PDGF-D inhibition by CR002 ameliorates tubulointerstitial fibrosis following experimental glomerulonephritis

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Abstract

Background. Arresting or regressing kidney scarring is of major clinical relevance. Platelet-derived growth factor D (PDGF-D) is widely expressed in fibrotic kidneys. Administration of the PDGF-D neutralizing fully human monoclonal antibody CR002 in the acute phase of progressive anti-Thy 1.1 glomerulonephritis reduced glomerular and secondary tubulointerstitial damage.

Methods. Using this model, we now assessed the effects of CR002 (n = 15) vs irrelevant control IgG (n = 17) administered on days 17, 28 and 35 after disease induction, i.e. after acute glomerular damage had subsided.

Results. In vitro, CR002 inhibited the PDGF-D- but not the PDGF-B-induced proliferation of rat renal fibroblasts. Following the first CR002 injection on day 17, exposure to therapeutic levels was maintained until day 49. Proteinuria in the CR002-treated group was transiently reduced between days 49 and 77 (%C0 19 to %C0 23% in comparison with the controls; P < 0.05). On day 100, CR002 treatment reduced the number of rats that had doubled their serum creatinine (CR002: 40 vs controls: 71%; P < 0.05). Compared with controls, the CR002 animals, on day 100, significantly lowered glomerular expression of vimentin and collagens as well as tubulointerstitial damage scores, interstitial fibrosis, vimentin and cortical PDGF-D mRNA levels.

Conclusions. PDGF-D antagonism, even after the phase of acute glomerular damage, exerts beneficial effects on the course of tubulointerstitial damage, i.e. the final common pathway of most renal diseases.

Keywords: CR002; fibroblasts; PDGF-D; renal fibrosis

Introduction

Both diabetic nephopathy and the majority of progressive glomerulonephritides are histologically characterized by glomerular mesangial cell proliferation and/or matrix accumulation [1,2]. In addition, all of these diseases progress to renal failure via secondary tubulointerstitial damage and fibrosis. Treatments targeting both of these processes would therefore be of major clinical relevance.

The platelet-derived growth factor (PDGF) family consists of four PDGF chains, PDGF-A–D, that bind to dimeric PDGF receptors composed of α- and/or β-receptor subunits. Whereas PDGF-A and -C bind only to the α-chain, PDGF-B is a ligand for all receptor types and PDGF-DD binds predominantly to the PDGF ββ-receptor [3,4]. All four PDGF isoforms, as well as both receptor subunits are expressed in the kidney, albeit in distinct spatial arrangements [5–7].

The role of both PDGF-B and -D chains in mediating mesangioproliferative changes in glomerular disease is now well established [3,4,8–11]. With respect to mediating fibrotic damage, some evidence implicates the actions of PDGF-B [12], but increasing evidence also implicates PDGF-D in various organs: (i) its renal interstitial expression increases in obstructive uropathy in both humans and mice [13], (ii) it is strongly up-regulated in an in vitro model of hepatic fibrogenesis [14], (iii) it is expressed by synovial fibroblasts and macrophages of patients with rheumatoid arthritis and osteoarthritis [15], and most importantly, (iv) in transgenic mice, overexpression of PDGF-D in the heart induced pronounced cardiac fibrosis development [16]. Finally, the receptor for both PDGF-B and -D, i.e. PDGF receptor-ββ, is also up-regulated in the fibrotic renal interstitium [13,17].

We have recently reported that specific antagonism of PDGF-D using a fully human monoclonal antibody (CR002) in a progressive model of mesangioproliferative glomerulonephritis, i.e. anti-Thy 1.1 nephritis in rats, potently reduced the early glomerular damage.
Despite cessation of the treatment on day 17 after disease induction, it also partially prevented the subsequent development of tubulointerstitial damage [18]. We now asked, whether such treatment would also be effective if initiated after the acute antibody-mediated phase of glomerular damage has subsided and first tubulointerstitial damage is already established. The treatment was therefore confined to days 17, 28 and 35 after disease induction.

