Specific Antagonism of PDGF Prevents Renal Scarring in Experimental Glomerulonephritis

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Abstract. Glomerular mesangial cell proliferation and/or mes- 
angial matrix accumulation characterizes many progressive 
renal diseases. Rats with progressive mesangioproliferative 
glomerulonephritis were treated from day 3 to day 7 after 
disease induction with a high-affinity oligonucleotide aptamer-
antagonist against platelet-derived growth factor-B chain 
(PDGF-B). In comparison with nephritic rats that received 
vehicle or a scrambled aptamer, treatment with the PDGF-B 
aptamer led to a significant reduction of mesangioproliferative 
changes, glomerular hypertrophy, podocyte damage, and glo-
merular macrophage influx on day 8. Both nephritic control 
groups subsequently developed progressive proteinuria and 
decreased renal function. On day 100, glomerulosclerosis, tu-
bulointerstitial damage, glomerular and interstitial accumu-
lation of types III and IV collagen, and overexpression of trans-
forming growth factor-β were widespread. All of these chronic 
changes were prevented in rats that received the PDGF-B 
aptamer, and their functional and morphologic parameters on 
day 100 were largely indistinguishable from non-nephritic rats. 
These data provide the first evidence for a causal role of PDGF 
in the pathogenesis of renal scarring and point to a new, highly 
effective therapeutic approach to progressive, in particular 
mesangioproliferative, renal disease.

Populations of patients with end-stage renal disease continue to 
grow in most Western countries (1). The most frequent causes 
of renal failure are diabetic nephropathy and glomerulonephrit-
ides (1). Both diabetic nephropathy and the majority of pro-
gressive glomerulonephritides, such as IgA nephropathy, mem-
branoproliferative glomerulonephritis, variants of idiopathic 
focal sclerosis, and lupus nephritis, are histologically charac-
terized by glomerular mesangial cell proliferation and/or ma-
trix accumulation (2,3). Of the various factors that affect mes-
angial cell behavior, platelet-derived growth factor-B chain 
(PDGF-B) seems to have a particularly important role (re-
viewed in references 4 and 5). First, mesangial cells produce 
PDGF, and various growth factors induce mesangial prolifer-
ation via induction of PDGF-B chain synthesis. Second, 
PDGF-B chain and its receptor are overexpressed in many 
glomerular diseases. Third, infusion of PDGF-B or glo-
merular transfection with a PDGF-B chain cDNA induces selective 
mesangial cell proliferation and matrix accumulation in vivo. 
Fourth, PDGF-B chain or β-receptor knockout mice fail to 
develop a mesangium. Finally, antagonism of PDGF-B chain 
with neutralizing antibodies can reduce mesangial cell prolif-
eration and matrix accumulation in a (reversible, nonprogres-
sive) rat model of mesangioproliferative nephritis, the anti– 
Thy-1.1 model.

Based on the above observations, specific antagonism of 
PDGF-B chain might represent a novel therapeutic approach to 
progressive renal diseases characterized by mesangial expan-
sion. However, despite the considerable information on PDGF 
in glomerular disease, all intervention studies so far have been 
confined to short-term observations and acute effects. Conse-
quently, it is unclear whether antagonism of PDGF in glomer-
ular disease indeed exerts long-term beneficial effects and, 
more important, whether it is safe, given that mesangioprolif-
erative changes frequently represent a response to injury (6) 
and as such may be analogous to a healing reaction.

We recently described a nuclease-resistant, high-affinity oli-
gonucleotide aptamer that specifically inhibits PDGF activity 
(7,8). This aptamer potently suppressed mesangial cell prolif-
eration and matrix accumulation in the anti–Thy-1.1 nephritis 
model (8). Furthermore, in comparison with our previous study 
with neutralizing anti-PDGF antibodies in anti–Thy-1.1 ne-
phritis (9), the aptamer antagonist offered the advantage of 
nonimmunogenicity (Drolet D, unpublished observations), 
which permits long-term observations. We investigated an 
anti–Thy-1.1 nephritis model, which, when left untreated, will 
progress to renal failure (10). Using this model, we addressed 
the question of whether transient antagonism of PDGF using 

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the specific aptamer antagonist affects the long-term evolution of progressive renal disease.

