

Inhibition of Mesangial Cell Proliferation and Matrix Expansion in Glomerulonephritis in the Rat by Antibody to Platelet-derived Growth Factor

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Summary

Platelet-derived growth factor (PDGF), a potent mitogen for mesenchymal cells in culture, is expressed in vivo in a variety of inflammatory conditions associated with cell proliferation, including atherosclerosis, wound repair, pulmonary fibrosis, and glomerulonephritis. However, it is not known if PDGF mediates the fibroproliferative responses that characterize these inflammatory disorders. We administered neutralizing anti-PDGF IgG or control IgG to rats with mesangial proliferative nephritis. Inhibition of PDGF resulted in a significant reduction in mesangial cell proliferation, and largely prevented the increased deposition of extracellular matrix associated with the disease. This suggests that PDGF may have a central role in proliferative glomerular disease.

Mesangial cell proliferation and matrix expansion characterize many types of glomerulonephritis (GN). The observations that platelet-derived growth factor (PDGF) is a potent mitogen for mesangial cells in culture (1) and is expressed in both experimental and human GN in which mesangial cell proliferation occurs (2–5) suggest that this factor may have an important role in mediating these changes.

One model in which PDGF has been studied is the mesangial proliferative GN in rats induced by antibody to the Thy-1 antigen, which is expressed by mesangial cells (2). The model is characterized by an acute complement-dependent loss of mesangial cells with disruption of the mesangial matrix (“mesangiolytic”) that is maximal at 24 h (2). The mesangial cell population, which is almost completely eliminated, undergoes a rebound proliferation that is accompanied by a marked upregulation of PDGF A and B chain mRNA in total glomerular RNA at 3 and 5 d after disease induction (2). When rats were depleted of either complement or platelets and were injected with anti-Thy-1 antibody, both the glomerular cell proliferation and increased glomerular PDGF expression were significantly inhibited (2, 3). However, despite demonstrating a strong association between glomerular PDGF expression and glomerular cell proliferation in mesangial proliferative GN, these studies do not determine whether PDGF plays a direct role in the pathogenesis of glomerular injury. We now report the effect of blocking PDGF in vivo in this model of nephritis utilizing a neutralizing polyclonal antibody to PDGF.

Materials and Methods

Experimental Protocol. Anti-Thy-1 GN was induced with goat anti-Thy-1 plasma in 150–200-g male Wistar rats (Simonsen, Gilroy, CA) as previously described (2). 8 h before the injection of anti-Thy-1 antibody, rats were injected with goat anti-PDGF IgG (60 mg/100 g body weight, i.p.) ($n = 6$) or equivalent quantities of nonimmune (control) goat IgG ($n = 6$) with repeated doses daily for 4 d. After disease induction, rats underwent renal biopsies at 2 and 4 d. Blood samples were collected for serum C3 levels (at 0, 2, and 4 d), leukocyte and platelet counts (day 4), and plasma anti-PDGF IgG levels (day 4, measured by ELISA [6]).

Anti-PDGF Antibody. The anti-PDGF IgG was raised in a goat immunized with PDGF purified from outdated human platelets and specifically neutralizes the mitogenic activity of rat PDGF and all dimeric forms of human PDGF (6).

Histology. The following antibodies were used for immunoperoxidase staining of methyl Carnoy's fixed, paraffin-embedded tissue: 19A2 (Coulter Immunology, Hialeah, FL), a mAb to the proliferating cell nuclear antigen (PCNA), which is a cell proliferation marker; ED-1 (Bioproducts for Science, Indianapolis, IN), a mAb to rat monocytes and macrophages; rabbit anti-rat collagen I and rabbit anti-rat laminin (Chemicon, Temecula, CA); rabbit anti-mouse collagen IV (Collaborative Research Inc., Bedford, MA); rabbit anti-mouse entactin (gift of A. Chung, Pittsburgh, PA); and rabbit anti-mouse heparan sulfate proteoglycan (gift of J. Couchman, Birmingham, AL) (2, 7). Immunofluorescence of snap-frozen tissue was also performed using FITC-conjugated rabbit anti-goat IgG and FITC-conjugated goat anti-rat C3 antibodies (Organon Teknica Corp., West Chester, PA) (8). Histological changes were quantitated as previously described (2, 7). PDGF B

chain mRNA was detected in formalin-fixed tissue using a digoxigenin-labeled antisense cRNA probe and quantitated as previously described (3).

