

Endogenous Fibroblast Growth Factor-2 Mediates Cytotoxicity in Experimental Mesangioproliferative Glomerulonephritis

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Abstract. Fibroblast growth factor-2 (FGF-2) is released from mesangial cells in experimental mesangioproliferative glomerulonephritis induced with anti-Thy 1.1 antibody. To investigate the functional role of released FGF-2, rats received either neutralizing anti-FGF-2 IgG or a functional peptide antagonist of FGF-2 (FGF₁₁₉₋₁₂₆) before or shortly after induction of anti-Thy 1.1 nephritis. In additional experiments, rats were treated with bolus injections of FGF-2 from 2 to 6 h after disease induction. The data showed that anti-FGF-2 therapy led to significant reductions of early mesangial cell injury (mesangiolysis, microaneurysm formation) and the subsequent mesangioproliferative changes (glomerular *de novo* expression of α -smooth muscle actin, mesangial cell proliferation, matrix

accumulation, and platelet influx). Conversely, injections of FGF-2 augmented both mesangial injury and the subsequent mesangioproliferative changes. Studies on the mechanisms underlying the amplification of mesangial cell injury by FGF-2 showed that anti-FGF-2 therapy reduced cell death at 2 and 8 h after disease induction by 58 and 54%, respectively. This was associated with significant reductions in the number of glomerular H₂O₂- and OH⁻-producing cells, as well as reduced glomerular production of nitric oxide. These data suggest that release of constitutively expressed FGF-2 after immune-mediated cell injury contributes to glomerular cell damage and thus identify FGF-2 as a novel mediator of cytotoxicity. (J Am Soc Nephrol 9: 792–801, 1998)

Glomerular mesangial cell injury, proliferation, and/or matrix accumulation characterize a large variety of human progressive renal diseases, including IgA nephropathy, para- or postinfectious glomerulonephritis, membranoproliferative glomerulonephritis, variants of idiopathic focal sclerosis, lupus nephritis, chronic renal transplant rejection, diabetic nephropathy, ischemic and hypertensive glomerular injury, and thrombotic microangiopathy (1–3). Mesangial damage may also contribute to the development of glomerulosclerosis independent of the underlying primary disease (4,5). Several pathogenetic factors have been identified that can lead to mesangial injury *in vivo*, including nephritogenic antibodies, immune complexes, complement, leukocytes, and hypertension. In contrast to these extrinsic mechanisms, no instances have yet been established in which intrinsic mesangial factors contribute to the glomerular injury. Identification of such factors will have relevance for novel therapeutic approaches to glomerular diseases.

Fibroblast growth factor-2 (FGF-2; basic FGF) is a widely expressed cytokine with pleiotropic activities (6,7). In contrast to most growth factors, FGF-2 is constitutively expressed at relatively high levels in a large variety of cells (6–8). FGF-2

lacks a signal peptide and is released mainly after cell injury (8,9). In glomerular mesangial cells, FGF-2 is constitutively expressed and released after (sub-)lethal mesangial cell injury (8,10). Both *in vitro* and *in vivo*, FGF-2 is mitogenic for mesangial cells (as well as endothelial cells) (8,11–15). Apart from its mitogenicity, FGF-2 may also modulate other aspects of glomerular cell behavior. Thus, we have recently shown that exogenous FGF-2 injections induce glomerulosclerosis in a model of membranous glomerulonephritis (12) and that this was associated with the induction of apoptosis in glomerular epithelial cells (16). In the present study, we have attempted to clarify the biological role of endogenous FGF-2 in modulating mesangial injury and cell behavior by antagonizing or amplifying its biological activity during the early phase of the anti-Thy 1.1 (or antithymocyte serum) glomerulonephritis model in rats. In this model injection of an antimetastatic cell antibody (anti-Thy 1.1/antithymocyte serum) results in complement-dependent lysis of the mesangial cells, followed by an overshooting reparative phase that resembles human mesangioproliferative nephritis (17,18).

Materials and Methods

Generation of a Neutralizing Anti-FGF-2 Antibody

A goat antihuman recombinant FGF-2 IgG antibody, purified by protein A-Sepharose chromatography (19), was tested for neutralizing activity by analyzing ³H-thymidine incorporation rates into NIH-3T3 fibroblasts (Table 1). *In vivo* studies showed that this anti-FGF-2 antibody reduced the initial vascular smooth muscle cell replication

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Table 1. Biological activity of anti-fibroblast growth factor (FGF)-2 IgG and FGF₁₁₉₋₁₂₆^a

FGF-2 (ng/ml) + anti-FGF-2 IgG (μg/ml)	0 + 0	5 + 0	5 + 100	5 + 200	5 + 300	5 + 400	5 + 500	5 + normal goat IgG (500 μg/ml)
³ H-Thymidine incorporation (cpm) into 3T3-fibroblasts	500	8800	5300	1300	4300	1100	1200	7500
FGF-2 (ng/ml) + FGF ₁₁₉₋₁₂₆ (ng/ml)	0 + 0	10 + 0	10 + 10	10 + 100	10 + 100	10 + 1000	10 + 1000	10 + scrambled peptide (1000 ng/ml)
³ H-Thymidine incorporation (cpm) into 3T3-fibroblasts	870 ± 100	4740 ± 1040	4830 ± 950	4100 ± 620	1890 ± 570	4550 ± 990	3400 ± 1320	
³ H-Thymidine incorporation (cpm) into mesangial cells	960 ± 370	3300 ± 1620	2790 ± 980	3030 ± 1040	1590 ± 800			

Thymidine incorporation into 3T3 fibroblasts and rat mesangial cells stimulated *in vitro* with FGF-2 in the presence or absence of neutralizing anti-FGF-2 IgG or FGF₁₁₉₋₁₂₆.

^a Means ± SD; n = 3 each.

by 50% in the carotid angioplasty model at 48 h (M. Reidy, unpublished data).