Materials and Methods

Aptamer-Based Antagonist against PDGF

The synthesis and characterization of the PDGF-B aptamer (NX1975) have been described in detail (7,8). Modifications of the original aptamer (7) involved substitutions of unmodified nucleotides with 2-fluoropyrimidines and 2′-O-methylpurines to improve nuclease resistance as well as coupling of the molecule to 40 kD polyethylene glycol (PEG) to prolong the in vivo half-time of the aptamer (8). The PDGF-B aptamer bound to rat and human recombinant PDGF-BB with the same affinity (Kd approximately 0.1 nM) (8). Based on photo cross-linking experiments (7), the aptamer makes a point contact with human PDGF-B chain at phenylalanine 84 (with isoleucine at position 83). Because this region is identical in mouse and human PDGF-B chain at phenylalanine 84 (with isoleucine at position 83). Because this region is identical in mouse and human PDGF-B chain, high-affinity binding of the aptamer to mouse PDGF-B chain is expected.

As a control aptamer, we used a sequence-scrambled analog of the aptamer conjugated to 40 kD PEG (8). The binding affinity of this scrambled aptamer (NX1976) for PDGF-BB (Kd approximately 1 μM) is 10,000-fold lower compared with the binding affinity of the PDGF-B aptamer (Kd approximately 0.1 nM) (8).

Experimental Model and Experimental Design

All animal studies were approved by the Institutional Review Board. Progressive anti-Thy-1.1 nephritis was induced in 12 male Wistar rats (Charles River, Sulzfeld, Germany), weighing 140 to 160 g at the start of the experiment by a right-sided uninephrectomy. One h later, the rats received a single intravenous bolus injection of 4 mg/kg monoclonal antibody 1-22-3 as described (10). From days 3 to 7 after disease induction, the rats received twice-daily intravenous injections of 5 mg/kg per d PDGF-B aptamer. The lower pole of the left kidney was biopsied on day 8. Because the morphologic changes at this time point of the disease show little interindividual variation (8), only randomly selected rats from each treatment group (see Figure 1) were investigated. After the renal biopsy, the rats remained untreated. Twenty-four-h urine collections were performed every 2 wk until the rats were killed on day 100. BP was measured by tail-cuff plethysmography on day 100. After this, the rats were killed and a serum sample as well as renal tissue were collected.

Two uninephrectomized, nephritic control groups were studied. Thirteen rats were treated from days 3 to 7 with 5 mg/kg per d scrambled aptamer while 6 rats received an equivalent volume of phosphate-buffered saline (PBS) twice daily from days 3 to 7. All other experimental parameters were identical to those described above. Food, in particular protein, intake in all nephritic groups was kept at similar levels by pair feeding the rats throughout the study period.

In addition to the three groups described above, two groups of non-nephritic yet uninephrectomized rats were studied. Three rats received the PDGF-B aptamer (5 mg/kg per d from days 3 to 7), and three rats received PBS only. Again, these rats were then followed until they were killed on day 100.

Renal Morphology

Tissue for light microscopy and immunoperoxidase staining was fixed in methyl Carnoy’s solution and embedded in paraffin. Four-μm sections were stained with the periodic acid-Schiff reagent and counterstained with hematoxylin. In the periodic acid-Schiff–stained sections, the number of mitoses within 30 to 50 glomerular tufts was determined. Furthermore, in renal sections obtained on day 8, irreversibly injured glomeruli were counted. These were defined as glomeruli in which the whole tuft was still replaced by microaneurysm(s) without evidence of cellular regeneration or completely obsolescent glomeruli. On day 100, the percentage of glomeruli that exhibited focal or global glomerulosclerosis was determined as described previously (11). Tubulointerstitial injury on days 8 and 100 was defined as inflammatory cell infiltrates, tubular dilation and/or atrophy, or interstitial fibrosis. Injury was graded according to Shih et al. (12) on a scale of 0 to 4 (0, normal; 0.5, small focal areas of damage; 1, involvement of <10% of the cortex; 2, involvement of 10 to 25% of glomeruli; 3, involvement of 25 to 75% of glomeruli; 4, involvement of ≥75% of glomeruli) with a scale of 0 to 4 (0, normal; 0.5, small focal areas of damage; 1, involvement of <10% of the cortex; 2, involvement of 10 to 25% of glomeruli).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PDGF-B Aptamer (n = 6)</th>
<th>Scrambled Aptamer (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β Immunostaining (% of area staining positively)</td>
<td>29.6 ± 1.1</td>
<td>34.4 ± 1.4</td>
</tr>
<tr>
<td>Glomerular cross sections</td>
<td>17.6 ± 0.7</td>
<td>33.9 ± 1.3b</td>
</tr>
<tr>
<td>Tubulointerstitium (0.09 mm²)</td>
<td>17.6 ± 0.7</td>
<td>33.9 ± 1.3b</td>
</tr>
<tr>
<td>Cortical TGF-β1 content (pg/mg protein)</td>
<td>170 ± 21</td>
<td>205 ± 24</td>
</tr>
</tbody>
</table>

