D (Vector Labs, Burlingame, CA) and color development with diaminobenzidine with nickel chloride for nuclear staining and otherwise without nickel. In the case of immunofluorescence staining, a secondary goat anti-rabbit antibody (Molecular Probes, Leiden, The Netherlands) was applied.

Expression of collagen IV, fibronectin, and the sGC β1-subunit was quantified using computer-assisted image-analysis software (MetaVue, Visitron Systems, Munich, Germany) in a blinded fashion. At least 50 glomerular cross sections were analyzed at 400-fold magnification. PCNA-positive nuclei, ED-1-positive cells, glomerular microaneurysms (13), as well as the number of all glomerular cells were counted separately in 50 consecutive glomeruli/section using 400-fold magnification in a blinded fashion. Mesangiolysis and platelet influx were assessed using 400-fold magnification in 50 consecutive glomeruli using a semiquantitative scoring system from 0 to 4, where 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% of the glomerular area mesangiolytic or positive for platelets, respectively. The same score was used for assessing collagen IV-positive areas only in the additional experiment comparing the effects of antihypertensive therapy with the placebo group. All results are given as means ± SD per glomerular cross section.

Immunohistochemical double staining. To determine the number of proliferating MC, double immunostaining for PCNA, a marker of cell proliferation, and for OX-7 (MC specific) was performed as described previously (12). The number of proliferating MCs is given as means ± SD per glomerular cross section.

Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling assay. Apoptotic cells were detected by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay as described previously (11). Cells were regarded as TUNEL positive if their nuclei were stained black and displayed typical apoptotic morphology with chromatin condensation. The number of apoptotic cells was counted in 50 sequentially selected glomeruli and is given as the means number ± SD per glomerular cross section.

Glomerular preparations and measurement of cGMP. Glomerular preparations were made as described elsewhere (12). Glomerular extracts were homogenized on ice in homogenization buffer containing 5 × 10−4 mmol IBMX (Sigma, St. Louis, MO) to block the activity of phosphodiesterases and snap-frozen in liquid nitrogen. Measurement of cGMP was performed in triplicate using a commercial radioimmunoassay kit (IBL, Hamburg, Germany) as described before (35).

Western blot analysis. Protein samples containing 20 μg of protein from isolated glomeruli were separated by 8% SDS-PAGE and blotted onto PVDF membranes. Blots were blocked for 12 h at 4°C in blocking solution containing 2% milk powder (Applichem, Darmstadt, Germany)/1% bovine serum albumin (Merck, Darmstadt, Germany). After being washed, blots were incubated with the primary antibody for 2 h, followed by horseradish peroxidase-conjugated anti-rabbit IgG (Amersham Biosciences, Buckinghamshire, UK). Immunoreactive bands were detected on the basis of chemiluminescence using an enhanced chemiluminescence kit. β-Actin was detected simultaneously as the loading control using an antibody from Abcam (Cambridge, UK). Quantitative analysis was performed using a computer-assisted system (AIDA Image Analyzer, Raytest, Straubenhardt, Germany).
**Miscellaneous measurements.** Urinary protein was measured colorimetrically using a commercial test kit (Bio-Rad, Hercules, CA) based on the Bradford dye-binding procedure (6, 30). Serum and urinary creatinine were measured using an autoanalyzer (Beckman Instruments, Brea, CA).

**Statistical analysis.** All values are expressed as means ± SD. Statistical significance (defined as a P < 0.05) was evaluated by Student’s t-test.

**RESULTS**

*sGC is expressed in the rat kidney and upregulated during experimental mesangial proliferative glomerulonephritis.* By immunostaining with a specific antibody against its β1-subunit, sGC localized mainly to MC (Fig. 1A), blood vessels, descending vasa recta, and interstitial cells (not shown). These findings are consistent with a previously published study in healthy rats (35). During anti-Thy1 nephritis, glomerular sGC was increased mainly during the later phase of MC proliferation, where it was confined predominantly to the mesangium (area positive for sGC was 36.3 ± 22% on day 6 vs. 4.9 ± 3.7% on day 0; P < 0.05; Fig. 1, A–C), but expression by other endogenous renal (for example podocytes) or infiltrating cells cannot be excluded. This increase in sGC protein was confirmed by Western blot analysis of isolated glomeruli from animals with anti-Thy1 disease on days 2 and 6 compared with healthy rats (Fig. 1, D and E).