Preparation of an Antagonistic FGF-2 Peptide

In a second approach, a synthetic peptide corresponding to amino acid 119 to 126 of human recombinant FGF-2 (FGF₁₁₉₋₁₂₆) was synthesized (Saxon Biochemicals, Hannover, Germany and Bachem, Heidelberg, Germany). In cultured endothelial cells, FGF₁₁₉₋₁₂₆ acted as a functional antagonist for FGF-2 (20). The ability of FGF₁₁₉₋₁₂₆ to neutralize the biological activity of FGF-2 was tested *in vitro* by analyzing the ³H-thymidine incorporation into FGF-2-stimulated NIH-3T3 fibroblasts, as well as rat mesangial cells (21) (Table 1). For control experiments, we also obtained an octapeptide composed of the same amino acids as FGF₁₁₉₋₁₂₆ but with a scrambled amino acid sequence (Table 1).

Experimental Design

All animal experiments were approved by the local review boards in Seattle, Washington, or Hannover, Germany.

Treatment with Neutralizing Goat Anti-FGF-2 IgG. Twelve male Wistar rats (Simonsen, Gilroy, CA) weighing 150 to 180 g received goat anti-FGF-2 IgG (five rats) or control IgG (five rats) 30 min before disease induction. Each rat was injected with 30 mg of IgG intraperitoneally and 30 mg of IgG intravenously. Mesangioproliferative glomerulonephritis was then induced by a tail vein bolus injection of polyclonal goat anti-rat thymocyte antiserum (17,18). Renal biopsies for histologic evaluation were obtained at days 2 and 4 and at sacrifice (day 7).

To investigate whether injection of the anti-FGF-2 IgG affected the subsequent glomerular binding of the antithymocyte antibody, eight additional rats were injected with anti-FGF-2 IgG (n = 4) or control IgG (n = 4) as described above. Thirty minutes later, all rats received the antithymocyte antiserum. This antiserum had been spiked with ¹²⁵I-labeled antithymocyte IgG (22). One hour after the antithymocyte serum injection, the kidneys were perfused free of blood (23), glomeruli were isolated by differential sieving (18), and glomerular radioactivity was determined in a gamma counter.

Treatment with FGF₁₁₉₋₁₂₆. Fifteen male Wistar rats (Charles River, Sulzfeld, Germany) weighing 150 to 170 g received an intravenous bolus injection of monoclonal anti-Thy 1.1 IgG₁ (21). One hour after induction of the disease, a continuous intravenous infusion of FGF₁₁₉₋₁₂₆ in PBS pH 7.4 (10 mg in 200 μl of PBS; five rats), scrambled peptide (10 mg in 200 μl of PBS; three rats), or PBS (five rats) was performed via the tail vein for the next 30 min. Additional intravenous bolus injections of FGF₁₁₉₋₁₂₆ (5 mg in 100 μl of PBS each), scrambled peptide (5 mg in 100 μl of PBS each), or PBS were administered at 3, 4, 5, and 6 h after disease induction. Renal biopsies for histologic evaluation were obtained at days 2 and 4 and at sacrifice (day 7). Serum and whole blood were obtained at 1 h after injection of anti-Thy 1.1 antibody for the determination of total hemolytic complement (CH₅₀) and platelet counts.

In a separate series of experiments, the very early events after the induction of the nephritis were investigated. Using the experimental design as described above, 36 rats received either PBS or FGF₁₁₉₋₁₂₆ injections and were studied at 2, 8, 24, and 96 h after disease induction (n = 6 per group and time point). At the four time points, renal tissue was obtained from the right kidney for histologic analyses. Also, at 8, 24, and 96 h the remaining left kidneys were perfused with ice-cold pyrogen-free saline, and glomeruli were isolated for the determination of glomerular nitric oxide (NO) production (see below), using standard techniques (17).

Injections of FGF-2. Twelve male Wistar rats (Charles River, Sulzfeld, Germany) weighing 140 to 160 g received an intravenous bolus injection of 0.8 mg/kg anti-Thy 1.1 IgG₁ (clone OX-7). A lower anti-Thy 1.1 IgG dose was chosen in this experiment to allow a better detection of disease amplification by FGF-2. Two, four, and six hours after disease induction, all rats received intravenous bolus injections of either 5 μ g of recombinant human FGF-2 (a kind gift of Amgen, Boulder, CO) or PBS. Renal biopsies for histologic evaluation were obtained at days 2 and 4 and at sacrifice (day 7).

Renal Morphology

Tissue for light microscopy and immunoperoxidase staining was fixed in methyl Carnoy's solution (17) and embedded in paraffin. Four-micrometer sections were stained with the periodic acid-Schiff reagent and counterstained with hematoxylin. In the periodic acid-Schiff-stained sections, the total number of cells per glomerular cross-section, as well as the number of mitoses within the glomerular tuft (extrapolated to mitoses per 50 glomerular cross-sections), was determined. Mesangiolytic was graded on a semiquantitative scale as follows: 0 = no mesangiolytic; 1 = segmental mesangiolytic; 2 = global mesangiolytic; and 3 = microaneurysm.

Immunofluorescence Staining

Immunofluorescence detection of glomerular rat complement C3c and C5b-9 was carried out on 4- μ m sections of frozen kidney tissue, using a direct immunofluorescence procedure. Primary antibodies included:

- Biotinylated (24) antibody 2A1, a murine monoclonal antibody against rat C5b-9 (25) (kindly provided by M. Schulze, Hannover, Germany).
- A biotinylated (24) IgG fraction of polyclonal rabbit-antihuman C3c (Dako, Glostrup, Denmark).

Biotin was then detected with streptavidin-FITC (Amersham, Braunschweig, Germany). In control sections, the primary antibody was substituted with equivalent concentrations of a biotinylated irrelevant murine monoclonal antibody or normal rabbit IgG. For the evaluation of immunofluorescent stains, glomerular cross-sections were graded semiquantitatively as either "0" (indistinguishable from control) or positive, according to the degree of glomerular fluorescence (0.5 = trace; 1 = weak; 2 = moderate; 3 = intense).

Immunoperoxidase Staining

Four-micrometer sections of methyl Carnoy's fixed biopsy tissue were processed by an indirect immunoperoxidase technique as described (17). Primary antibodies included:

- α -SM1, a murine monoclonal antibody to an NH₂-terminal synthetic decapeptide of α -smooth muscle actin (kind gift of G. Gabbiani, Geneva, Switzerland) (26).
- 19A2 (American Biotech, Plantation, FL), a murine IgM monoclonal antibody against human proliferating cell nuclear antigen (PCNA), which is expressed by actively proliferating cells and correlates with the cell proliferation as assessed by the conventional method of ³H-thymidine incorporation (27).
- ED1 (Bioproducts for Science, Indianapolis, IN), a murine monoclonal IgG antibody to a cytoplasmic antigen present in monocytes, macrophages, and dendritic cells (28).
- PL-1, a murine monoclonal antibody against rat platelets (kind gift of W. Timens, Gröningen, The Netherlands) (29).

