Antagonism of PDGF-D by Human Antibody CR002 Prevents Renal Scarring in Experimental Glomerulonephritis

Tammo Ostendorf,* Song Rong,* Peter Boor,* Stefanie Wiedemann,* Uta Kunter,* Ulrike Haubold,* Claudia R.C. van Roeyen,* Frank Eitner,* Hiroshi Kawachi,† Gary Starling,‡ Enrique Alvarez,‡ Glennda Smithson,‡ and Jürgen Floege*†

*Division of Nephrology, Rheinisch-Westfälische Technische Hochschule University of Aachen, Aachen, Germany; †Department of Cell Biology, Institute of Nephrology, Niigata School of Medicine, Niigata, Japan; and ‡CuraGen Corporation, Branford, Connecticut

Glomerular mesangial cell proliferation and/or matrix accumulation characterizes many progressive renal diseases. PDGF-D was identified recently as a novel mediator of mesangial cell proliferation in vitro and in vivo. This study investigated the long-term consequences of PDGF-D inhibition in vivo. Rats with progressive mesangioproliferative glomerulonephritis (uninephrectomy plus anti-Thy-1.1 antibody) received the PDGF-D-neutralizing, fully human mAb CR002 on days 3, 10, and 17 after disease induction. Glomerular mesangioproliferative changes on day 10 were significantly reduced by anti–PDGF-D treatment as compared with control antibody. Eight weeks after disease induction, anti–PDGF-D therapy significantly ameliorated focal segmental glomerulosclerosis, podocyte damage (de novo desmin expression), tubulointerstitial damage, and fibrosis as well as the accumulation of renal interstitial matrix including type III collagen and fibronectin. Treatment with anti–PDGF-D also reduced the cortical infiltration of monocytes/macrophages on day 56, possibly related to lower renal fibrosis as well as the accumulation of renal interstitial matrix including type III collagen and fibronectin. Treatment with anti–PDGF-D also reduced the cortical infiltration of monocytes/macrophages on day 56, possibly related to lower renal fibrosis as well as the accumulation of renal interstitial matrix including type III collagen and fibronectin. Treatment with anti–PDGF-D also reduced the cortical infiltration of monocytes/macrophages on day 56, possibly related to lower renal fibrosis as well as the accumulation of renal interstitial matrix including type III collagen and fibronectin. Treatment with anti–PDGF-D also reduced the cortical infiltration of monocytes/macrophages on day 56, possibly related to lower renal fibrosis as well as the accumulation of renal interstitial matrix including type III collagen and fibronectin.

The number of patients with ESRD continues to grow in most Western countries (1). Besides diabetic nephropathy, which underlies up to 35% of ESRD cases, glomerulonephritides in particular the most common type IgA nephropathy, account for approximately 20% of ESRD cases in most Western countries. Both diabetic nephropathy and the majority of progressive glomerulonephritides are histologically characterized by glomerular mesangial cell proliferation and/or matrix accumulation (2,3). Ample evidence now is available to link the PDGF system to these processes. The role of PDGF-B chain in mediating mesangioproliferative changes is well established (4–6), but the role of PDGF-D has been elucidated only recently (7–10). Like PDGF-B, PDGF-D signals through the PDGF-β receptor (7,8). PDGF-B binds to all three known PDGF receptors with high affinity (11); in contrast, PDGF-D binds predominately to the PDGF-β receptor (7,8). This suggests that PDGF-B, in comparison with PDGF-D, may signal through at least partially different pathways.

Several recent studies link PDGF-D to renal disease. PDGF-D induces mesangial cell proliferation in vitro and is overexpressed in mesangioproliferative glomerulonephritis in vivo (9). Adenoviral expression of PDGF-D in the liver induced prominent mesangioproliferative nephritis in mice (10). PDGF-β receptor knockout mice fail to develop a mesangium (12), and, finally, specific antagonism of PDGF-D chain with the fully human mAb CR002 reduced mesangial cell proliferation and matrix accumulation in a (reversible, nonprogressive) rat model of mesangioproliferative nephritis, the anti–Thy-1.1 model (9).

On the basis of these observations, specific antagonism of PDGF-D might represent a novel therapeutic approach to progressive renal diseases that are characterized by mesangial expansion. However, the only intervention study so far has been confined to short-term observations (9). Consequently, it is unproved whether antagonism of PDGF-D in glomerular disease exerts long-term beneficial effects. In this study, we investigated the long-term consequences of PDGF-D antagonism using a progressive anti–Thy-1.1 nephritis model similar to the model of Cheng et al. (13). This model is induced by uninephrectomy and anti–Thy-1.1 antibody administration and, when left untreated, progresses to renal failure.