**BAY 41–2272 specifically stimulates sGC activity in glomerular cells in vivo.** To demonstrate specific intracellular sGC stimulation of glomerular cells in vivo by BAY 41–2272, glomerular cGMP levels of isolated glomeruli after a 6-day treatment were compared with placebo-treated anti-Thy1 rats. BAY 41–2272 treatment caused a fourfold increase in glomerular cGMP levels, as indicated in Fig. 2A. In contrast, Western blot analysis of glomerular protein extracts on day 6 of anti-Thy1 disease indicated that treatment with BAY 41–2272 did not increase protein levels of sGC (Fig. 2, B and C). This was also confirmed by immunostaining (Fig. 1C). Since sGC stimulation is known to decrease systemic blood pressure and inhibit platelet aggregation, we performed measurement of systolic blood pressure using tail plethysmography and assessed bleeding time before death on day 6. As a result of sGC stimulation, a >15% reduction of systemic blood pressure in rats with sGC stimulation was found; however, this reduction did not reach the level of significance compared with placebo-treated rats (89 ± 16 vs. 106 ± 10 mmHg; P = 0.07). In addition, sGC stimulation resulted in a significantly prolonged bleeding time compared with placebo (7.42 ± 2.61 vs. 2.31 ±
showed a decreased rate of total glomerular proliferation in rats proliferating cells with a monoclonal antibody against PCNA by sGC stimulation via BAY 41–2272 in vivo. Identification of proliferative glomerulonephritis in humans and in the anti-Thy1 hyperproliferative response is the hallmark of mesangial proliferative glomerulonephritis. Since the MC both groups (Table 1).

Microaneurysms 1.5 vs. 16.0; \( P < 0.05 \) (Fig. 2). Figure 2A shows an example of this double staining, with arrows indicating black PCNA-positive nuclei (proliferation marker) surrounded by an OX-7-positive cytoplasm (MC). In addition, glomerular cell number on day 6 was significantly decreased in anti-Thy1 nephritic rats with sGC stimulation compared with placebo-treated rats (46.9 ± 5 vs. 66.5 ± 6.5; \( P = 0.001 \); Fig. 3D). On day 2, when non-MC proliferation is predominant, no difference in glomerular or MC proliferation was detected between BAY 41–2272- and placebo-treated rats (Fig. 3, A, B, and D).

Stimulation of sGC using BAY 41–2272 does not affect mesangiolysis. To exclude alteration of the disease induction process by the treatment, application of BAY 41–2272 or placebo was started no earlier than 1 h after antibody induction of the anti-Thy1 model. In addition, assessment of mesangiolysis on day 2 via a scoring system verified equivalent disease induction in all rats independently of the treatment (2.9 ± 0.9 in placebo- vs. 3.1 ± 0.7 in BAY 41–2272-treated anti-Thy1 rats; Table 1). Mesangiolysis was also unchanged during the later time course of anti-Thy1 disease on day 6 (Table 1). Since microaneurysms are thought to develop as a consequence of mesangiolysis and the subsequent repair reaction, the percentage of microaneurysm formation in kidneys of sGC-stimulated or placebo-treated rats was assessed, but it was equivalent in both groups (Table 1).

Stimulation of sGC inhibits MC proliferation in experimental mesangial proliferative glomerulonephritis. Since the MC hyperproliferative response is the hallmark of mesangial proliferative glomerulonephritis in humans and in the anti-Thy1 model, we examined whether this response would be affected by sGC stimulation via BAY 41–2272 in vivo. Identification of proliferating cells with a monoclonal antibody against PCNA showed a decreased rate of total glomerular proliferation in rats with sGC stimulation on day 6 compared with nontreated rats (12 ± 2.7 vs. 16 ± 1.9; \( P < 0.05 \)) (Fig. 3A). Most of the proliferating glomerular cells on day 6 are of MC origin. Double staining for proliferating MCs (PCNA and OX-7 positive) demonstrated that sGC stimulation significantly inhibits MC proliferation compared with placebo-treated rats (8.5 ± 1.8 vs. 11.8 ± 3; \( P < 0.05 \)) (Fig. 3B). Figure 3C shows an example of this double staining, with arrows indicating black PCNA-positive nuclei (proliferation marker) surrounded by an OX-7-positive cytoplasm (MC). In addition, glomerular cell number on day 6 was significantly decreased in anti-Thy1 nephritic rats with sGC stimulation compared with placebo-treated rats (46.9 ± 5 vs. 66.5 ± 6.5; \( P = 0.001 \); Fig. 3D). On day 2, when non-MC proliferation is predominant, no difference in glomerular or MC proliferation was detected between BAY 41–2272- and placebo-treated rats (Fig. 3, A, B, and D). Since glomerular cell number is balanced by both cellular proliferation and cell death, apoptotic cells per glomerular cross section were investigated using the TUNEL assay, but no