- Affinity-purified polyclonal goat antihuman/bovine type IV collagen (Southern Biotechnology, Birmingham, AL).
- An affinity-purified IgG fraction of polyclonal rabbit anti-rat fibronectin (Southern Biotechnology).
- An IgG fraction of polyclonal guinea pig anti-rat type I collagen (30) (kindly provided by L. Iruela-Arispe, Seattle, WA).

For all biopsies, negative controls consisted of substitution of the primary antibody with equivalent concentrations of an irrelevant murine monoclonal antibody or normal rabbit or goat IgG. Evaluation of all slides was performed by an observer, who was unaware of the origin of the slides.

To obtain mean numbers of proliferating cells or infiltrating leukocytes in glomeruli, more than 30 consecutive cross-sections of glomeruli containing more than 20 discrete capillary segments were evaluated, and mean values per kidney were calculated. For the evaluation of the immunoperoxidase stains for α -smooth muscle actin, type IV collagen, fibronectin, and platelets, each glomerular area was graded semiquantitatively, and the mean score per biopsy was calculated. Each score reflects changes mainly in the extent rather than the intensity of staining and depends on the percentage of the glomerular tuft area showing focally enhanced positive staining: 0 = absent staining; 0.5 = 1 to 5%; 1 = 5 to 25%; 2 = 25 to 50%; 3 = 50 to 75%; 4 = >75% (31,32).

Immunohistochemical Double-Staining

Double-immunostaining was performed as reported previously (4) by first staining the sections for proliferating cells with 19A2, the IgM monoclonal antibody to PCNA, using an indirect immunogold procedure. Sections were then incubated with the IgG₁ monoclonal antibody α -SM1 against α -smooth muscle actin, which is present in activated mesangial cells (18). Cells were identified as proliferating mesangial cells if they showed positive nuclear staining for PCNA and if the nucleus was completely surrounded by cytoplasm positive for α -smooth muscle actin. Negative controls included omission of either of the primary antibodies, in which case no double-staining was noted.

Immunohistochemical Detection of Glomerular Oxygen Radical Production

As described elsewhere (33), glomerular *in situ* production of hydrogen peroxide was determined using 6- μ m sections of frozen tissue that were incubated for 30 min at 37°C in 0.05 M Tris buffer, pH 7.0, containing 7.5 mg/L 3,3'-diaminobenzidine (Sigma Chemical Corp., St. Louis, MO) and 1.7 mM nickel chloride. Thereafter, sections were fixed in 4% paraformaldehyde and counterstained with methyl green. Glomerular superoxide anion production was assessed using the same method, except that the incubation buffer additionally contained 1 μ M sodium azide and 5 mM MnCl₂.

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling Staining for the Detection of Glomerular Cell Death

In situ detection of cell death was performed using terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling, as described (34). Briefly, using 4- μ m sections of formaldehyde-fixed tissue, nuclear proteins were stripped using proteinase K, and the slides were then incubated with terminal deoxynucleotidyl transferase (Pharmacia, Freiburg, Germany) and biotin-14-dATP (Life Technologies, Eggenstein, Germany). Incorporated biotinylated ATP was detected using the ABC kit (Vector, Burlingame, CA) and 3,3'-

diaminobenzidine plus nickel (see above). Positive controls included a DNase control (DNase 1; Sigma), in which all nuclei exhibited positive staining. Negative controls included omission of biotin-14-ATP, in which case no nuclear staining could be detected.

Glomerular NO Production

Glomeruli were seeded at a density of 2000/well into 24-well plates (Nunc, Roskilde, Denmark) in Dulbecco's modified Eagle's medium (without phenol red), supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM/ml L-glutamine (all from Life Technologies), 50 µmol/L tetrahydrobiopterin (Calbiochem, Bad Soden, Germany), and 0.1% low endotoxin bovine serum albumin (Sigma). Three wells of glomeruli per preparation were then incubated at 37°C and 5% CO₂ for 48 h. As positive controls, two additional wells with 2000 glomeruli each were incubated in the above medium plus 1 µg/ml lipopolysaccharide. Glomerular NO production was indirectly assessed by measuring the stable metabolite nitrite in the glomerular supernatants via the Griess reaction (Promega, Leiden, The Netherlands). In pilot experiments, we had been unable to detect significant

amounts of nitrate in the glomerular supernatants, because reduction of nitrate to nitrite using glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and nitrate reductase (35) did not yield higher values in the Griess reaction than direct measurement of nitrite (data not shown). Therefore, nitrate concentrations in the glomerular supernatants were not routinely measured.

Miscellaneous Measurements

Plasma CH₅₀ levels were measured using standard techniques (36). Peripheral blood platelet counts were determined after lysis of erythrocytes with 1% ammonium oxalate. Urinary protein was measured using the Bio-Rad protein assay (Bio-Rad Laboratories, Munich, Germany) and bovine serum albumin (Sigma) as a standard.

Statistical Analysis

All values are expressed as means ± SD. Statistical significance (defined as *P* < 0.05) was evaluated using the unpaired *t* test.