Materials and Methods

**Fully Human PDGF-D MAb CR002**

Generation and specificity of fully human PDGF-DD mAb CR002 was described in detail previously (9,14).

**Experimental Model and Experimental Design**

All animal studies were approved by the local review boards. Male Wistar rats (n = 29) that weighed 180 g were purchased from Charles

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ISSN: 1046-6673/1704-1054
Approximately 400 selected rats each of groups 1 and 2 for histologic evaluation (15). Results too problematic (data not shown). Food, in particular protein doses induced complement depletion, making interpretation of the antibody dose in the nephritic groups was selected because higher/11001

1. Nephritic cortical tissue of each rat was used to isolate RNA (see below). 10003

2. Nephritic renal tissues for histologic evaluation were collected. The remaining 10003

vein, and 24-h urine collections were performed weekly until the rats 10003

were treated with the anti–PDGF-D mAb CR002 or control IgG2

European Collection of Animal Cell Cultures, Salisbury, England). Rats 10003

duced by a right-sided uninephrectomy followed 1 h later with a single 10003

of anti–Thy-1.1 mAb (clone OX-7; 10003

River (Sulzfeld, Germany). Progressive anti–Thy-1.1 nephritis was 10003

induced by a right-sided uninephrectomy followed 1 h later with a single 10003

intravenous bolus injection of 1 mg/kg anti–Thy-1.1 mAb (clone OX-7; 10003

European Collection of Animal Cell Cultures, Salisbury, England). Rats 10003

were treated with the anti–PDGF-D mAb CR002 or control IgG2 (Fitzgerald Industries International Inc., Concord, MA) on days 3, 10, and 17 after disease induction. Treatment consisted of intraperitoneal injections of the antibodies dissolved in 800 µl of 20 mM Tris-HCl/100 mM NaCl (pH 7.4). The timing of antibody treatment was chosen to target mesangial cell proliferation, which occurs between days 5 and 10 after disease induction. CR002 and control IgG2 serum levels were measured on days 16, 28, and 56 (Table 1).

Four groups of uninephrectomized rats were studied:

1. Nephritic + control IgG (n = 9): 1 mg/kg body wt control IgG2

2. Nephritic + CR002 (n = 10): 1 mg/kg body wt CR002

3. Nonnephritic + control IgG (n = 5)

4. Nonnephritic + CR002 IgG (n = 5)

The antibody dose in the nephritic groups was selected because higher doses induced complement depletion, making interpretation of the results too problematic (data not shown). Food, in particular protein intake, in all groups was kept at similar levels by pairs feeding the rats. Intravital renal biopsies were obtained on day 10 from five randomly selected rats each of groups 1 and 2 for histologic evaluation (15). Approximately 400 µl of venous blood was drawn weekly from a tail vein, and 24-h urine collections were performed weekly until the rats were killed on day 56. BP was measured by tail-cuff plethysmography on days 2, 28, and 55. After the rats were killed, serum samples and renal tissues for histologic evaluation were collected. The remaining cortical tissue of each rat was used to isolate RNA (see below).

Cortical RNA Extraction and Analyses

Total RNA was extracted from renal cortex using the RNeasy Mini Kit (Qiagen, Hilden, Germany). cDNA syntheses and real-time quantitative PCR were performed as described previously (9). Sequences of primers and probes that were used in this study are listed in Table 2. mRNA for each sample was normalized to glyceraldehyde-3-phosphate dehydrogenase.

Renal Morphology

Tissue for light microscopy and immunoperoxidase staining was fixed in methyl Carnoy’s solution as well as in formalin and embedded in paraffin. Four-micrometer sections were stained with the periodic acid-Schiff reagent and counterstained with hematoxylin. In these sections of day 10 biopsies, the number of mitoses within 50 to 100 glomerular cross-sections of consecutive cortical glomeruli each, which contained >20 discrete capillary segments, was evaluated. On day 56, the percentage of glomeruli that exhibited focal or global glomerulosclerosis was determined as described (16). Tubulointerstitial injury on day 56 was graded according to Shih et al. (17) on a scale of 0 to 4 as described previously (6).