Materials and methods

Fully human PDGF-D monoclonal antibody CR002
Generation and specificity of the fully human PDGF-DD mAb CR002 was described previously [10].

Cell culture experiments
To examine the mitogenic effect of PDGF-D on rat renal interstitial fibroblasts, NRK-49F cells [German National Resource Center for Biological Material (DSMZ), Braunschweig, Germany] were seeded in 96-well plates (Nalge Nunc, Naperville, IL, USA), grown to subconfluency and growth-arrested for 72 h in serum-free medium. Cells were then stimulated for 24 h with recombinant human PDGF-DD (100 ng/ml; produced as described previously [4]) or recombinant human PDGF-BB (10 ng/ml; Sigma-Aldrich, Deisenhofen, Germany) with or without the addition of the neutralizing PDGF-D antibody CR002 (1 µg/ml) or control IgG2 (1 µg/ml) immediately after adding the mitogens. DNA synthesis was determined by a 5-bromo-2'-deoxyuridine (BrdU) incorporation assay according to the manufacturer's instructions (Roche, Mannheim, Germany). The experiments were performed in quadruplicates.

Experimental design
All animal experiments were approved by the local review boards. Animals were held in rooms with constant temperature and humidity, 12 h/12 h light cycles, and had ad libitum access to drinking water (ozone-treated and acidified). Daily fluid intake was measured, and the different treatment groups were pair-fed (normal chow diet).

Progressive mesangio proliferative glomerulonephritis was induced in 32 male Wistar rats weighing 200 g (Charles River, Sulzfeld, Germany) by unilateral nephrectomy followed by injection of 1 mg/kg monoclonal anti-Thy 1.1 antibody (clone OX-7; European Collection of Animal Cell Cultures, Salisbury, England) as described previously [18]. On day 3, rats were randomized according to proteinuria into two groups: the first group received control IgG2 (5 mg/kg body weight; n = 17) and the second was treated with fully human anti-PDGF-D monoclonal antibody CR002 (5 mg/kg body weight; n = 15). The antibodies were dissolved in 20 mM Tris-HCl/100 mM NaCl, pH 7.4 and administered by intraperitoneal injections on days 17, 28 and 35 after disease induction. To start treatment in established tubulointerstitial fibrosis, we chose day 17 since the first changes in tubulointerstitium are observed already in the first week of progressive anti-Thy1.1 nephritis [19] and since the acute immune-mediated phase of glomerular damage subsides around day 10. Longer treatment was not attempted given concerns about the potential immunogenicity of the neutralizing human antibody in rats. Non-nephritic control groups with a uninephrectomy were not included in the present study, as we have shown recently that CR002 treatment over 3 weeks has no significant effects under these conditions [18]. No rat treated with CR002 died during the experiment. In animals that received control IgG2, two rats had to be sacrificed due to rapid progression of the disease on days 24 and 95 after disease induction (the second was included into the study).

Venous blood samples (drawn from a tail vein or the inferior vena cava at sacrifice) and 24 h urine collections were performed on days 3, 35, 42, 49, 56, 77, 90 and 100. Blood pressure was measured by tail cuff plethysmography on days 2, 35, 56, 73 and 99. Following sacrifice, serum samples as well as renal tissues for histological evaluation were collected. The remaining cortical tissue of each rat was used to isolate RNA.

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-micrometre sections were stained with the Periodic acid-Schiff (PAS) reagent and counterstained with haematoxylin. In PAS-stained sections of day 100 biopsies, the percentage of glomeruli exhibiting focal or global glomerulosclerosis was determined as described [9]. Tubulointerstitial injury on day 100 was graded on a scale of 0-4 as described previously [9].