### Table 1. Microaneurysm formation, mesangiolysis, number of glomerular monocytes/macrophages, glomerular accumulation of platelets, and creatinine clearance were not affected by sGC stimulation in the anti-Thy1 model on day 2 or 6

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 2</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>sGC-stimulated</td>
</tr>
<tr>
<td>Microaneurysms</td>
<td>1.5±0.2</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>Mesangiolysis (score 0–4)</td>
<td>2.9±0.9</td>
<td>3.1±0.7</td>
</tr>
<tr>
<td>Platelet accumulation (score 0–4)</td>
<td>3.7±0.2</td>
<td>3.4±0.5</td>
</tr>
<tr>
<td>Macrophages/monocytes, ( n )</td>
<td>3.6±1.4</td>
<td>3.6±1.2</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min</td>
<td>80.5±27</td>
<td>104.1±54</td>
</tr>
</tbody>
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Values are means ± SD expressed per 50 glomerular cross sections except for creatinine clearance. sGC, soluble guanylyl cyclase. The no. of glomerular microaneurysms was counted after sections were stained for JG-12. Platelet accumulation within glomeruli was assessed by semiquantitative scoring system after sections were stained for PL-1. The no. of monocytes/macrophages was counted after sections were stained for ED-1.
significant differences were identified between the groups on either days 2 or 6 (Fig. 3E).

Stimulation of sGC inhibits matrix expansion in anti-Thy1 nephritis. Since matrix accumulation usually accompanies mesangial proliferative glomerulonephritis, leading to renal fibrosis, we also assessed glomerular matrix expansion in the anti-Thy1 model, as indicated by collagen IV and fibronectin immunostaining in response to sGC regulation. sGC stimulation significantly reduced glomerular collagen IV (area positive for collagen IV was 22 ± 9/1006 vs. 32.7 ± 6%; P = 0.05; Fig. 4A) and glomerular fibronectin (area positive for fibronectin was 13.4 ± 5.4 vs. 29 ± 3.8%; P = 0.001; Fig. 4B) on day 6 of anti-Thy1 nephritis compared with placebo-treated rats. Figure 4 shows representative images of collagen IV immunostaining in BAY 41–2272-treated animals (Fig. 4C) compared with placebo-treated rats (Fig. 4D). No differences were apparent on day 2, when hardly any matrix expansion had yet occurred.

Stimulation of sGC reduces proteinuria during anti-Thy1 nephritis. Since proteinuria is considered an important indicator as well as a progression factor for renal disease, we also assessed proteinuria and creatinine clearance during experimental renal disease. As a consequence of sGC stimulation, BAY 41–2272-treated animals demonstrated decreased proteinuria on days 2 (19 ± 11 vs. 38.1 ± 16.5 mg/24 h; P < 0.05) and 6 (25.6 ± 8.8 vs. 53.1 ± 17.8 mg/24 h; P < 0.05) (Fig. 4E), whereas no differences could be found regarding renal function by creatinine clearance measurement (Table 1).

Antihypertensive effects of sGC stimulation do not affect glomerular cell proliferation. Although the antihypertensive effect of sGC stimulation by BAY 41–2272 in our particular experiment did not reach a level of significance compared with placebo-treated anti-Thy1 animals (P = 0.07), the effect of a decrease in systolic blood pressure per se on development of anti-Thy1 nephritis was tested. Although the standard triple antihypertensive treatment used in this control experiment resulted in a significant ~35% decrease of blood pressure on day 6, no differences in the glomerular proliferative response and matrix accumulation were detected compared with placebo-treated rats [PCNA-positive cells per glomerular cross section were 14.9 ± 1.6 vs. 14.2 ± 2.7, not significant (NS); glomerular score for collagen IV 2.6 ± 0.4 vs. 2.8 ± 0.2, NS].
sGC stimulation does not affect accumulation of inflammatory cells. Since other reports (10, 33) demonstrated that platelet aggregation can be inhibited by sGC stimulation via BAY 41–2272, we also investigated the glomerular accumulation of platelets (PL-1 positive) and monocytes/macrophages (ED-1 positive), which was equivalent in the BAY 41–2272 and placebo group at all time points during anti-Thy1 nephritis (Table 1).