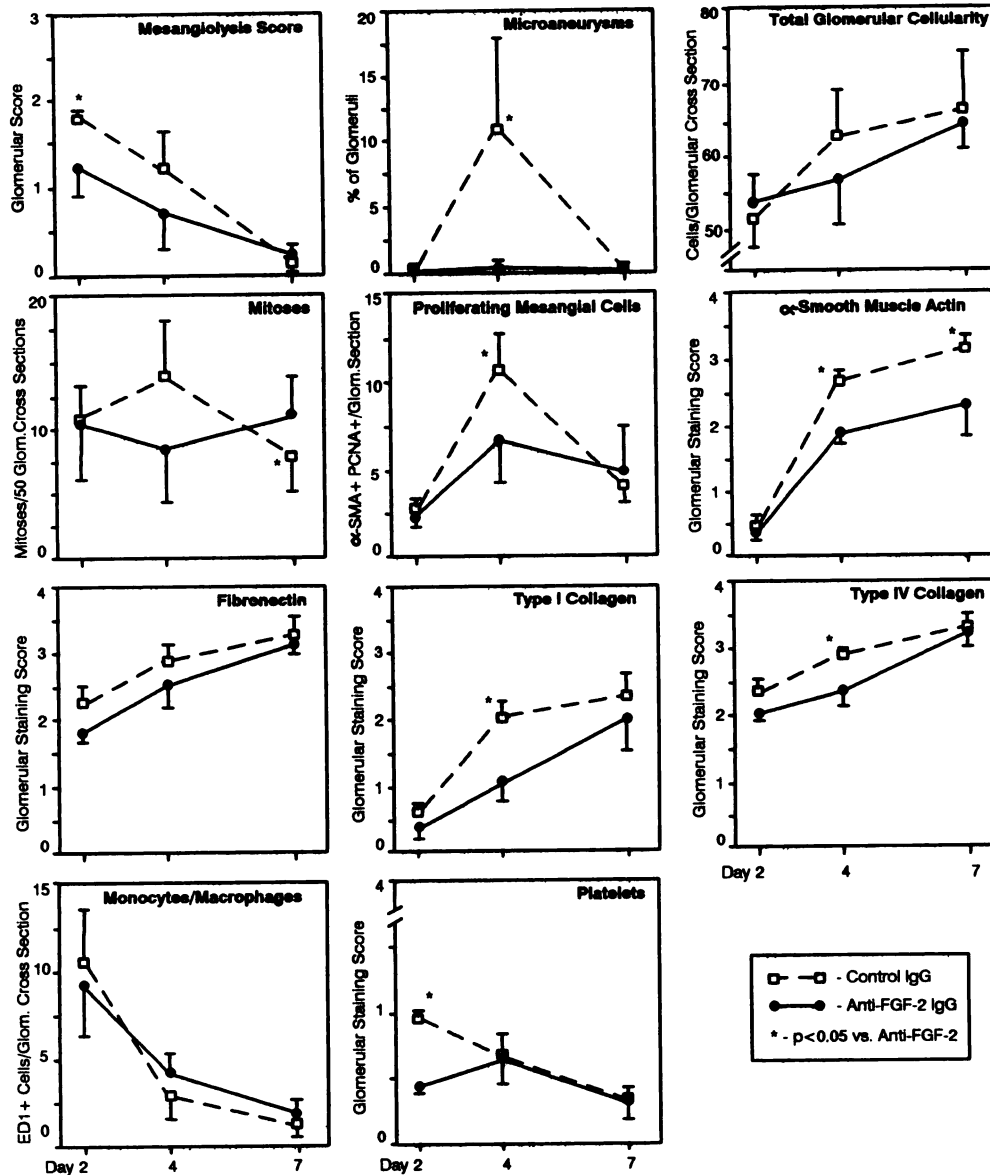


Figure 1. Anti-fibroblast growth factor-2 (anti-FGF-2) IgG treatment. Glomerular changes in rats with anti-Thy 1.1 mesangioproliferative glomerulonephritis treated 30 min before disease induction with either neutralizing anti-FGF-2 IgG (*n* = 5) or irrelevant control IgG (*n* = 5). Values are means ± SD. "αSMA + PCNA+/Glom. Section" indicates the number of cells double-immunostained for both α-smooth muscle actin and proliferating cell nuclear antigen per glomerular cross-section.