*a* PDGF-B, platelet-derived growth factor-B chain; TGF, transforming growth factor.

*b* P < 0.005 *versus* nephritic rats that received PDGF-B aptamer.
kidney. The stains for a (clone 1A4) to described previously (11). Primary antibodies were identical to those processed by a direct or indirect immunoperoxidase technique as scrambled aptamer, or PBS alone. *, P versus a PBS, phosphate-buffered saline.

Figure 2. Proteinuria in rats that received the PDGF aptamer, the scrambled aptamer, or PBS alone. *, P < 0.05; **, P < 0.01.

Effects of treatment with the PDGF-B aptamer on serum urea concentrations on day 100 after disease induction

Table 2. Effects of treatment with the PDGF-B aptamer on serum urea concentrations on day 100 after disease induction

<table>
<thead>
<tr>
<th>Group</th>
<th>Nephritic</th>
<th>Non-Nephritic</th>
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<tr>
<td>Serum urea (mmol/L)</td>
<td>PDGF-B APTamer</td>
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<tr>
<td>8.0 ± 0.3</td>
<td>11.8 ± 1.5</td>
<td>11.0 ± 1.2b</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>1.46 ± 0.07</td>
<td>1.13 ± 0.11b</td>
</tr>
</tbody>
</table>

a PBS, phosphate-buffered saline.
b P < 0.05 versus nephritic rats that received PDGF-B aptamer.

Immunostaining for TGF-β

In randomly selected subgroups of animals (see Table 1), immunostaining for TGF-β was performed on frozen sections fixed in acetone for 5 min. Mean renal functional and histologic changes including SD in the subgroups analyzed were comparable to those of the whole groups (data not shown). A mouse monoclonal antibody against TGF-β1 to -β3 (Genzyme Diagnostics, Cambridge, MA) was used. Bound antibody was detected using an alkaline phosphatase anti-alkaline phosphatase detection system (Dako, Hamburg, Germany). Controls included the omission of the first or second antibody in each section, in which case no staining was observed.

Determination of Renal Cortical TGF-β1 Content

Slices of renal cortex were obtained on day 100 from six nephritic rats that were treated with the PDGF-B aptamer and six nephritic rats that were treated with the scrambled aptamer. Whole cortical tissue was homogenized in 1 ml of a lysis buffer (50 mM HEPES [pH 7.5], 150 mM NaCl, 1.5 mM MgCl2, 1 mM ethyleneglycol-bis(β-aminoethyl ether) N,N,N′-tetraacetic acid, 10% glycerol, 1% Triton X-100, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride) at 4°C. After incubation for 5 min, lysates were centrifuged at 4°C for 15 min at 10,000 × g. The TGF-β1 concentrations in the lysates were determined using a commercially available enzyme-linked immunosorbent assay (ELISA; Quantikine TGF-β1; R&D Systems, Wiesbaden, Germany). The protein concentrations in the lysates were determined by the method of Lowry et al. (13).

Determination of Renal Collagen Content

It has been shown in rat renal tissue that measurements of hydroxyproline concentration provide a reliable assessment of total using a point-counting method. For this, a grid composed of 121 dots was superimposed on glomeruli (range, 30 to 50; magnification of 100-fold) or 0.09-mm² fields of cortical tubulointerstitium (range, 30 to 40) and the percentages of dots overlying stained areas were counted. In the case of immunostaining for desmin, edges of glomerular tufts were scored semiquantitatively depending on the percentage of edge showing positive staining: 0, to 5% stained; I, 5 to 25%; II, 25 to 50%; III, 50 to 75%; IV, >75%.