Statistics. Values are expressed as mean \pm SD. The one-tailed student's *t* test was used to test the hypothesis that anti-PDGF treatment would reduce cell proliferation, total glomerular cellularity, or matrix expansion relative to controls.

Results and Discussion

In this study, neutralizing anti-PDGF IgG or control IgG was administered to rats with mesangial proliferative GN. Two time points were selected for study, representing the day of initial proliferation (day 2) and the day of peak proliferation (day 4). Later time points were not studied due to the concern that the rats would develop autoantibodies to the administered goat IgG.

Anti-PDGF IgG treatment was well tolerated, and serum C3 levels (measured at 0, 2, and 4 d) and platelet and leukocyte counts (at 4 d) were normal and not different from controls. Whereas anti-PDGF IgG levels were undetectable in control rats, plasma anti-PDGF IgG levels in treated rats were 4.6 ± 0.7 mg/ml at day 4, concentrations that are 20–30 times that required to inhibit the mitogenic activity of PDGF on rat smooth muscle cells *in vitro* (6).

Previous studies have demonstrated that the initial injury (i.e., mesangiolytic) in this model is dependent on delivery and binding of anti-Thy-1 antibody to the mesangial cell followed by complement activation (9). Anti-PDGF IgG treatment did not prevent this initial injury, as both control and anti-PDGF IgG-treated rats had equivalent mesangiolytic (2.6 \pm 0.5 vs. 2.9 \pm 0.2, respectively, scale of 0–4+; *p* = NS) with an equal reduction in total glomerular cellularity at day 2 (Table 1). Anti-PDGF IgG treatment also did not inhibit the glomerular macrophage infiltration at either day 2 (9.1

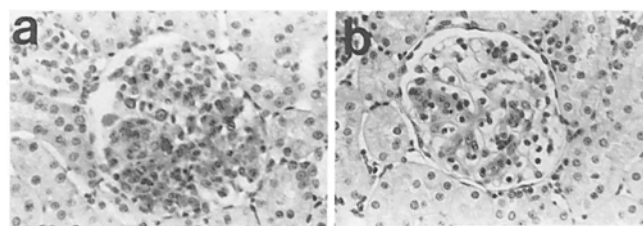


Figure 1. Compared with control rats with mesangial proliferative GN (a), anti-PDGF IgG-treated rats with GN (b) had significantly less glomerular cellularity at day 4 (periodic acid/Schiff reagent with hematoxylin counterstain, $\times 240$).

± 2 vs. 9.2 ± 1) or day 4 (8.0 ± 2 vs. 8.8 ± 1 ED-1+ cells/glomerular cross-section in control vs. anti-PDGF IgG-treated rats, respectively).

Effect on Glomerular Cell Proliferation. The initial glomerular cell proliferation (i.e., PCNA+ cells) was not affected by anti-PDGF IgG treatment (Table 1). In contrast, at day 4, during the peak phase of cell proliferation, a 57% reduction in cell proliferation was observed (*p* < 0.005), and was associated with a significant reduction in total glomerular cellularity as compared with controls (Table 1 and Fig. 1). Most of the proliferating cells in this model have been shown to be mesangial cells (2, 8), and this was supported in the current study in which >85% of the PCNA+ cells in both groups excluded the monocyte-macrophage marker, ED-1, by double immunolabeling.

One concern was that anti-PDGF IgG treatment might result in significant PDGF/anti-PDGF immune complexes in glomeruli that would prolong the mesangiolytic and delay the proliferative response. However, both control and anti-PDGF IgG-treated rats had equivalent mesangial staining of IgG at 2 and 4 d (2+, scale of 0–4+) with negative staining of C3 by immunofluorescence. Similarly, incubation of both resting and proliferating mesangial cells in culture with anti-PDGF IgG (5 mg/ml) resulted in no significant ^{51}Cr release compared with control IgG in the presence of complement (data not shown). Finally, injection of equivalent doses of anti-PDGF IgG into normal rats (*n* = 2) as used in the experimental study resulted in no detectable mesangial injury in biopsies obtained at days 2 and 4.