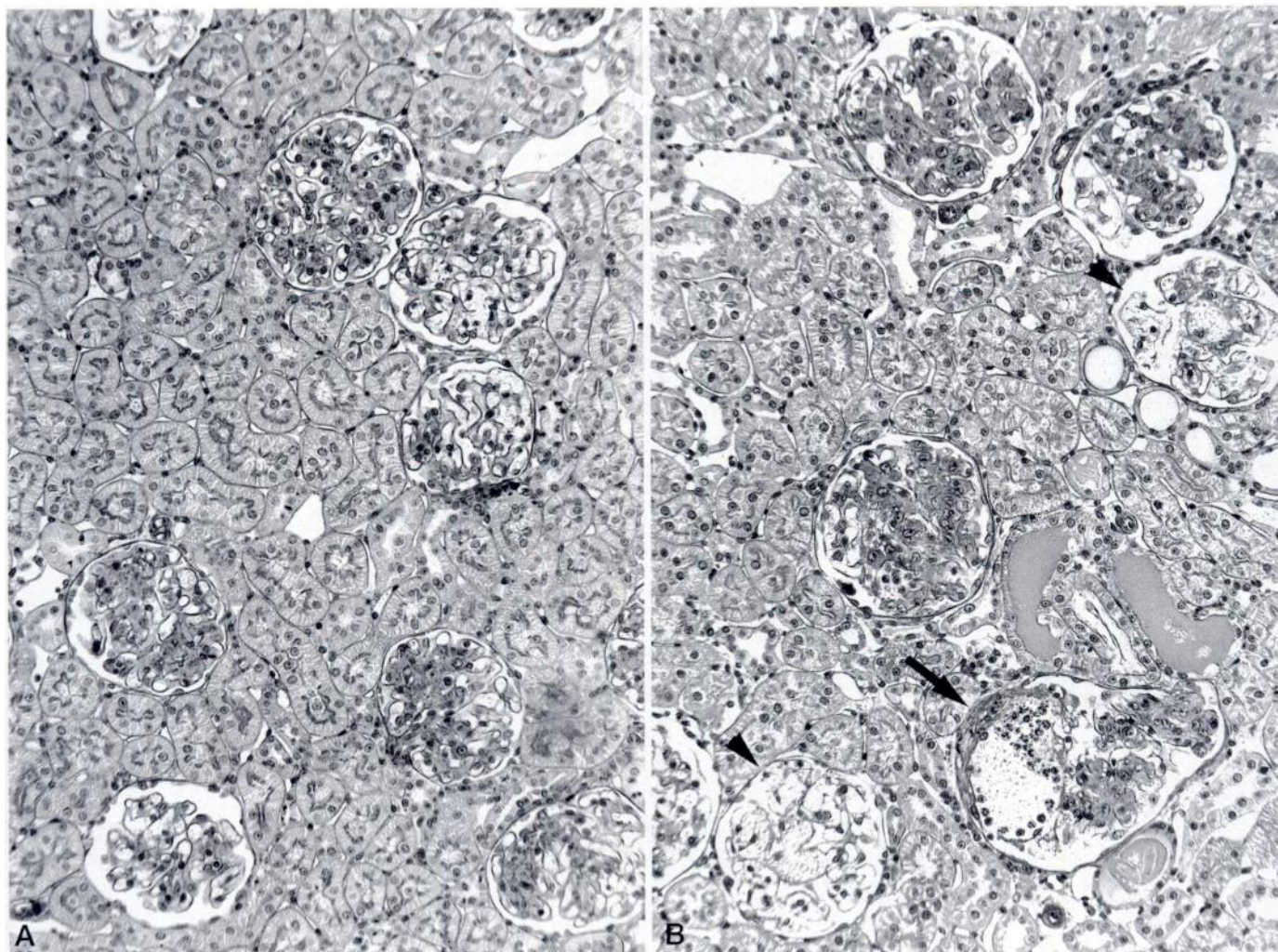


Figure 2. Anti-FGF-2 IgG treatment. Renal cortical changes at day 4 of anti-Thy 1.1 nephritis in rats treated with neutralizing anti-FGF-2 IgG (A) or irrelevant control IgG (B). Glomerular microaneurysm formation (arrow) is completely prevented in anti-FGF-2 IgG-treated rats. There is also less mesangiolysis in Panel A compared with Panel B (arrowheads). Periodic acid-Schiff stain. Magnification, $\times 200$.

Results

Treatment with Neutralizing Goat Anti-FGF-2 IgG

Compared to rats treated with control IgG, treatment of nephritic rats with anti-FGF-2 IgG led to a reduction of the glomerular mesangiolysis score and completely prevented the most severe consequence of mesangiolysis, *i.e.*, glomerular microaneurysm formation (Figures 1 and 2).

Counting of total glomerular cell numbers and mitotic figures per glomerular cross-section revealed a reduction in the anti-FGF-2 IgG-treated group at day 4 (Figure 1). Treatment with anti-FGF-2 IgG significantly reduced the glomerular *de novo* expression of α -smooth muscle actin, a specific marker of activated mesangial cells (18), as well as the maximum number of proliferating mesangial cells (Figure 1).

Glomerular immunostaining for types I and IV collagen and fibronectin increased with the duration of the disease, as described previously (37). Immunostaining scores for types I and IV collagen at day 4 were significantly lower in the rats that had received anti-FGF-2 IgG than in those that had received control IgG (Figure 1).

Treatment with anti-FGF-2 IgG had no significant influence on the glomerular monocyte/macrophage influx in anti-Thy 1.1 nephritis (Figure 1). The induction of disease also led to an early glomerular influx of platelets, which was significantly reduced in the anti-FGF-2 IgG-treated rats (Figure 1).

Assessment of glomerular antithymocyte antibody binding at 1 h showed no significant difference between anti-FGF-2 IgG-treated rats and control IgG-treated rats ($12,160 \pm 1595$ cpm/76,000 glomeruli in anti-FGF-2 IgG rats *versus* $11,680 \pm 1199$ cpm/76,000 glomeruli in control IgG rats; $n = 4$ each).

Treatment with an Antagonistic FGF-2 Peptide (FGF₁₁₉₋₁₂₆)

As shown in Figure 3, treatment of nephritic rats with FGF₁₁₉₋₁₂₆ had similar effects as treatment with anti-FGF-2 IgG. Compared to rats treated with PBS, treatment with FGF₁₁₉₋₁₂₆ led to a significant reduction of the mesangiolysis score at day 2, completely prevented the glomerular formation of microaneurysms at day 4, and led to significant reductions of glomerular mitoses at days 2 and 4, as well as decreasing the

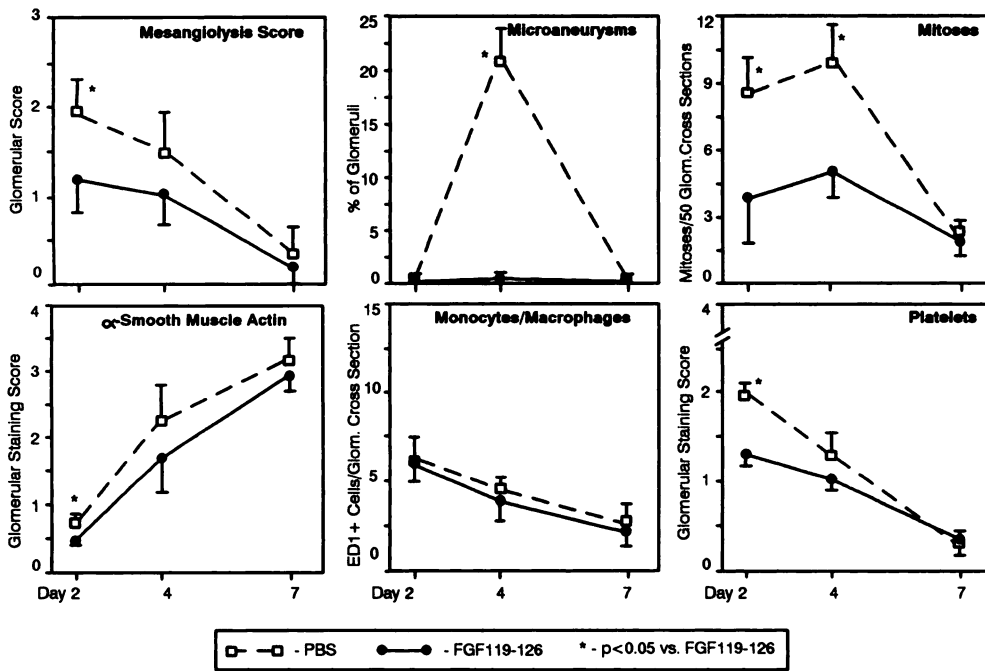


Figure 3. FGF₁₁₉₋₁₂₆ treatment. Glomerular changes in rats with anti-Thy 1.1 mesangioproliferative glomerulonephritis at days 2, 4, and 7 treated from 1 to 6 h after disease induction with either FGF₁₁₉₋₁₂₆ peptide (n = 5) or PBS (n = 5). Values are means ± SD.