For the evaluation of total collagen content, renal tissues were stained with Sirius red and evaluated by computer-based morphometry using the analySIS v3.1 software (Soft Imaging System GmbH, Münster, Germany). The percentage of positively stained area in each tissue was calculated separately for glomeruli and in 20 interstitial fields representing almost the whole cortical area 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-micrometre sections of methyl Carnoy’s fixed renal tissues were processed as previously described [10]. Primary antibodies included a murine monoclonal antibody (clone 1A4) to α-smooth-muscle actin (α-SMA); a murine monoclonal IgG antibody (clone ED1) to monocytes, macrophages and dendritic cells; affinity purified polyclonal goat antibodies against human type I and type III collagens (Southern Biotechnology, Birmingham, Alabama, USA); a murine monoclonal antibody against porcine vimentin (clone V9, Dako, Glostrup, Denmark); a murine monoclonal IgG against human E-cadherin (Dako Cytomation, Glostrup, Denmark) plus appropriate negative controls as described previously [18].

The stains for cortical type I and type III collagens, α-SMA, infiltrating monocytes/macrophages (ED1), vimentin and, E-cadherin were evaluated by computer-based morphometry as described for Sirius red (mentioned previously).
Late PDGF-D inhibition ameliorates renal fibrosis

Since renal fibroblasts have been implicated in the development of tubulointerstitial fibrosis, we tested whether CR002 would block rat renal fibroblast proliferation in response to PDGF-D. Figure 1 shows that PDGF-D, like PDGF-B, significantly induced proliferation of rat renal fibroblasts. Addition of CR002 specifically inhibited the effect of PDGF-D, but not that of PDGF-B. Irrelevant control IgG did not affect the PDGF-D or -B-induced fibroblast proliferation (data not shown).

Serum levels obtained after injection of CR002 into nephritic rats

Seven days after the last i.p. injection (i.e. on day 42) the mean concentration of circulating CR002 was 4.00 ± 0.45 μg/ml and no rat had to be excluded due to insufficient serum level. Compared with day 42, circulating CR002 concentrations decreased 2- and 8-fold on days 49 and 56, respectively (Figure 2) and 100-fold on day 100 (0.035 ± 0.003 μg/ml). The concentrations reached on both days 42 and 49
All differences between CR002 and IgG treated groups are not significant.

Table 2. Body weight and parameters of kidney function in nephritic rats treated with irrelevant IgG (IgG, n = 17) and CR002 (anti-PDGF-D Ab, n = 15).

<table>
<thead>
<tr>
<th>Day after disease induction</th>
<th>Parameter</th>
<th>Group</th>
<th>3</th>
<th>35</th>
<th>42</th>
<th>49</th>
<th>56</th>
<th>77</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (g)</td>
<td>IgG</td>
<td>201 ± 4</td>
<td>347 ± 9</td>
<td>366 ± 9</td>
<td>377 ± 9</td>
<td>389 ± 10</td>
<td>429 ± 10</td>
<td>445 ± 12</td>
<td>426 ± 12</td>
</tr>
<tr>
<td></td>
<td>CR002</td>
<td>205 ± 6</td>
<td>352 ± 8</td>
<td>365 ± 9</td>
<td>382 ± 9</td>
<td>395 ± 9</td>
<td>437 ± 10</td>
<td>456 ± 12</td>
<td>436 ± 12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum creatinine (µmol/l)</td>
<td>IgG</td>
<td>0.38 ± 0.02</td>
<td>0.42 ± 0.03</td>
<td>0.39 ± 0.03</td>
<td>0.30 ± 0.03</td>
<td>0.32 ± 0.3</td>
<td>0.28 ± 0.03</td>
<td>0.25 ± 0.03</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CR002</td>
<td>0.37 ± 0.02</td>
<td>0.41 ± 0.04</td>
<td>0.41 ± 0.02</td>
<td>0.34 ± 0.03</td>
<td>0.29 ± 0.02</td>
<td>0.30 ± 0.03</td>
<td>0.30 ± 0.03</td>
<td>0.24 ± 0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Creatinine clearance (ml/min/100 g)</td>
<td>IgG</td>
<td>0.03 ± 0.02</td>
<td>0.02 ± 0.03</td>
<td>0.03 ± 0.02</td>
<td>0.04 ± 0.03</td>
<td>0.05 ± 0.02</td>
<td>0.06 ± 0.03</td>
<td>0.07 ± 0.04</td>
<td>0.08 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>CR002</td>
<td>0.02 ± 0.02</td>
<td>0.02 ± 0.03</td>
<td>0.02 ± 0.02</td>
<td>0.03 ± 0.03</td>
<td>0.04 ± 0.02</td>
<td>0.05 ± 0.03</td>
<td>0.06 ± 0.04</td>
<td>0.07 ± 0.05</td>
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</tbody>
</table>