BAY 41–2272 treatment in normal healthy rats does not affect either renal histology or function. To investigate whether sGC stimulation alters renal histology and function in normal rats, five additional healthy rats each were treated with either BAY 41–2272 or placebo. Despite a 6-day treatment, no difference in the cGMP levels of glomeruli from BAY 41–2272- or placebo-treated rats (1.9 ± 0.8 vs. 1.3 ± 0.3 relative glomerular, sGC β1-subunit protein/β-actin, NS) (not shown). Serum creatinine (0.182 ± 0.02 vs. 0.182 ± 0.02 mg/dl, NS), creatinine clearance (2.9 ± 0.5 vs. 3.1 ± 0.2 ml/min, NS), as well as proteinuria (6.5 ± 0.7 vs. 7.8 ± 2.2 mg/24 h, NS) were equal in BAY 41–2272- and placebo-treated rats. Renal histology as assessed by periodic acid-Schiff staining was completely normal and unremarkable in both groups. These findings indicate that BAY 41–2272 treatment did not cause any obvious renal side effects in normal healthy rats.

**DISCUSSION**

Considering the controversial and promiscuous actions of NO in renal cells and disease, we sought to examine whether direct, NO-independent, and specific stimulation of intracellular sGC may mimic part of typical NO-induced actions or cause completely different effects (28). In the present study, we demonstrate that the NO-sensitive enzyme sGC is tightly regulated in glomeruli during mesangial proliferative glomerulopathy.
ulonephritis and that direct and specific pharmacological stimulation of sGC early on causes various beneficial effects in this disease model.

Consistent with previous studies (22, 35), we could demonstrate endogenous expression of sGC protein in the normal rat kidney and glomerulus. In addition, glomerular sGC protein was upregulated predominantly at the late proliferative phase in anti-Thy1 nephritis, and immunostaining revealed a typical mesangial pattern. Despite endogenously stimulated expression of sGC during the late proliferative phase of the anti-Thy1 model, selectively the functional activity of sGC could be markedly increased by daily oral treatment of rats with a selective sGC stimulator (BAY 41–2272) from day 1 on. Treatment with this highly potent compound increased glomerular cGMP levels as the downstream messenger molecule but not sGC expression levels, clearly demonstrating that BAY 41–2272 reached its intracellular pharmacological target in vivo. These findings are consistent with previously published data on BAY 41–2272 and its closely related compounds (31–33) demonstrating NO-independent, but heme-dependent, enzyme activity mediated via a selective binding site located on the α-subunit of sGC. Whereas these compounds can enhance stimulatory effects of NO on sGC, NO antagonism, as provided by in vivo application of l-NAME in a low-NO model of hypertension (33), did not interfere with its enzyme-stimulating potency. In contrast to the results in anti-Thy1 nephritic rats, we could not detect any increase in glomerular cGMP levels after BAY 41–2272 treatment in normal healthy rats. While, clinically, some bleeding tendency at death (during surgery) indicated BAY 41–2272 action in these animals, no renal phenotype was apparent in these rats as assessed by histological and functional parameters. The reason for these unaltered glomerular cGMP levels by BAY 41–2272 treatment in normal rats is most likely the detection limit of the assay. While glomerular cGMP levels in anti-Thy1 nephritic rats were increased ~20-fold, glomerular cGMP levels in normal healthy rats were only in the femtomolar range, where potential differences are hard to detect and may be especially vulnerable to some degradation that cannot be prevented during the glomerular isolation process.

A major finding of the present study was that sGC stimulation reduces glomerular cell proliferation in anti-Thy1 nephritis. In this model, glomerular cell proliferation at the late proliferative phase (as represented by day 6 biopsies) is predominantly confined to the mesangium, whereas more than one-half of the proliferating glomerular cells at the early time point on day 2 are of endothelial and monocytes/macrophage origin (13). Our data suggest that the antiproliferative effect of sGC stimulation is exclusively confined to MCs, since double staining for PCNA- and OX-7-positive, proliferating MCs revealed a clear-cut decrease in the BAY 41–2272-treated rats associated with a reduced glomerular cell number on day 6 but no effect in the early phase of disease on day 2. In addition, microaneurysm formation being dependent on endothelial cell repair and the degree of mesangiolysis was not altered by this treatment. The reduction of glomerular hypercellularity is likely due to a sole inhibition of MC proliferation, because glomerular cell removal via apoptosis, the major pathway of resolution in this disease (3), was not altered by sGC stimulation. The fact that the decrease in systemic blood pressure per se does not interfere with the time course of mesangial proliferative glomerulonephritis is supported by the results of our control experiment using a standard triple regimen as an antihypertensive treatment as well as former studies in this particular nephritis model (37). Since platelet depletion in this model is associated with inhibition of glomerular cell proliferation (16) and sGC stimulation is involved in inhibition of platelet activation/aggregation, we cannot completely exclude indirect antiproliferative effects via sGC-stimulated platelets. Nevertheless, this interpretation is not supported by the results of our study, because BAY 41–2272 did not influence glomerular platelet aggregation during anti-Thy1 nephritis.