glomerular staining scores for α-smooth muscle actin and platelets at day 2 after disease induction (Figure 3). Similar to anti-FGF-2 IgG-treated rats, treatment with FGF₁₁₉₋₁₂₆ did not affect glomerular monocyte/macrophage counts (Figure 3). No significant effects of FGF₁₁₉₋₁₂₆ on the total hemolytic activity of complement, CH₅₀, or peripheral blood platelet counts were detected in the treated nephritic rats at 24 h compared with the PBS-treated nephritic rats (data not shown). All data obtained in the rats that had received scrambled peptide instead of FGF₁₁₉₋₁₂₆ were within the range of PBS-treated, nephritic rats (data not shown).

To analyze the mechanism(s) underlying the beneficial effects of FGF₁₁₉₋₁₂₆ in the anti-Thy 1.1 nephritis, an additional series of experiments was carried out, in which the very early

events after disease induction were studied. The data showed that FGF₁₁₉₋₁₂₆ treatment led to significant reductions of: (1) glomerular cell death as identified by the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling method at 2 and 8 h after disease induction; (2) the number of glomerular hydrogen peroxide and superoxide anion-producing cells at 2 and/or 8 h; and (3) glomerular *in vitro* nitrite production at 8 h (Figures 4 through 6). Glomerular deposition of C3c and C5b-9 at 24 h was similar in both PBS- and FGF₁₁₉₋₁₂₆-treated rats and ranged from 2+ to 3+ (C3c) and 1+ to 2+ (C5b-9).

Treatment with Recombinant Human FGF-2

To examine the effects of an opposite approach, *i.e.*, amplification of FGF-2 effects, nephritic rats received repeated bolus

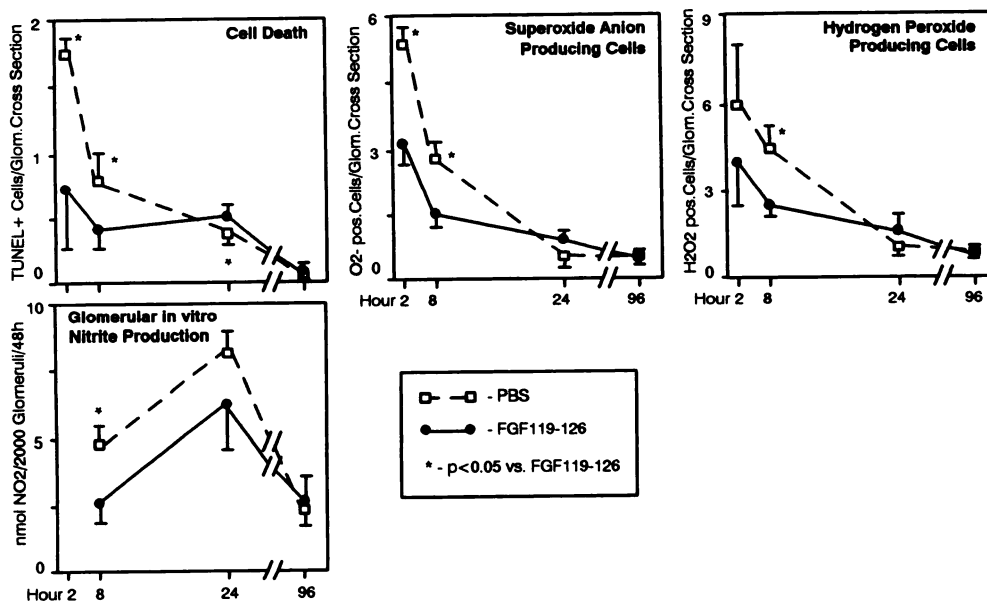


Figure 4. FGF₁₁₉₋₁₂₆ treatment: early changes. Early glomerular changes and 48-h *in vitro* glomerular nitrite production rates in rats with anti-Thy 1.1 mesangioproliferative glomerulonephritis at 2, 8, 24, and 96 h treated from 1 to 6 h after disease induction with either FGF₁₁₉₋₁₂₆ peptide (n = 6) or PBS (n = 6). Values are means ± SD.

injections of FGF-2. To better allow the detection of augmented injury, the dose of the nephritic anti-Thy 1.1 antibody was reduced to 80% of the regular dose (see above). Under these circumstances, FGF-2 injections at 2, 4, and 6 h after disease induction led to significant increases in the mesangiolysis score, the number of microaneurysms, glomerular mitoses, the *de novo* expression of glomerular α -smooth muscle actin, and glomerular platelet influx at days 2 and/or 4 after disease induction (Figure 7).

Discussion

We have previously shown that FGF-2 is released from mesangial cells during the early phase of anti-Thy 1.1 nephritis as a consequence of antibody and complement-mediated mesangial cell injury (8). Given that FGF-2 is mitogenic for cultured rat mesangial cells (14), we had hypothesized that release of stored mesangial cell FGF-2 may contribute to the onset of the glomerular cell activation, proliferation, and matrix accumulation in this mesangioproliferative glomerulone-