Electron Microscopy

Maximally 1-mm³ large tissue pieces fixed in 4% phosphate-buffered formaldehyde were embedded in araldite. Ultrathin sections were stained with lead citrate and viewed in an electron microscope (Zeiss EM10; Zeiss, Oberkochen, Germany). Podocytic foot process width on day 8 was determined in two to five glomeruli of three nephritic rats that were treated with PDGF aptamer and three rats that received scrambled aptamer.

Immunoperoxidase Staining

Four-μm sections of methyl Carnoy’s fixed biopsy tissue were processed by a direct or indirect immunoperoxidase technique as described previously (11). Primary antibodies were identical to those described previously (11) and included a murine monoclonal antibody (clone 1A4) to α-smooth muscle actin (α-SMA); a murine monoclonal IgG antibody (clone ED1) to a cytoplasmic antigen present in monocytes, macrophages, and dendritic cells; a murine monoclonal antibody (clone PGF-007) to PDGF-B chain; a murine monoclonal antibody (clone D33) against human muscle desmin; affinity-purified polyclonal goat anti-human/bovine type IV collagen IgG preabsorbed with rat erythrocytes; plus appropriate negative controls. In addition, slides were stained with an affinity-purified polyclonal goat antibody against human type III collagen (Southern Biotechnology, Birmingham, AL). All slides were evaluated by an observer who was unaware of the origin of the slides.

To obtain mean numbers of infiltrating monocytes/macrophages in glomeruli, we evaluated more than 30 consecutive cross sections of glomeruli (range, 30 to 100) and calculated mean values per kidney. To obtain total counts of infiltrating monocytes/macrophages in the renal interstitium, we analyzed more than 40 grid fields (range, 40 to 60), measuring 0.09 mm² each, and, again, obtained mean counts per kidney. The stains for α-SMA, types III and IV collagen, PDGF B, and transforming growth factor-β TGF-β (see below) were evaluated

Table 2. Effects of treatment with the PDGF-B aptamer on serum urea concentrations on day 100 after disease induction

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a PBS, phosphate-buffered saline.
b P < 0.05 versus nephritic rats that received PDGF-B aptamer.
Renal cortical hydroxyproline concentrations therefore were determined using previously described methods (14,15). Renal collagen content was expressed as μg per mg protein as determined by the method of Lowry et al. (13).

Miscellaneous Measurements

Urinary protein was measured using the Bio-Rad Protein Assay (Bio-Rad Laboratories GmbH, Munich, Germany) and bovine serum albumin (Sigma, Deisenhofen, Germany) as a standard. Serum urea concentration was measured using an autoanalyzer (Beckman Instruments GmbH, München, Germany).

Statistical Analyses

All values are expressed as mean ± SEM. Statistical significance (defined as $P < 0.05$) was evaluated using Kruskal-Wallis tests or Mann-Whitney $U$ tests.

Results

Acute Effects of the PDGF-B Aptamer in Rats with Anti–Thy-1.1 Nephritis

In renal biopsies obtained from nephritic rats on day 8 after disease induction, treatment with the PDGF-B aptamer significantly reduced the number of glomerular mitoses in comparison with PBS-treated rats (Figure 1). This reduction seemed to be due largely to reduced mesangial cell proliferation as suggested by our previous data (8) and by the finding that the PDGF-B aptamer significantly ameliorated the glomerular de novo expression of $\alpha$-SMA (Figure 1), a specific marker of mesangial cell activation (16). Treatment with the PDGF-B aptamer also reduced the glomerular accumulation of type IV collagen and the influx of monocytes/macrophages (Figure 1).

In all cases, treatment with the scrambled aptamer had no effect on the morphologic changes (Figure 1). Finally, the
number of irrevocably injured glomeruli was significantly lower in nephritic rats that received the PDGF-B aptamer (4.4 ± 0.8% versus 18.1 ± 4.4% in those that received scrambled aptamer and 13.9 ± 4.0% in those that received PBS; \( P < 0.01 \) versus PDGF-B aptamer group in both cases).