For the evaluation of total collagen, renal tissues were stained with Sirius red and evaluated by computer-based morphometry (Soft Imaging System GmbH, Münster, Germany). The percentage of positively stained area in each tissue was calculated in 20 fields, with each field having an area of 0.37 mm². In all analyses, the investigator was unaware of the origin of the slides.

Immunoperoxidase Staining

Four-micrometer sections of methyl Carnoy’s–fixed biopsy tissue were processed as described previously (16). Primary antibodies included a murine mAb (clone 1A4) to α-smooth muscle actin (α-SMA); a murine monoclonal IgG antibody (clone ED1) to monocytes, macrophages, and dendritic cells; a murine mAb (clone D33) against human muscle desmin; affinity-purified polyclonal goat anti-human/bovine type IV collagen IgG; affinity-purified polyclonal goat antibodies against human types I and III collagen (Southern Biotechnology, Birmingham, AL); an affinity-purified IgG fraction of a polyclonal rabbit anti-rat fibronectin antibody (Chemicon, Temecula, CA); a murine mAb

<table>
<thead>
<tr>
<th>Groups</th>
<th>Human mAb Serum Level (µg/ml), Day 16</th>
<th>Human mAb Serum Level (µg/ml), Day 28</th>
<th>Human mAb Serum Level (µg/ml), Day 56</th>
<th>Rat Anti-Human IgG (AU), Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephritic + IgG2 (n = 9)</td>
<td>10 ± 21</td>
<td>1 ± 0.7</td>
<td>0.3 ± 0.1</td>
<td>155 ± 227</td>
</tr>
<tr>
<td>Nephritic + CR002 (n = 10)</td>
<td>2 ± 0.6</td>
<td>5 ± 3</td>
<td>1 ± 1</td>
<td>110 ± 113</td>
</tr>
<tr>
<td>Nonnephritic + IgG2 (n = 5)</td>
<td>153 ± 46</td>
<td>34 ± 25</td>
<td>19 ± 34</td>
<td>162 ± 182</td>
</tr>
<tr>
<td>Nonnephritic + CR002 (n = 5)</td>
<td>123 ± 25</td>
<td>144 ± 61</td>
<td>22 ± 14</td>
<td>76 ± 80</td>
</tr>
<tr>
<td>Nephritic + PBS (n = 10)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>202 ± 148²</td>
</tr>
</tbody>
</table>

²Data are arbitrary optical units per 10 µl of serum; all differences between groups are not significant by ANOVA and Bonferroni t tests. AU, arbitrary units; ND, not determined.

³Four sera in the PBS group were taken on days 26, 34, 35, and 43 at the time of death of the animals.

Table 1. Human mAb levels achieved in vivo on days 16, 28, and 56 after disease induction as well as ELISA data on rat anti-human IgG in serum on day 56

Table 2. Primers and probes

<table>
<thead>
<tr>
<th>Gene (Rat)</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Taqman Probe</th>
<th>GenBank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>5'-ACAAAGATGTTGGAAGCTGCTTCG-3'</td>
<td>5'-AGAAGGCAGCCTGCTTGAAACC-3'</td>
<td>5'-CCGATTGGCCCGCTATCGGACG-3'</td>
<td>AF10860</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>5'-ACACAGGTCTCGACTCCCAT-3'</td>
<td>5'-AGAATGCTCGGAGTATG-3'</td>
<td>5'-ACGATTTCGCTACCGCAACTTACC-3'</td>
<td>X15906</td>
</tr>
<tr>
<td>Type I collagen</td>
<td>5'-GAAGGCGAACAGTGTACCC-3'</td>
<td>5'-CGTACGCTTGGCCAAGTTC-3'</td>
<td>5'-ACAGCAGGCTTGATGGCTGC-3'</td>
<td>Z78229</td>
</tr>
</tbody>
</table>

⁴GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
Serum Measurements of mAb CR002 and Rat Anti-Human IgG