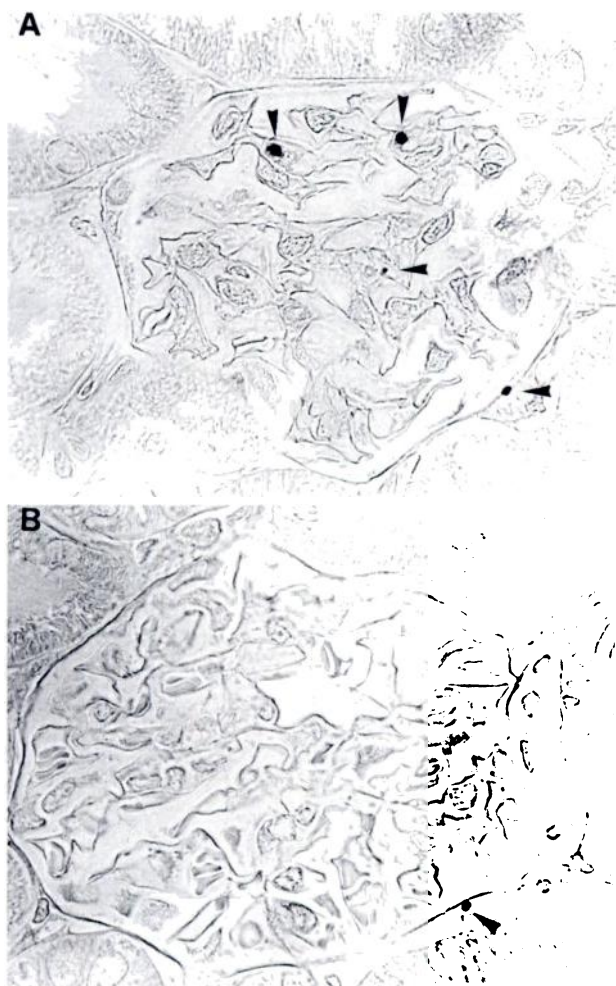


Figure 5. Glomerular cell death (as detected by TUNEL) at 2 h after induction of anti-Thy 1.1 nephritis. Positive nuclear staining (arrowheads) is observed in three intraglomerular cells in the PBS-treated rat (A), whereas no intraglomerular cell is labeled in the FGF₁₁₉₋₁₂₆-treated rat (B). Magnification, $\times 1000$.

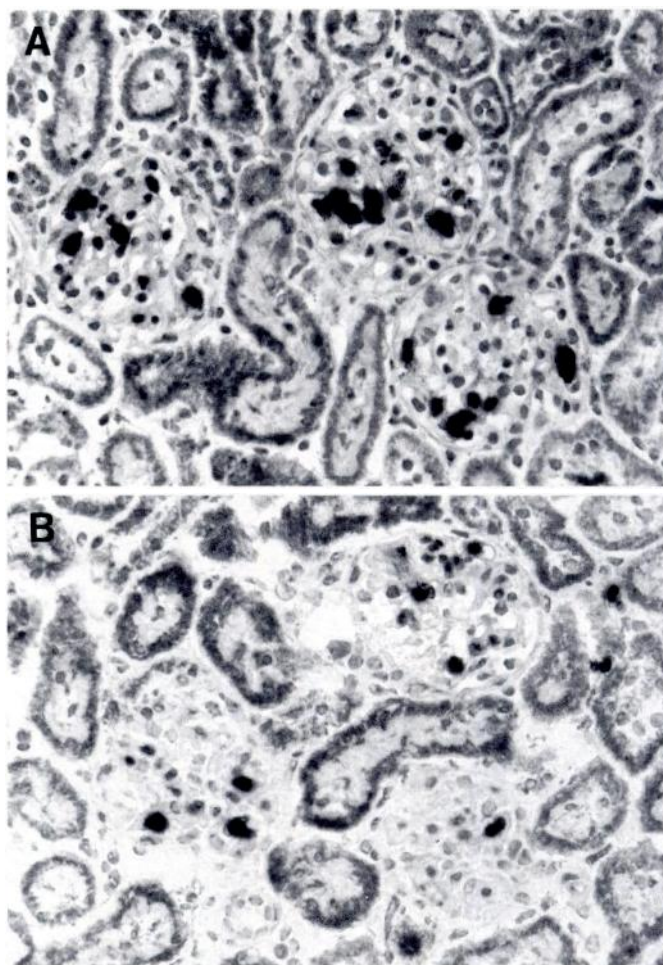


Figure 6. *In situ* detection of glomerular H₂O₂-producing cells at 2 h after induction of anti-Thy 1.1 nephritis. Compared with the PBS-treated rat (A), fewer glomerular cells exhibit positive staining in the FGF₁₁₉₋₁₂₆-treated rat (B). Some peroxidase activity is present in tubules. Magnification, $\times 400$.

phritis model. Indeed, the present study appeared to confirm this hypothesis because specific antagonism of FGF-2 *in vivo* exerted beneficial effects on all of these parameters (*i.e.*, it reduced glomerular α -smooth muscle actin expression, cell proliferation, and collagen accumulation).

The unexpected finding of the present study was that anti-FGF-2 therapy ameliorated the initial mesangiolysis and completely prevented its most severe consequence, *i.e.*, the formation of glomerular microaneurysms. In contrast, in our previous studies with neutralizing anti-PDGF antibody or heparins, *i.e.*, other inhibitors of mesangial cell growth, initiation of therapy before the time of mesangiolysis reduced subsequent mesangioproliferative changes at the price of persistent or even augmented mesangiolysis and microaneurysm formation (R. Johnson and J. Floege, unpublished observations; see also references 21, 38, and 39). Therefore, the major effect of FGF-2 antagonism in the present study is unlikely to relate to an inhibition of FGF-2-mediated mesangial cell proliferation. Rather, the data suggest that anti-FGF-2 therapy reduced the extent of the initial mesangial injury and cell death and thereby