The observation that mesangial cell proliferation was reduced by anti-PDGF IgG treatment at day 4 but not at day 2 has several potential interpretations, including: (a) the possibility that numerous growth factors, including PDGF, are involved in the initial cell proliferation such that mesangial cells are maximally stimulated even when PDGF is inhibited; (b) the possibility that inadequate anti-PDGF IgG was present locally to block PDGF before its binding to the mesangial cell at day 2; and (c) the possibility that responsiveness to PDGF requires upregulation of the PDGF receptor. PDGF receptor β subunit expression is known to be upregulated in this disease (2), and in this study, PDGF receptor β subunit immunostaining was markedly increased in glomeruli at day 4 relative to day 2 (data not shown).

Other possible growth regulatory molecules that could mediate the initial mesangial cell proliferation include factors

Table 1. Effect of Anti-PDGF IgG Treatment on Total Glomerular Cellularity and Proliferating (PCNA+) Cells in Mesangial Proliferative GN.

	Total cells	Proliferating (PCNA+) cells
Normal	77 \pm 1.8	0.9 \pm 0.2
Mesangial proliferative GN, day 2		
Control	53 \pm 5	10.4 \pm 1
Anti-PDGF	53 \pm 6	10.0 \pm 2
Mesangial proliferative GN, day 4		
Control	89 \pm 13	13.4 \pm 4
Anti-PDGF	77 \pm 10*	5.7 \pm 1†

Values are expressed as the mean number \pm SD of cells per glomerular cross-section. For comparison, values for normal Wistar rats (*n* = 6) are shown (2).

* *p* < 0.05 relative to control rats.

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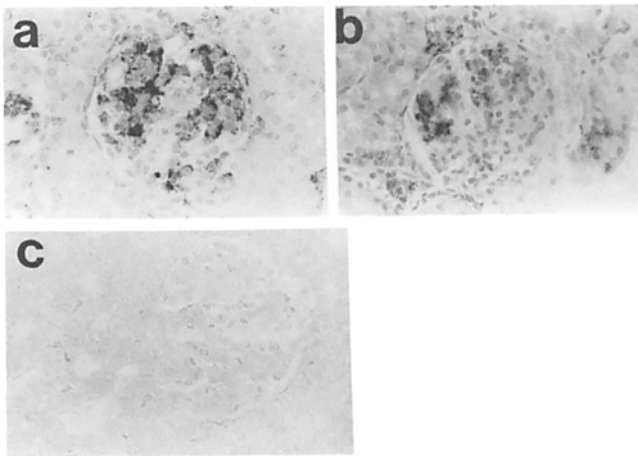


Figure 2. Whereas the expression of PDGF B chain mRNA was often diffuse in glomeruli of control rats with mesangial proliferative GN (a), in anti-PDGF IgG-treated rats with GN, expression was often segmental (b). Hybridization with a sense probe for PDGF B chain mRNA was always negative (c) ($\times 240$).

released by platelets, since thrombocytopenic animals show a decrease in mesangial cell proliferation in this model at day 3 (8) and basic fibroblast growth factor (bFGF), which is released by a variety of damaged cells, and is a mitogen for mesangial cells in culture (10). Recently, we have shown that mesangial cells produce bFGF, and release bFGF during acute mesangiolysis (i.e., the first 24 h) in this model (J. Floege et al., manuscript submitted for publication). This may also be relevant to the PDGF-mediated response, given that bFGF will induce both PDGF expression by mesangial cells in culture (10) and an increase in PDGF receptors on smooth muscle cells (our unpublished observations).

Glomerular Source of PDGF. Although the source of the glomerular PDGF may partially originate from platelets, activated macrophages, or endothelial cells, our previous studies (2, 3) suggest that most of the PDGF is expressed by mesangial cells, where it may function as an autocrine growth factor.