All differences between CR002 and IgG treated groups are not significant.

were similar to those measured previously in experiments demonstrating pharmacological effects [18] and thus likely to be therapeutically effective.

No production of rat anti-human IgG that could have neutralized the effect of CR002 was detected in sera obtained on days 49 and 100 (data not shown).

**PDGF-D antagonism transiently reduces proteinuria and retards progressive renal failure**

CR002 treatment led to significant reductions in proteinuria when compared with the group receiving irrelevant IgG, on days 49, 56 and 77 (Figure 2). At later time points, differences were no longer significant and considerable variability in the extent of proteinuria was noted. Albuminuria on day 100 also did not differ between the two groups (84 ± 5 mg/day in IgG-treated rats vs 77 ± 6 in CR002-treated rats; not significant).

Serum creatinine concentrations and creatinine clearances did not differ significantly throughout the study, although the means were constantly lower (creatinine) or higher (creatinine clearance) in the CR002-treated group vs the IgG group (Table 2).

To account for the large variability in renal failure progression between different rats, we also calculated the number of rats that doubled their serum creatinine in the course of the experiment (i.e. >92 µmol/l at day 100). This analysis showed that 6 of 15 rats receiving CR002, vs 12 of 17 rats receiving IgG, doubled their serum creatinine (P < 0.05). No statistically significant differences were found at earlier time points. In addition, 47% of the rats in the IgG group vs 20% in the CR002 group lost >50% of glomerular filtration rate between days 35 and 100 (P = 0.053).

Since both groups received pair-feeding, body weight was similar in both groups (Table 2). The measured daily water intake also did not differ between the two groups at various time points throughout the study (data not shown).

Systolic and diastolic blood pressures were constantly elevated between days 36 and 100, but did not differ significantly throughout the study (day 100: systolic pressure 161 ± 5 vs 153 ± 7 mmHg in IgG- and CR002-treated rats, respectively; diastolic pressure 124 ± 5 vs. 122 ± 7 mmHg in IgG- and CR002-treated rats, respectively).

**PDGF-D antagonism reduces overall tubulointerstitial damage on day 100**

Widespread glomerular and tubulointerstitial damage with considerable glomerulosclerosis and tubulointerstitial fibrosis developed in both groups. However, when compared with rats receiving irrelevant IgG,
the CR002-treated group exhibited significantly lower tubulointerstitial damage scores (Figure 3). The development of focal segmental glomerulosclerosis was also reduced in CR002-treated rats, but the difference was not significant (66 ± 4 vs 57 ± 4% of glomeruli in IgG- and CR002-treated animals, respectively).

**PDGF-D antagonism reduces glomerular mesangial cell activation and matrix accumulation on day 100**

The glomerular de novo expression of α-SMA and interstitial types of collagen (i.e. types I and III) as well as overexpression of vimentin is characteristic of a myofibroblast-like phenotype acquisition of mesangial cells. As shown in Figures 4 and 5, Sirius red staining as well as immunostaining for types I and III collagen and vimentin were significantly reduced in CR002-treated rats as compared with rats receiving irrelevant IgG by 33, 49, 35, and 20%, respectively. Glomerular α-SMA expression was reduced by PDGF-D antagonism by 39% (Figures 4 and 5). Glomerular monocyte/macrophage infiltration was not affected by CR002 treatment on day 100 (0.11 ± 0.04 vs 0.05 ± 0.03% of stained glomerular area in IgG- and CR002-treated animals, respectively; the high mean value in the IgG group is due to a single outlier animal).