Circulating levels of anti-PDGF-DD (CR002) mAb were measured by ELISA. Briefly, 96-well ELISA plates (Corning, Acton, MA) were coated with 1 μg/well recombinant PDGF-D in bicarbonate buffer (pH 9.6) at 4°C overnight. After washing, plates were blocked with 200 μl/well blocking buffer (Pharmingen Diluent; BD Pharmingen, San Diego, CA) for 1 h and washed, and 100 μl/well sample or standard was added to each well. CR002 in rat serum served as a standard. Plates were incubated at room temperature for 1 h and washed, and goat anti-human IgG κ coupled to horseradish peroxidase (HRP) and diluted in PBS that contained 0.5% BSA and 0.1% Tween-20 was added to each well at 100 μl/well. After the plates were washed, 100 μl/well substrate was added (BD Pharmingen TMB reagent A&B), and the plates incubated for 5 min in the dark. The reaction was stopped by the addition of 100 μl/well 1 N HCl, and the optical density was determined at 450 nm using a microplate reader (TECAN; Research Triangle Institute, Research Triangle Park, NC). Concentrations of CR002 in serum samples were calculated using Softmax Pro software (Molecular Devices, Sunnyvale, CA).

For assessment of whether CR002 treatment induced the formation of rat anti-human IgG, ELISA plates were coated with 0.22 μg/well human IgG (Dianova) in PBS at 4°C overnight. After washing and blocking with 2% BSA/PBS-Tween (0.05%) for 1 h, wells were incubated with 10 μl of rat serum in PBS for 1 h. Subsequently, plates were washed and incubated with biotinylated goat anti-rat IgG (2 mg/ml, 1:10,000; Dianova), followed by washing and by incubation with streptavidin-coupled HRP for 1 h. After washing, substrate solution that contained 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid; Sigma, Taufkirchen, Germany) was added to the wells for 30 min, and optical density was determined at 405 nm in a microplate reader (Dynatech, Chantilly, VA). The optical densities were compared with values that were obtained in wells with sera of healthy, nontreated rats. As a control, rat anti-human IgG also was measured in the sera of an extra group of nephritic rats (n = 10) that were treated on days 3, 10, and 17 with PBS only.

Miscellaneous Measurements

Urinary albumin levels were determined by ELISA (Nephrat; Exocell, Philadelphia, PA). Serum creatinine was determined by an autoanalyzer. BP measurements were performed by the tail-cuff method, using a programmed sphygmomanometer (Softrom Co., Tokyo, Japan).

Urinary levels of α-1-microglobulin were measured by Western blot: Rat urine (1 μg protein/lane) was loaded and separated by SDS-PAGE using 4% acrylamide for the stacking and 12.5% for the resolving gel. Protein was transferred to nitrocellulose membranes (pore size 0.45 μm; Schleicher & Schuell, Keene, NH) and probed with a polyclonal antibody against rat α-1-microglobulin (rabbit polyclonal antibody; AgriSera, Vännäs, Sweden). The primary antibody was detected using HRP-conjugated goat anti-rabbit IgG (Vector Laboratories Inc., Burlingame, CA). The negative control consisted of substitution of the primary antibody with equivalent concentrations of normal rabbit IgG. Bound antibodies were visualized by the ECL system (Amersham Pharmacia Biotech, Freiburg, Germany). The intensity of the identified 27-kD band was quantified by densitometry using the system of Bio- step GmbH (Jahnshdorf, Germany), corrected for the relative intensity of a nonspecific band and subsequently calculated to correspond to 1 nmol of urinary creatinine in the samples. The analysis software was from TotalLab (Phoretix International, Newcastle, UK).

Statistical Analyses

All values are expressed as means ± SD. Statistical significance (defined as P < 0.05) was evaluated using ANOVA and Bonferroni t test or paired t test where appropriate.

Results

Acute Effects of PDGF-D Antagonism in Rats with Progressive Glomerulonephritis

On day 10 after disease induction, treatment with CR002 significantly reduced the number of glomerular mitoses in comparison with the control antibody (Figure 1A). CR002 significantly ameliorated the glomerular de novo expression of α-SMA (Figure 1B), a specific marker of mesangial cell activation (21).

Chronic Effects of PDGF-D Antibody CR002 in Rats with Progressive Glomerulonephritis

Albuminuria, Renal Function, BP, and Body Weight. As shown in Table 3, albuminuria on days 49 and 56 after disease induction in rats that received either PDGF-D antibody CR002 or irrelevant IgG2 on day 3 (n = 5 per group).
Table 3. Effects of PDGF-D antibody CR002 treatment on serum creatinine and albuminuria