Table 2. Effect of Anti-PDGF IgG Treatment on Extracellular Matrix Accumulation in Mesangial Proliferative GN

	Normal	Control	Anti-PDGF
Type IV collagen	0.9 \pm 0.8	3.1 \pm 0.7	2.1 \pm 0.4*
Type I collagen	0.03 \pm 0.02	2.5 \pm 0.6	1.6 \pm 0.4*
Laminin	0.8 \pm 0.4	2.6 \pm 0.6	1.8 \pm 0.3*
Entactin/nidogen	0.2 \pm 0.1	2.5 \pm 0.7	1.6 \pm 0.4*
Heparan sulfate proteoglycan	1.8 \pm 0.5	3.1 \pm 0.6	2.6 \pm 0.6

Semiquantitative immunohistochemical scores for various ECM components (scale of 0–4+ [7]) in the mesangium of rats with mesangial proliferative GN that had been treated with nonimmune IgG (control, $n = 6$) or with anti-PDGF IgG ($n = 6$). For comparison, the values in normal Wistar rats ($n = 6$) are shown.

* $p < 0.01$ vs. control rats.

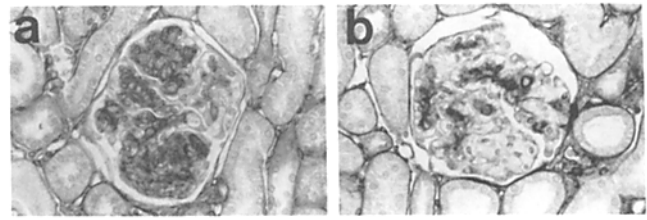


Figure 3. A diffuse increase in laminin was present in the mesangium of control rats with GN (a), and was significantly reduced in rats with GN that had received anti-PDGF IgG (b) ($\times 240$).

We therefore performed in situ hybridization to determine the effect of anti-PDGF IgG treatment on PDGF B chain mRNA expression at day 4. An increase in PDGF B chain mRNA could be detected in mesangial regions in both control and anti-PDGF IgG-treated rats (Fig. 2). However, the amount of PDGF B chain mRNA in the glomeruli was lower in anti-PDGF IgG-treated animals (1.68 ± 0.4 vs. 1.24 ± 0.2 in control vs. anti-PDGF-treated rats, scale of 0–3+, one-tailed Student's t test; $p < 0.05$ [3]). The reduction in glomerular expression of PDGF B chain mRNA in the anti-PDGF IgG-treated rats is consistent with an inhibition of mesangial cell proliferation and an interruption of PDGF-mediated induction of its own expression in mesangial cells (10).

Effect of Anti-PDGF IgG Treatment on Extracellular Matrix (ECM) Accumulation. Previous studies have demonstrated that in this model mesangial cell proliferation is accompanied by an expansion of several ECM proteins in the mesangium (7, 11). In this study, control rats with anti-Thy-1 GN also had a diffuse increase in glomerular staining at day 4 for various ECM proteins, including types I and IV collagen, laminin, and entactin (Table 2). In contrast, glomeruli from anti-PDGF IgG-treated rats with GN showed significantly less, and often only segmental, increases in staining (Table 2 and Fig. 3).

The reduction in immunostaining for the various ECM components in anti-PDGF IgG-treated rats may reflect, in part, the reduction in mesangial cell number. Alternatively, anti-PDGF treatment may be affecting TGF- β production within the glomeruli. PDGF induces mesangial cells to express TGF- β (12). TGF- β , in turn, induces mesangial cells to produce a variety of ECM components (13, 14). TGF- β is also increased in glomeruli of rats with anti-Thy-1 GN, and treatment of rats with anti-Thy-1 GN with anti-TGF- β antibody inhibits expansion of the mesangial matrix (11). Thus, it is possible that the beneficial effects of anti-PDGF treatment on ECM expansion in anti-Thy-1 GN may reflect inhibition of PDGF-mediated stimulation of mesangial cell production of TGF- β .

In conclusion, the current study provides the first direct in vivo evidence for a role for PDGF as a growth stimulatory molecule in GN. Treatment of rats with mesangial proliferative GN with a neutralizing anti-PDGF antibody significantly reduced mesangial cell proliferation and matrix expansion at 4 d. The observation that the inhibition was only partial (i.e., 60%) suggests that other growth factors may be involved in this proliferative response, or that there was insufficient antibody available at the cellular level to effect a total response.

The fact that this degree of proliferation and matrix expansion could be reduced by inhibiting a single growth factor

suggests that PDGF may play a crucial role in progressive glomerular injury.

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