**Fig. 3.** Tubulointerstitial damage was significantly ameliorated by CR002. PAS-stained sections from IgG-treated (A) or CR002 treated (B) animals. Inhibition of PDGF-D improved the tubulointerstitial damage score (C). Data are means ± SEM. *P < 0.05, vs IgG group. Magnification 100×.

**Fig. 4.** Sirius red staining (A, B) and specific immunohistochemistry for collagen type I (C, D) and III (E, F), vimentin (G, H) and α-SMA (I, J) in IgG-treated control animals (images A, C, E, G, I) or CR002-treated animals (B, D, F, H, J). Magnification 100×.
**PDGF-D antagonism reduces tubulointerstitial matrix accumulation on day 100**

Tubulointerstitial fibrosis was assayed by a global marker (i.e. Sirius red) and by the expression of specific matrix molecules. Figures 4 and 5 show that CR002 treatment, when compared with irrelevant IgG, significantly reduced the tubulointerstitial Sirius red-positive area (~20%) as well as the renal cortical areas covered by type I collagen (~28%), type III collagen (~32%), and vimentin (~41%). Renal cortical mRNA levels of type I and III collagens did not differ between the two groups (Table 3). We also assessed the renal cortical hydroxyproline content, which was 1328 ± 232 mg/g cortical kidney tissue (CR002 group; P = 0.09, IgG vs CR002).

Expression of α-SMA decreased by 33% but failed to reach statistical significance. Again, monocyte/macrophage infiltration on day 100 was not affected by CR002 treatment (0.52 ± 0.09 vs 0.41 ± 0.05% of stained glomerular area in IgG- and CR002-treated animals, respectively; the higher mean value in the IgG group is due to a single outlier animal). There was no difference in E-cadherin between the groups (0.85 ± 0.11% vs 0.70 ± 0.09% of stained area in IgG- and CR002-treated animals, respectively).

**PDGF-D antagonism down-regulates cortical PDGF-D but not -B or PDGFR-β mRNA on day 100**

Treatment with CR002 showed no effect on cortical mRNA levels of PDGF-B and PDGFR-β, whereas PDGF-D levels were significantly down-regulated on day 100 (Table 3).

To investigate, whether the renal PDGF-D/PDGFR-β system was stimulated in our model, we used renal cortical RNA samples of our previous study.

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**Fig. 5.** Quantitative assessment of glomerular and tubulointerstitial damage on day 100. Panels show the quantification of Sirius red stain (A, B), the expression of type I (C, D) and type III (E, F) collagens, vimentin (G, H) and α-SMA (I, J). Upper graphs show the results from the glomerular compartment (A, C, E, G, I), the lower graphs the results from the tubulointerstitial compartment (B, D, F, H, J). The Y-axis gives the relative area (%) of positively stained tissue. Data are means ± SEM. *P < 0.05, vs IgG group.
Late PDGF-D inhibition ameliorates renal fibrosis

Table 3. Renal cortical mRNA expression of type I and III collagens, PDGF-B, -D and PDGFR-β in nephritic rats treated with CR002 (anti-PDGF-D Ab, n = 15) relative to nephritic animals treated with irrelevant IgG (IgG, n = 17) at day 100

<table>
<thead>
<tr>
<th>Relative mRNA expression</th>
<th>IgG</th>
<th>CR002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I collagen</td>
<td>1.18 ± 0.18</td>
<td>1.27 ± 0.20</td>
</tr>
<tr>
<td>Type III collagen</td>
<td>1.15 ± 0.17</td>
<td>1.01 ± 0.15</td>
</tr>
<tr>
<td>PDGF-B</td>
<td>1.11 ± 0.12</td>
<td>0.91 ± 0.07</td>
</tr>
<tr>
<td>PDGF-D</td>
<td>1.09 ± 0.11</td>
<td>0.77 ± 0.08*</td>
</tr>
<tr>
<td>PDGFR-β</td>
<td>1.10 ± 0.14</td>
<td>1.01 ± 0.10</td>
</tr>
</tbody>
</table>

* P < 0.05, CR002 vs IgG group.