Both nephritic rats that received the PDGF-B aptamer and those that received the scrambled aptamer were normotensive on day 4, and no significant difference was noted between the groups (data not shown).

With the exception of glomerular \( \alpha \)-SMA, treatment with the PDGF-B aptamer lowered all parameters investigated in nephritic rats to levels comparable to those observed in PBS-treated non-nephritic rats (Figure 1). Mild focal tubulointerstitial injury was present in some nephritic rats but was not affected by the different treatment modalities (Figure 1).

**Chronic Effects of the PDGF-B Aptamer in Rats with Anti-Thy-1.1 Nephritis**

**Proteinuria, BP, and Renal Function.** As shown in Figure 2, treatment of nephritic rats with the PDGF-B aptamer from days 3 to 7 prevented the subsequent development of proteinuria, whereas treatment with the scrambled aptamer was ineffective. Levels of proteinuria observed in the nephritic PDGF-B aptamer group were indistinguishable from those observed in non-nephritic rats. BP in nephritic rats that received the PDGF-B aptamer were normal on day 100 (101 ± 5 mmHg) and lower than in the nephritic rats that received scrambled aptamer (115 ± 10 mmHg), although statistical significance was not reached. Serum urea concentrations were increased and creatinine clearances decreased in the nephritic groups that received PBS or scrambled aptamer, whereas those of the PDGF-B aptamer groups were indistinguishable from non-nephritic rats (Table 2).

**Glomerulosclerosis and Tubulointerstitial Damage.** Light microscopic changes, i.e., the extent of focal segmental glomerulosclerosis and tubulointerstitial damage, were significantly reduced on day 100 in nephritic rats that were treated with the PDGF-B aptamer as compared with those that received scrambled aptamer or PBS alone (Figure 3). Chronic changes in non-nephritic rats were mild and their extent was comparable to those observed in nephritic rats that received the PDGF-B aptamer (Figure 3D).

To investigate potential mechanisms by which the PDGF-B aptamer reduced the frequency of glomerulosclerosis, we assessed glomerular hypertrophy via the measurement of the cross-sectional glomerular area and podocyte damage via their de novo expression of the cytoskeletal protein desmin (17). As shown in Figure 4, in nephritic rats that received the PDGF-B aptamer, both of these parameters were reduced to levels observed in non-nephritic rats on days 8 and 100 of the study period. By electron microscopy, no areas of foot process fusion or detachment from the glomerular basement membrane were noted. However, foot process width in nephritic rats that were treated with the PDGF-B aptamer was reduced as compared with those that received scrambled aptamer (0.64 ± 0.03 \( \mu \)m versus 0.77 ± 0.14 \( \mu \)m; \( n = 3 \) each). Glomerular counts of mitoses and \( \alpha \)-SMA staining scores on day 100 were low and not significantly different in all nephritic groups (data not shown). Furthermore, the glomerular PDGF-B staining scores on day 100 in the aptamer-treated group were low (0.68 ± 0.04; \( n = 12 \)) and not significantly different from the scrambled aptamer-treated rats (0.61 ± 0.04; \( n = 13 \)).

**Renal Extracellular Matrix Accumulation.** Renal matrix accumulation was assessed by immunostaining for basement membrane, i.e., type IV, and interstitial type, i.e., type III, collagen. In nephritic rats, treatment with the PDGF-B aptamer reduced the accumulation of both collagen types to similar levels as those observed in non-nephritic controls (Figure 5). Furthermore, the total renal collagen content, as assessed via the analysis of hydroxyproline content, was also decreased in PDGF-B aptamer-treated nephritic rats in comparison to the other nephritic groups (Table 3).