Besides its antiproliferative effect, sGC stimulation also markedly inhibited extracellular matrix accumulation, the second hallmark of renal disease progression, as indicated by collagen IV as well as fibronectin staining. Previous studies have frequently demonstrated a link between glomerular cell proliferation and matrix accumulation (8, 15), although glomerular matrix expansion and MC proliferation can also be dissociated (39). Further studies will have to differentiate between whether this antifibrotic effect of sGC stimulation is due to its antiproliferative action or possibly independent of it.

While the specific mechanisms mediating these antiproliferative and antifibrotic effects of sGC stimulation have to be investigated in future studies, cGMP produced in response to NO and natriuretic peptides has been demonstrated to regulate various genes involved in cellular proliferation and matrix production (24). In vascular smooth muscle cells, MC, and fibroblasts, mainly antiproliferative effects of cGMP involve inhibition of growth factor-induced Erk-1/2 activity, inhibition of the early growth response gene-1 (egr-1) (28), reduction of endothelin-1 synthesis, modulation of cell-cycle regulatory genes, and increased expression of MAP kinase phosphatase-1 (24). In addition, the NO-cGMP pathway has also been shown to inhibit expression of genes mediating matrix synthesis directly or indirectly via transforming growth factor-β, the major profibrotic cytokine. Studies in cultured MC demonstrated that the NO-cGMP system is able to downregulate the profibrotic mediator connective tissue growth factor (17) as well as thrombospondin-1, the activator of the transforming growth factor-β procyclotyke complex after glucose stimulation (25, 38).

sGC stimulation also significantly reduced proteinuria, which is considered an important indicator and progression factor in renal disease, at both early and later time points of anti-Thy1 disease. This antiproteinuric effect does not seem to be dependent on its antiproliferative and antifibrotic action, because on day 2 no other parameter besides proteinuria was affected by sGC stimulation. Since the development of proteinuria is frequently caused by alterations of the basement membrane or podocytes, the protective effect on proteinuria in this model may rather reflect stimulation of the sGC system in podocytes than in the mesangium. Although information on the role of the cGMP signaling cascade is very limited, the sGC-cGMP system is expressed in podocytes and can be stimulated by NO as demonstrated by in vitro studies (20, 22).

These various beneficial effects of BAY 41–2272 therapy could have been caused by a general interference with the disease inducing antibody binding or with the injury induction that follows antibody binding, especially since NO has been shown to be important in mediating MC injury in vitro and in this model (23, 29). To avoid interference with antibody
binding, BAY 41–2272 treatment was initiated after maximal antibody binding had already occurred (14, 16). The equal degree of mesangiolysis, microaneurysm formation, cellular proliferation/matrix accumulation, and influx of inflammatory cells early on day 2 in both groups further supports the conclusion that sGC stimulation by BAY 41–2272 neither affected disease induction/MC injury unspecifically, via interference with antibody binding, nor specifically, such as suggested for inhibition of NO synthase by NO2-monomethyl-L-arginine (L-NMMA) treatment (23). An interesting question is whether sGC stimulation can successfully prevent inhibition of mesangiolysis in a situation where NO production is inhibited via L-NMMA; this may be investigated in the future. Nevertheless as discussed before, unspecific interference with the disease induction process must be excluded, when a treatment such as L-NMMA with alteration of MC injury is started before disease induction, as done in the study by Narita et al. (23). On the other hand, initiation of treatment after antibody binding may come too late if NO and sGC are already important for mesangiolysis within the first hours after disease induction. Although the beneficial effects of sGC stimulation seen in this model system are similar to earlier studies with NO donors (28, 36) and contrary to some studies using NO synthase inhibitors (4, 9, 26, 34), the design of our study is not suitable for differentiating the diverse NO-mediated pathobiological effects on kidney disease. In addition, the protective effects gained by pharmacological sGC stimulation cannot be just extrapolated for the situation of the endogenous sGC in anti-Thy1 nephritis, where a specific sGC blockade due to the lack of available inhibitors will not be easily achievable.

In conclusion, we established specific pharmacological stimulation of sGC within glomerular cells as an effective therapeutic strategy in experimental mesangial proliferative glomerulonephritis, exerting antiproliferative, antifibrotic, and antiproteinuric properties, whereas effects regarding mesangiolysis and apoptosis were lacking. Treatment with an orally available sGC stimulator such as BAY 41–2272 thus may become a potential novel form of treatment for mesangial proliferative glomerulonephritis in humans, the most common form of glomerulonephritis in the Western world.

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