PDGF, platelet-derived growth factor; PDGFR, receptor for PDGF.

in the same model obtained on day 56 [18]. In comparison with PDGF-D mRNA levels in uninephrectomized rats (0.36 ± 0.05 relative mRNA expression), PDGF-D mRNA levels were elevated 7.4-fold and 4.3-fold in phosphate-buffered saline (PBS)-treated (2.67 ± 0.36 relative mRNA expression) or IgG-treated (1.54 ± 0.40 relative mRNA expression) nephritic uninephrectomized rats, respectively (both P < 0.05). Similarly, expression of PDGFR-β mRNA increased 3.0-fold and 2.6-fold in PBS- or IgG-treated rats, respectively (P < 0.05 vs. uninephrectomized rats; data not shown). A 3.3-fold and 3.2-fold up-regulation of PDGF-D (1.42 ± 0.19 relative mRNA expression) and PDGFR-β mRNA (3.10 ± 0.50 relative mRNA expression), respectively, is observed already at day 10 in nephritic uninephrectomized rats if compared with nephrectomized rats (both P < 0.05; unpublished data).

Discussion

In our previous study, we showed that administration of the CR002 antibody against PDGF-D, on days 3, 10 and 17 after induction of anti-Thy 1.1 nephritis, ameliorated not only glomerular damage but also the secondary interstitial fibrosis [18]. Since PDGF-D is overexpressed in both rodent and human fibrotic renal interstitium [6,13], we asked whether PDGF-D antagonism would be beneficial if initiated after the acute phase of mesangio proliferative nephritis. Since the increase of glomerular mesangial cell proliferation in the anti-Thy 1.1 nephritis model subsides at around day 10 after disease induction, CR002 was administered on days 17, 28 and 35. Due to concerns regarding the immunogenicity of the fully human antibody in rats, a longer treatment duration was not attempted.

We first verified in vitro that PDGF-D acts as a potent mitogenic stimulus in rat interstitial fibroblasts and that this can be reversed by adding CR002 (Figure 1). Our findings extend our previous findings in mouse NIH 3T3 fibroblasts [10] as well as recent data showing that PDGF-D induces proliferation of rat vascular fibroblasts, primary mouse cardiac fibroblasts and human synovial fibroblasts [15,16,20].

The major finding of the present study was that treatment with CR002 on days 17, 28 and 35 transiently decreased proteinuria, slowed progression of renal failure and ameliorated glomerular as well as tubulointerstitial fibrotic changes at day 100. These findings appear particularly notable given that most effects of CR002 persisted 65 days after the last treatment and ~50 days after circulating CR002 concentrations fell below the therapeutic level. Given our in vitro data in renal fibroblasts, it appears likely that PDGF-D antagonism acted by at least transiently retarding fibroblast proliferation, and thus, the development of tubulointerstitial fibrosis. Similar collagen type I and III mRNA levels, but a difference in immunostaining of these long-lived collagens on day 100, are consistent with such a transient effect of CR002 treatment. Our data imply that continuous antagonism of PDGF-D from day 17 to 100 might be even more effective if immunological problems of the antibodies can be avoided. However, it needs to be pointed out that in other experimental models of renal fibrosis using a large variety of monotherapeutic approaches, generally with continuous treatment, renal fibrosis was reduced by a similar order of magnitude, usually 30–50% [21]. PDGF-D inhibition in the late phase of progressive mesangio proliferative glomerulonephritis was not as effective as the early inhibition [18]. A direct comparison, however, is difficult since the compartments and cell types mainly affected by CR002 are different and also, the severity of the disease in the later course is much more pronounced.