The renal expression of the profibrotic cytokine TGF-\( \beta \) on day 100 after disease induction was increased in rats that received the scrambled aptamer or PBS. Immunoreactivity was present in the expanded interstitium, some infiltrating cells, some tubular cells, and glomerular cells and/or extracellular matrix (Figure 6B). TGF-\( \beta \) immunostaining was mostly decreased in globally sclerosed glomeruli. In rats that were treated with the PDGF aptamer, the staining pattern was comparable to that observed in normal rats in that some glomerular cells, very few tubular cells, the renal interstitium, and occasional infiltrating cells stained positively (Figure 6A). Quantitative evaluation of the renal cortical TGF-\( \beta \) expression confirmed the marked reduction in PDGF aptamer-treated rats, in particular in the renal tubulointerstitium (Table 1). Specific determination of the whole cortical TGF-\( \beta \)1 content by ELISA revealed a lesser content in PDGF aptamer-treated rats as compared with
Figure 5. Renal expression of type III and IV collagen on day 100 after disease induction. (A) Renal type III collagen expression in a rat that received PDGF aptamer is confined to interstitial areas. (B) Type III collagen in a scrambled aptamer-treated rat is present in some glomeruli (arrow) and the widened interstitium. (C) Type IV collagen expression in a PDGF aptamer-treated rat shows a normal glomerular and interstitial distribution except for occasional mesangial matrix expansion (arrow). (D) Type IV collagen expression in a scrambled aptamer-treated rat is increased in both glomeruli and the renal interstitium. (E) Quantitative evaluation of renal type III and IV collagen immunostaining. *, P < 0.05; **, P < 0.01.
those that received scrambled aptamer, which, however, failed to reach statistical significance (Table 1). These data suggest that TGF-β2 and/or -β3 overexpression, which was not detected in the TGF-β1–specific ELISA, might also have contributed to the increased renal TGF-β immunostaining in rats that received scrambled aptamer.

Renal Monocyte/Macrophage Infiltration. Figure 7 shows that glomerular monocyte/macrophage counts on day 100 after disease induction were low and in a comparable range in all nephritic groups. In contrast, renal interstitial monocyte/macrophage counts were increased in nephritic rats that received scrambled aptamer or PBS in comparison with those that received PDGF-B aptamer.

Discussion

In the present study, we first established that under our experimental conditions the PDGF-B aptamer was as potent in reducing acute mesangioproliferative changes as in our previous study (8), in which a different nephritis model had been investigated (reversible anti–Thy-1.1 nephritis induced by monoclonal antibody OX7 in rats with two kidneys). The data show that in both models, the PDGF-B aptamer led to comparable reductions of mesangioproliferative changes and glomerular monocyte/macrophage influx. Because it is well established that the acute phase in the anti–Thy-1.1 nephritis model is PDGF dependent (8), these data, in concert with our recent in vitro data (8) as well as recent data in the PDGF-dependent carotid restenosis model (18), substantiate the notion that the aptamer acted in vivo by specifically antagonizing PDGF-B chain.
We next asked whether the acute amelioration of mesangio-proliferative changes would extend to the prevention of progressive renal damage. Because some evidence is available to invoke PDGF-B chain in the pathogenesis of renal interstitial fibrosis (19), we confined the period of PDGF antagonism to the phase of acute glomerular damage to avoid therapeutic effects on secondary pathomechanisms of renal failure, such as interstitial fibrosis. The central finding of the present study was that transient yet potent inhibition of PDGF-B chain during the acute phase of mesangio-proliferative nephritis almost completely prevented the subsequent development of proteinuria, glomerulosclerosis, tubulointerstitial damage, and renal fibrosis. These observations provide a strong argument against concerns that inhibition of overshooting mesangial cell growth and matrix production after injury might represent inhibition of healing and thus aggravate glomerular damage. Rather, our findings place the mesangial overexpression of PDGF-B chain at a very early and possibly key position in the cascade of events that eventually leads to renal failure in diseases associated with mesangio-proliferative changes.

By which mechanism(s) did the transient inhibition of PDGF exert beneficial long-term effects? PDGF antagonism was unlikely to have affected the extent of early mesangial damage, because treatment was started 3 d after disease induction. At this time point, immune injury in the anti–Thy-1.1 nephritis model is already past its maximum (8,20). Treatment-related effects on other important variables that contribute to progression of renal damage, such as systemic hypertension or dietary protein intake (21–23), are also unlikely because normotension was maintained during the phase of PDGF antagonism and because pair feeding was performed throughout the study period. It also seems unlikely that the PDGF-B aptamer acted directly on tubular cells after its glomerular filtration, because in the acute phase of the anti–Thy-1.1 nephritis, no tubular overexpression of PDGF-B has been noted (4,5). Finally, on day 100, no long-term effects of PDGF-B aptamer treatment on glomerular cell proliferation, mesangial cell activation, or glomerular PDGF expression persisted. This suggests that ongoing mesangial cell activation and proliferation are not required for the development of progressive renal damage.