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Creatinine (μmol/L) Day 49</th>
<th>Albuminuria (mg/24 h) Day 49</th>
<th>Serum Creatinine (μmol/L) Day 56</th>
<th>Albuminuria (mg/24 h) Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephritic + IgG2 (n = 9)</td>
<td>41 ± 3</td>
<td>8 ± 10</td>
<td>37 ± 4</td>
<td>7 ± 10</td>
</tr>
<tr>
<td>Nephritic + CR002 (n = 10)</td>
<td>34 ± 3</td>
<td>4 ± 5</td>
<td>34 ± 4</td>
<td>7 ± 10</td>
</tr>
<tr>
<td>Nonnephritic + IgG2 (n = 5)</td>
<td>34 ± 2</td>
<td>0.2 ± 0.1</td>
<td>35 ± 2</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Nonnephritic + CR002 (n = 5)</td>
<td>35 ± 3</td>
<td>0.4 ± 0.4</td>
<td>33 ± 5</td>
<td>0.7 ± 0.9</td>
</tr>
</tbody>
</table>

aP < 0.05 versus nephritic rats that received IgG2.

Table 4. Effects of PDGF-D antibody CR002 treatment on systolic BP and body weighta

<table>
<thead>
<tr>
<th>Groups</th>
<th>Systolic BP (mmHg) Day 2</th>
<th>Systolic BP (mmHg) Day 28</th>
<th>Systolic BP (mmHg) Day 55</th>
<th>Body Weight (g) Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephritic + IgG2</td>
<td>102 ± 11 (n = 5)</td>
<td>120 ± 8 (n = 6)</td>
<td>134 ± 15b (n = 7)</td>
<td>376 ± 34 (n = 9)</td>
</tr>
<tr>
<td>Nephritic + CR002</td>
<td>118 ± 6 (n = 2)</td>
<td>115 ± 14 (n = 5)</td>
<td>132 ± 14 (n = 7)</td>
<td>402 ± 41 (n = 10)</td>
</tr>
<tr>
<td>Nonnephritic + IgG2</td>
<td>108 ± 1.7 (n = 2)</td>
<td>118 ± 11 (n = 5)</td>
<td>127 ± 5 (n = 5)</td>
<td>422 ± 14 (n = 5)</td>
</tr>
<tr>
<td>Nonnephritic + CR002</td>
<td>104 ± 3 (n = 3)</td>
<td>110 ± 15 (n = 5)</td>
<td>125 ± 9 (n = 5)</td>
<td>410 ± 68 (n = 5)</td>
</tr>
</tbody>
</table>

aAll BP and body weight differences between treatment groups at each time point (ANOVA and Bonferroni t test) are NS.  
bStatistically significant difference (P < 0.05, paired t test) within a treatment group between BP on days 2 and 55.

induction was 10- to 40-fold higher in nephritic rats as compared with uninephrectomized, nonnephritic rats. Treatment of nephritic rats with the neutralizing PDGF-D antibody on days 3, 10, and 17 tended to reduce albuminuria on day 49 but not on day 56 after disease induction, compared with the nephritic group that was treated with irrelevant IgG. However, variability within groups was high on day 49, and differences between groups failed to reach significance. Albuminuria at earlier time points was not different between both nephritic groups. Assessment of total proteinuria yielded very similar findings (data not shown). Excretion of a low molecular weight protein, α1-microglobulin, in the urine of nephritic rats on day 56 after disease induction was similar in all groups and not significantly different from that observed in normal rats (data not shown).

Serum creatinine concentrations on days 49 and 56 increased in nephritic rats that received control IgG in comparison with nonnephritic rats (Table 3). In contrast, creatinine levels on days 49 and 56 were not different from those of nonnephritic rats in the group that was treated with neutralizing PDGF-D antibody (Table 3).

BP were normal in all rats on day 2 after disease induction and increased thereafter until day 55, with a significant increase within the nephritic control IgG–treated group (Table 4). Treatment with CR002 did not affect BP levels (Table 4).

Despite pair feeding, nephritic control rats on day 56 tended to exhibit lower body weights than nonnephritic rats, suggesting increased catabolism in the former groups. Treatment with CR002 led to higher, albeit not normalized, body weights (Table 4).