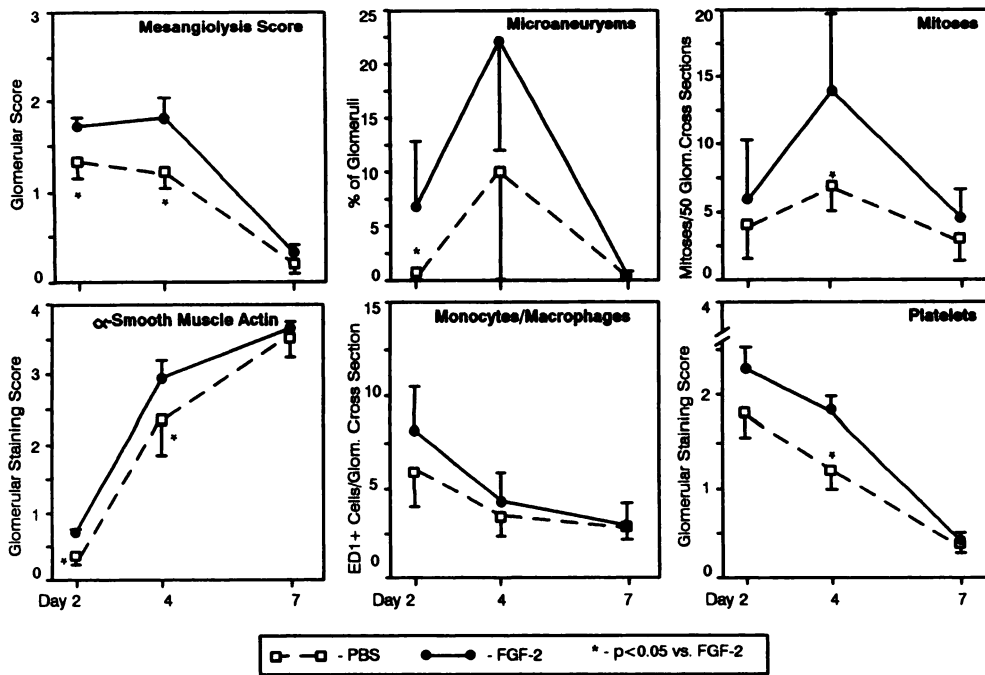


Figure 7. FGF-2 treatment. Glomerular changes in rats with anti-Thy 1.1 mesangioproliferative glomerulonephritis at days 2, 4, and 7 treated from 2 to 6 h after disease induction with either FGF-2 bolus injections ($n = 6$) or PBS ($n = 6$). Note that the dose of the nephritogenic anti-Thy 1.1 antibody was reduced to 80% of the regular dose in these experiments. Values are means \pm SD.

led to a diminution of the subsequent need for a regenerative glomerular proliferative response. Consistent with this hypothesis are the findings in FGF-2-injected nephritic rats, in which mesangiolysis and subsequent mesangial proliferation were augmented (in contrast to normal rats, in which FGF-2 did not affect glomerular morphology; reference 8).

Under our study conditions, anti-FGF-2 therapy could have reduced mesangial cell injury in anti-Thy 1.1 nephritis in a number of ways:

1. Interference with glomerular binding of the nephritogenic anti-Thy 1.1 antibody. This is unlikely given our glomerular anti-Thy 1.1 IgG binding data and given the identical glomerular influx of monocytes/macrophages in both of the experiments with anti-FGF-2 IgG and FGF₁₁₉₋₁₂₆.
2. Hemodynamic effects. FGF-2 can induce an NO-dependent vasodilation (40) and, consequently, release of glomerular FGF-2 might lower intraglomerular pressure. Lowering of intraglomerular pressure, for example, by angiotensin-converting enzyme inhibition, has been shown to reduce injury in the anti-Thy 1.1 nephritis model (41). In the present study, we observed the opposite, *i.e.*, that FGF-2 augmented injury. Furthermore, glomerular capillary pressure at 24 h after induction of anti-Thy 1.1 nephritis has been shown to increase (42). Although no micropuncture studies have been performed in the current study, these observations do not support a predominantly hemodynamic action of FGF-2.
3. Effects on systemic complement levels. Complement depletion prevents the development of anti-Thy 1.1 nephritis (18). Anti-FGF-2 therapy, however, did not affect CH₅₀ levels or glomerular complement deposition.
4. Effects on platelets. Platelets mediate some of the mesangial cell proliferation and activation, but not the mesangiolysis, in anti-Thy 1.1 nephritis (17,18). Consequently, it is possible that anti-FGF-2 therapy may have reduced glomerular

cell activation and proliferation indirectly through the reduction of the glomerular platelet influx (Figures 1 and 3). However, the amelioration of the early mesangial injury in anti-FGF-2-treated rats cannot be attributed to this reduced platelet influx (but instead is likely to underlie it).

The above considerations have led us to investigate the effects of anti-FGF-2 therapy on other mediators of the glomerular cytotoxic injury in anti-Thy 1.1 nephritis. Previous studies have demonstrated that NO mediates the mesangiolysis in anti-Thy 1.1 nephritis (43). Cytotoxic injury induced by NO is effected by direct inhibition of mitochondrial respiration and by peroxynitrite, a reaction product of NO and reactive oxygen species (44). Thus far, only few data are available to link FGF-2 to the production of NO and reactive oxygen species. Apart from inducing NO-dependent vasodilation (40), FGF-2 has been shown to induce expression of inducible NO synthase in mesangial cells (44). In bovine articular chondrocytes, the formation of oxygen radicals was augmented by FGF-2 (45). The present study extends these data by showing that anti-FGF-2 therapy in anti-Thy 1.1 nephritis reduced glomerular NO production and oxygen radical formation (both likely to be derived from infiltrating leukocytes based on the time kinetics of the changes). Studies in a pheochromocytoma cell line have also demonstrated that FGF-2, apart from its potential role in inducing oxygen radical and/or NO-mediated cell death (*e.g.*, via inducing NO synthase), can prime these cells to greatly increase their susceptibility to peroxynitrite-induced apoptosis (46). Both of these observations can explain how anti-FGF-2 therapy led to 58% (2 h) and 54% (8 h) reductions of glomerular cell death in early anti-Thy 1.1 nephritis (Figure 4).

In conclusion, our study is the first to demonstrate that FGF-2 released from glomerular cells after immunologic injury modulates the very early phase of mesangioproliferative glomerulonephritis via an amplification of cytotoxic injury, and

thus defines a novel function of FGF-2. Because endogenous FGF-2 appears to be just one mediator of cytotoxic damage in the anti-Thy 1.1 model (in addition to antibody, complement, and NO), and because anti-FGF-2 therapy in the current study was confined to the first hours of the disease, it was not unexpected that the effects of anti-FGF-2 therapy were blunted at day 7. Therefore, the importance of FGF-2 in mediating mesangial cell proliferation in later phases of the disease remains to be investigated. The early amplification of cytotoxic damage appears to be at least partially due to the effects of FGF-2 on the generation of NO and reactive oxygen species, and/or direct effects of FGF-2 on apoptosis. On the basis of these observations, anti-FGF-2 therapy may have a particular role in the many renal diseases associated with ongoing mesangiolysis and glomerular cell proliferation (3). Our findings also offer potentially new insights into early mechanisms operative in inflammatory disorders in general, because FGF-2 is widely and constitutively expressed in most tissues (6,7) and may therefore contribute to cytotoxic injury at other sites as well.

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