Possibly the most important consequence of the treatment with PDGF-B aptamers was the reduction of early glomerular hypertrophy. This likely resulted from a direct antiproliferative effect of the PDGF-B aptamer on mesangial cells (8) as well as from antifibrotic actions of PDGF antagonism (reviewed in references 4 and 5). How does reduction of glomerular hypertrophy relate to the prevention of renal failure and progressive structural damage? Glomerular hypertrophy has been recognized as a strong predictor for progressive renal disease in both experimental and human studies (2,24). We recently showed that glomerular hypertrophy is associated closely with podocyte damage (11), which is regarded as a crucial event in the development of glomerulosclerosis (25). To investigate whether PDGF antagonism in the present study also affected podocyte damage, we assessed the podocytic de novo expression of desmin, a marker of podocyte damage (17), and electron microscopic podocyte changes. These data showed that PDGF antagonism in the current nephritis model reduced not only mesangio-proliferative changes but also the associated (secondary?) podocyte as well as irreversible glomerular damage. The effect of PDGF-B antagonism on podocyte damage was probably an indirect one, because podocytes do not express PDGF receptors in vivo and glomerular epithelial cells in vitro do not respond to even high PDGF-B concentrations (26–31). Apart from its role in the pathogenesis of glomerulosclerosis, podocyte damage is also a central mechanism in the development of proteinuria (32). Our observation that treatment with the PDGF-B aptamer prevented the development of progressive proteinuria therefore provides further, albeit indirect, evidence for a treatment-related decrease in podocyte damage. Proteinuria in turn is viewed as an important determinant of tubulointerstitial damage, which is associated strongly with progressive renal failure (33). Therefore, by reducing early glomerular hypertrophy and podocyte damage, transient antagonism of PDGF may have exerted the various beneficial long-term effects noted in the present study.

One mechanism by which the prevention of progressive proteinuria in the PDGF aptamer-treated nephritic rats translated into reduced renal scarring may involve the action of TGF-β. In the kidney, TGF-β has been established to be of central importance in the mediation of fibrotic changes (34,35). Our observation that transient PDGF antagonism in vivo resulted in a long-term reduction of tubulointerstitial TGF-β expression thus provides a molecular basis by which interstitial fibrosis was reduced. Whereas in glomerular mesangial cells a direct link between the expression of PDGF-B chain and subsequent TGF-β induction has been established (36), the relationship between antagonism of PDGF-B chain and reduced tubulointerstitial TGF-β expression seems to be indirect. Thus, it is likely that, analogous to observations in other models of renal interstitial damage, the tubulointerstitial TGF-β overexpression resulted from proteinuria-induced activation of renal tubular cells and the subsequent induction of peritubular inflammatory changes (37). Alternatively, it is conceivable that the tubulointerstitial overexpression of TGF-β was mediated by mechanisms different from proteinuria, such as the leakage of proinflammatory substances from inflamed glomeruli via the urine or through the glomerular stalk.
In conclusion, these data identify PDGF-B chain as a growth factor for which transient, specific antagonism prevents the development of progressive renal scarring. In this respect, the role of PDGF-B chain seems more central than in vascular disease, where the same aptamer potently suppressed the formation of a neointima in the rat carotid restenosis model yet had a smaller long-term protective effect after the cessation of treatment (18). A possible correlate for these observations is provided by mice that are genetically deficient for PDGF-B chain, which develop no mesangium while the gross morphology of vascular smooth muscle cells is normal (38). The present data therefore suggest that antagonism of PDGF may be a useful therapeutic approach to renal diseases characterized by mesangio-proliferative changes. PDGF-B chain is a particularly attractive therapeutic target, because a transgenic mouse line, which expresses high levels of circulating PDGF antagonist during adult life only, does not exhibit phenotypic abnormalities (39). This suggests that it may be safe to block PDGF-B chain in adult life, where it does not seem to have central physiologic roles.

Acknowledgments

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