Glomerulosclerosis and Tubulointerstitial Damage. By day 56, nephritic rats that were treated with control IgG had developed widespread glomerulosclerosis (Figure 2A). Treatment with CR002 markedly reduced the frequency of sclerotic glomeruli (Figure 2, B and C). Comparison of the serum levels of CR002 (Table 1) and the percentage of sclerotic glomeruli in each animal showed a clear correlation between the level of CR002 exposure at day 56 and glomerular damage (P = 0.027). To assess more specifically podocyte damage, which occurs Figure 2. Renal morphologic changes on day 56 after disease induction. (A and B) Periodic acid-Schiff–stained renal section of a nephritic rat that received control IgG, showing widespread glomerulosclerosis and focal tubular atrophy, interstitial infiltration, and fibrosis (A), or a rat that received CR002 on days 3, 10, and 17, showing some residual mesangial expansion, occasional glomerulosclerosis and no tubulointerstitial damage (B). (C) Frequency of focal segmental glomerulosclerosis (FSGS) and tubulointerstitial damage scores. Data are means ± SD. *P < 0.05 versus nephritic rats that received control IgG2. Nephritic, rats with anti-Thy-1.1 nephritis after previous uninephrectomy; UNX, nonnephritic rats that had received a uninephrectomy only. Magnification, ×100.
secondary to mesangial injury in anti-Thy-1.1 nephritis (6), we studied renal sections for the de novo expression of desmin, a nonspecific injury marker in podocytes (22). As shown in Figure 3, nephritic animals that received control IgG showed desmin at the edge of the glomerular tuft on average in 50% of the glomerular circumference. Treatment with CR002 led to a significant reduction of staining scores in comparison with the nephritic control (Figure 3).

Nephritic rats that received control IgG exhibited tubulointerstitial damage that affected on average 10 to 25% of the cortex (Figure 2C). Treatment with CR002 led to a significant reduction of scores in comparison with the nephritic group that was treated with control IgG (Figure 2C). Renal interstitial fibrosis also was assessed by Sirius red staining. Figure 4 shows that PDGF-D antagonism significantly reduced renal interstitial fibrosis in comparison with nephritic rats that had received control IgG only.

The development of interstitial fibrosis was studied further by investigation of the expression of specific matrix proteins. As shown in Figure 5A, nephritic rats that received control IgG showed interstitial foci of type III collagen accumulation, involving approximately 5% of the renal cortex. Treatment with CR002 significantly reduced accumulation of type III collagen in comparison with the nephritic control (Figure 5, B and C). Similarly, renal interstitial accumulation of type I collagen was reduced by an average of 30 to 40% in the CR002-treated nephritic group as compared with the nephritic control (data not shown). Finally, the tubulointerstitial accumulation of fibronectin was significantly reduced in CR002-treated nephritic rats as compared with the nephritic control (Figure 5D). Quantification of renal cortical mRNA levels for type I collagen and fibronectin failed to reveal significant differences between the groups (data not shown).

Tubulointerstitial Complement Activation and Epithelial-to-Mesenchymal Transition. To assess the mechanisms that are involved in reduced tubulointerstitial damage after PDGF-D antagonism, we investigated tubulointerstitial complement activation, which mediates most of the “toxicity” of proteinuria (23), and epithelial-to-mesenchymal transition (EMT) (24). During EMT, renal tubular cells lose expression of tubular markers, such as E-cadherin, and start to express markers of (myo-)fibroblasts, such as vimentin or α-SMA (24–26). As illustrated in Figure 6, treatment of rats with CR002 significantly reduced tubulointerstitial deposition of C5b-9 in comparison with nephritic control rats.

Staining of kidneys for E-cadherin revealed a >50% decrease in the expression of E-cadherin on the basolateral part of tubular epithelial cells in nephritic control rats as compared with nonnephritic rats (Figure 7, A, B, and D). Previous treatment with CR002 largely restored the normal expression pattern in nephritic rats (Figure 7, C and D). In the case of vimentin, increased tubular and tubulointerstitial expression was noted in the cortex of nephritic control rats compared with nonnephritic rats (Figure 7, E, F, and H). CR002 treatment led to a significant reduction of tubulointerstitial vimentin expression on day 56 in the group of nephritic rats (Figure 7, F, G, and H).
Finally, increased expression of tubulointerstitial α-SMA and desmin in the nephritic control group was reduced by CR002 treatment, albeit not significantly for desmin (data not shown).

Renal Monocyte/Macrophage Infiltration. Tubulointerstitial monocyte/macrophage counts increased more than two-fold in nephritic control rats as compared with nonnephritic rats on day 56 after disease induction (Figure 8). Treatment of nephritic rats with CR002 led to a significant reduction of tubulointerstitial monocyte/macrophage infiltration in comparison with nephritic control rats (Figure 8).