Our study is the first to specifically inhibit one of the two ligands of the PDGF β-receptor, i.e. PDGF-B and -D, in a model of renal tubulointerstitial damage. In the case of PDGF-B, no specific antagonism has been tested so far. However, it has been shown that PDGF-B, like PDGF-D, is overexpressed in fibrotic renal interstitium and that the prolonged administration of high doses of PDGF-B can induce renal interstitial fibrosis [9,12,17,22]. Differences in the pro-fibrotic activity of PDGF-B and -D might be due to the fact that only PDGF-B can activate the PDGF αα-receptor, which is very widely expressed in fibrotic renal tissue [23,24]. Interestingly, however, heart-specific overexpression of PDGF-D in mice caused fatal cardiac fibrosis, whereas PDGF-B led to a non-lethal phenotype with only focal fibrosis [16]. Also, in mice, hepatic overexpression of PDGF-D caused a more profound glomerulopathy than overexpression of PDGF-B [11]. At least in mesangial cells, we recently showed that both PDGF-isoforms activated the cells almost exclusively via the β-receptor despite the presence of both PDGF α- and β-receptors on these cells [25]. Collectively these observations identify PDGF-D as a more potent agonist of the PDGF β-receptor, whereas the role of the PDGF α-receptor in vivo is less clear.

Several studies have investigated the effects of imatinib (STI-571), a receptor tyrosine kinase blocker, in models of renal disease. Imatinib is widely used in...
cancer therapy as a blocker of the c- abl kinase, but also blocks signal transduction of the PDGF-receptor tyrosine kinase [26]. Imatinib retarded the development of experimental diabetic nephropathy [22], ameliorated experimental chronic allograft nephropathy [27] and the course of renal fibrosis after unilateral ureter ligation [28]. It was also heart- and renoprotective in a model of hypertensive end-organ damage [29], and attenuated pulmonary and liver fibrosis in different models [30,31]. However, whether this beneficial effect of imatinib was indeed mediated via reduction of PDGF signalling remains uncertain, since Wang et al. [28] demonstrated that renal fibroblasts express c- abl kinase, that the major pro-fibrotic cytokine TGF-β acts via c- abl kinase, and that imatinib can interfere with this process. More relevant in the context of the present study is the observation that specific inhibition of the PDGF-B chain by anti-sense mRNA in a rat model of liver fibrosis significantly attenuated the overexpression of type I collagen and α-SMA [32].

De novo expression of vimentin and α-SMA and the tubular epithelial loss of E-cadherin expression are markers of epithelial–mesenchymal transition (EMT), i.e. a phenotypic change of tubular epithelial cells to (myo-) fibroblast-like cells. EMT is believed to play an important role in kidney fibrosis [33,34]. Late PDGF-D antagonism ameliorated EMT, although it did not influence the loss of E-cadherin, but downregulated the tubular de novo vimentin expression (Figure 4 G–J). Thus, late PDGF-D inhibition did not interfere with the process of EMT as potently as the early inhibition [18].

PDGF-D antagonism in the present study did not affect the glomerular or tubulointerstitial monocyte/macrophage influx on day 100. It presently remains unclear whether PDGF-D has a direct chemotactic role for macrophages similar to that of PDGF-B [35,36]. Our previous data [18] as well as those of Uutela et al. [37] suggest that PDGF-D is capable of recruiting monocytes/macrophages into the kidney and skin, respectively. However, the PDGF-receptor tyrosine kinase inhibitor imatinib also had inconsistent effects in this respect, since it reduced macrophage infiltration in diabetic nephropathy [22] but not in rat renal interstitial fibrosis [28].

An interesting finding is that CR002 did not affect the renal cortical mRNA levels of PDGF-B and PDGFR-β on day 100 but led to a persistent decrease of PDGF-D mRNA. This finding is consistent with a lower number of fibroblasts in the CR002-treated group, since fibroblasts are a major source of PDGF-D in the fibrotic kidney [13].

In conclusion, our data are the first to suggest that specific antagonism of PDGF-D may not only be beneficial in the phase of active, immune-mediated glomerular injury, but also in later phases where progression of renal disease has become independent of the initiating condition. PDGF-D antagonism by CR002 thereby targets two clinically important processes and offers the hope of providing therapeutic efficacy to patients in various phases of progressive glomerular disease. Our study should serve to spark subsequent studies in this field.

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