Deposition of CR002 in Renal Tissue and Formation of Rat Anti-Human IgG Antibody. As shown in Table 1, no rat anti-human IgG, which might have neutralized the activity of CR002, was detectable in any group. All values were below those of a nephritic group that was treated with PBS only. Furthermore, no intrarenal deposition of human IgG was noted in any group on day 56 (data not shown).

Discussion

In this study, we extend our previous work, which showed that specific antagonism of PDGF-D in rat mesangioproliferative glomerulonephritis reduces mesangial cell proliferation, glomerular influx of monocytes/macrophages, and fibronectin accumulation (9). This first study on PDGF-D in renal disease was confined to a model of acute damage, which is not suitable to study long-term sequelae of PDGF-D antagonism, as spontaneous healing of the glomerular changes occurs in “standard” anti-Thy-1.1 nephritis independent of treatment (27). We therefore aggravated anti-Thy-1.1 nephritis by performing a uninephrectomy before disease induction. Using the monoclonal
Thy-1.1 antibody clone 1-22-3, Cheng et al. (13) already reported that this combination will override the glomerular healing capacity, and both glomerular and subsequently tubulointerstitial scarring occurs. We established that usage of another clone of monoclonal Thy-1.1 antibody, OX-7, also results in chronic renal failure. The data of our study confirm that at 8 wk after disease induction, widespread evidence of irreversible renal damage is present in the new model.

After establishing the model, we first demonstrated that in chronic anti–Thy-1.1, analogous to the short-term, reversible model (9), the anti–PDGF-D antibody CR002 acutely reduced mesangial cell proliferation, as evidenced by lower numbers of glomerular mitotic figures and reduced expression of the mesangial cell–specific α-SMA. The major finding of our study was that inhibition of PDGF-D during the acute phase of anti–Thy-1.1 nephritis markedly reduced both glomerular and tubulointerstitial scarring on day 56 after disease induction. PDGF-D inhibition also prevented an increase of serum creatinine on day 49, although the increase in the nephritic IgG control group was not very pronounced. On day 56, the serum creatinine in the nephritic control group was not significantly different from those of all other groups. Albuminuria was not reduced during the course of the disease in the anti–PDGF-D–treated group. Similar effects (a reduction of glomerular and tubulointerstitial scarring in combination with unchanged proteinuria) also has been noted in rats that had puromycin aminonucleoside nephrosis and were treated with inhibitors of complement (28,29) and in rats that underwent 5/6 nephrectomy and were treated with a high dose of angiotensin I–converting enzyme inhibitor (30). Furthermore, in a nearly identical model of mesangioproliferative glomerulonephritis, compared with that used in our study, complement inhibition also reduced tubulointerstitial damage with no change in proteinuria (31). Our results demonstrate that 1 mg/kg body wt CR002 administered at weekly intervals was sufficient to antagonize the biologic activity of PDGF-D in vivo. Phase I trials in normal male volunteers are currently under way. One goal of these studies will be to evaluate pharmokinetic properties of CR002 and to do preliminary studies to explore the relationship between CR002 dose and CR002 bound versus free PDGF-D in the serum.

The mechanisms by which PDGF-D antagonism prevented chronic renal failure in our model probably include the specific reduction of mesangial expansion during the course of anti–Thy-1.1 nephritis. Mesangial expansion in combination with glomerular hyperfiltration and hypertrophy as a result of previous uninephrectomy (32) will lead to secondary podocyte stretch and damage, a key mechanism in the development of

Figure 7. Renal tubular expression of E-cadherin and interstitial expression of vimentin. (A) Section of a nonnephritic, uninephrectomized rat showing widespread tubular expression of E-cadherin. (B) Section of a nephritic rat that received control IgG2 showing E-cadherin expression by a small number of tubular epithelia. (C) Section of a nephritic rat that received CR002 on days 3, 10, and 17, showing widespread tubular expression of E-cadherin, similar to that observed in nonnephritic, uninephrectomized rats. (D) Morphometric evaluation of E-cadherin–positive area in the renal cortex. (E) Section of a nonnephritic, uninephrectomized rat showing vimentin expression mainly in smooth muscle cells of peritubular capillaries and by some interstitial cells. (F) Section of a nephritic rat that received control IgG2 showing markedly increased tubular and tubulointerstitial expression of vimentin. (G) Section of a nephritic rat that received CR002 on days 3, 10, and 17 showing moderate tubulointerstitial vimentin expression. (H) Morphometric evaluation of vimentin-positive area in the renal cortex. Data are means ± SD. *P < 0.05 versus nephritic rats that received control IgG2. Magnifications: ×100 in A through C; ×200 in D through F.

Figure 8. Tubulointerstitial influx of monocytes/macrophages on day 56 after disease induction. Data are means ± SD. *P < 0.05 versus nephritic rats that received control IgG2.
glomerulosclerosis (33). Podocyte damage indeed was reflected in our study by the widespread de novo expression of desmin, a nonspecific marker of podocyte injury (22). CR002 likely acted by reducing glomerular expansion and secondary podocyte damage, as evidenced by lower desmin expression at the glomerular edge. Reduced glomerular damage in nephritic rats that were treated with the PDGF-D antibody may be one of the reasons that subsequent tubulointerstitial damage also was ameliorated. Alternatively, it is conceivable that the persistence of CR002 in vivo (Table 1) directly affected the onset of tubulointerstitial fibrosis. At least on a descriptive level, Taneda et al. (34) showed that PDGF-D is produced de novo in interstitial cells in areas of tubulointerstitial fibrosis in mice with unilateral ureteral obstruction. In follow-up studies, we therefore are addressing the question of whether PDGF-D antagonism also may have a therapeutic role in renal fibrosis in general, i.e., independent of the action on glomerular disease.

Amelioration of tubulointerstitial scarring in nephritic rats that were treated with PDGF-D antibody was associated with a reduction in three key events, which are considered to be of importance in the pathophysiology of renal fibrosis. First, CR002-treated rats exhibited lower tubulointerstitial deposition of the membrane attack complex C5b-9. On the basis of elegant studies in various proteinuric models (23,28,35,36), it now is well established that proteinuria per se can induce complement activation in the tubular system, leading to tubular damage and subsequent peritubular inflammation and fibrosis. Second, CR002 prevented the tubular loss of E-cadherin expression and reduced the de novo expression of vimentin and \( \alpha \)-SMA in renal myofibroblasts. Both of these features are characteristic of EMT, which is viewed as a key event contributing to chronic renal interstitial fibrosis (24–26). Finally, CR002 led to reduced tubulointerstitial monocyte/macrophage influx, which is likely to contribute also to lesser chronic damage. In this regard, it is important to note that PDGF-D was linked recently to monocyte/macrophage infiltration in the skin (37).

Our study is one of the rare examples in which specific growth factor inhibition prevented glomerular and tubulointerstitial scarring. It thereby lends a further basis to clinical trials that aim to intervene in the PDGF system in progressive renal disease. Using a similar model of chronic anti-Thy-1.1 nephritis, we have shown that specific and transient antagonism of PDGF-B also can prevent irreversible renal damage in this model (6). This raises the interesting question of whether (and, if so, how) PDGF-B and -D might interact in vivo. Kinetic studies in the acute, reversible anti-Thy-1.1 nephritis model suggested that PDGF-B is an early auto- and paracrine mechanism involved in mesangio proliferative nephritis, whereas PDGF-D seemed to play a role at somewhat later stages and, more important, may also act as an endocrine mechanism (9). The questions of whether PDGF-D represents a “back-up system” for PDGF-B and whether combined inhibition of the two isoforms or, alternatively, blockade of the common PDGF-\( \beta \) receptor might be more effective than antagonism of either growth factor alone remain unresolved. In this respect, it is notable that treatment with Imatinib (STI 571), a nonspecific PDGF receptor tyrosine kinase inhibitor, in acute anti-Thy-1.1 nephritis was no more effective than inhibition of either PDGF-B or -D alone (9,15,38). However, a comparison of the efficacies of these compounds was never performed in a head-to-head manner.

**Conclusion**

Our study extends the evidence linking PDGF-D to the pathogenesis of renal damage in mesangio proliferative nephritis and lays the basis for clinical studies to address the question of whether PDGF-D antagonism might represent a novel approach to diseases such as IgA nephropathy or other nephritides that are characterized by augmented mesangial cell proliferation and expansion.

**Acknowledgments**

This study was supported by a grant (SFB 542/C7) of the German Research Foundation to J.F. and T.O. F.E. is a recipient of a stipend of the German Kidney Foundation (Deutsche Nierengesellschaft). U.K. is a recipient of a Lise-Meitner stipend from the Nordrhein-Westfalen region. The excellent technical help of Gabriele Dietzel, Andrea Cosler, and Sharon Arrol is gratefully acknowledged.

**References**


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