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Platelet-derived growth factor (PDGF) and PDGF receptor are induced in mesangial proliferative nephritis in the rat

(glomerulonephritis)

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ABSTRACT **We investigated whether platelet-derived growth factor (PDGF), or its receptor (PDGF-R), was up-regulated in a rat model of mesangial proliferative glomerulonephritis. A marked increase in both PDGF A- and B-chain mRNA could be demonstrated in glomerular RNA by Northern blot analysis 3 and 5 days after disease induction, corresponding to the time of mesangial cell proliferation. PDGF-R β-subunit mRNA and protein were also increased in glomeruli in mesangial proliferative nephritis, being maximal at day 5. The principal cells expressing PDGF B-chain appeared by immunostaining to be a subpopulation of mesangial cells; in contrast, the majority of the mesangial cells expressed the PDGF-R β-subunit protein. Both complement depletion and platelet depletion significantly reduced cell proliferation and expression of both PDGF and PDGF-R. Thus, in mesangial proliferative nephritis there is a platelet- and complement-mediated induction of PDGF A and B chain and PDGF-R β-subunit gene transcription and protein synthesis. The finding that the majority of PDGF is produced by the mesangial cell supports the role of PDGF as an autocrine growth factor in glomerulonephritis.**

Mesangial cell proliferation occurs in many types of glomerulonephritis (GN), but the growth factors responsible for cell proliferation *in vivo* are largely unknown. One candidate is platelet-derived growth factor (PDGF), which is present in platelets, macrophages, and the mesangial cell itself (1, 2). PDGF has been reported to be a "complete" mitogen for mesangial cells in culture (2); others have shown that PDGF may act as a "competence" factor in the proliferation induced with interleukin 1 or insulin growth factor 1 (3, 4). The observation that many growth factors induce PDGF production by mesangial cells suggests that PDGF may also act as an autocrine growth factor (5). This is supported by the observation that the mesangial cell proliferation induced by epidermal growth factor can be inhibited with anti-PDGF antibody (5). A similar role for PDGF has been reported in interleukin 1-mediated fibroblast proliferation (6). Given the potential importance of PDGF in glomerular cell proliferation, we examined the glomerular expression of PDGF and its receptor (PDGF-R) in a rat model of mesangial proliferative GN.

MATERIALS AND METHODS

Disease Model and Manipulations. Mesangial proliferative GN was induced in rats with goat anti-rat thymocyte plasma (ATS) (7–10). Six groups of Wistar rats (n = 10–12 rats per group) (Simonsen Laboratories, Gilroy, CA) were studied: group 1, normal rats; groups 2–4, rats with anti-Thy 1 GN that were sacrificed 1, 3, or 5 days after disease induction; groups 5 and 6, rats with anti-Thy 1 GN that were either complement depleted or platelet depleted and sacrificed 3 days after disease induction. Complement depletion was induced in rats with cobra venom factor (CVF) (*Naja naja kaounthia*; Diamedix, Miami; ref. 9). Serum C3 levels at the time of injection of ATS and at sacrifice were <10% of baseline values. Platelet depletion was performed in eight rats with polyclonal goat anti-platelet IgG as described (7). Thrombocytopenia (platelet counts, <25,000 per mm^3) was achieved in all rats at the time of injection of ATS and was maintained until sacrifice 3 days later.

Histology and Immunocytochemistry. Methyl Carnoy's fixed tissue was processed and 3-μm sections were stained with the periodic acid/Schiff reagent with hematoxylin counterstain. Additional sections from six animals in each group were immunostained by an avidin/biotin indirect immunoperoxidase method (7) with the following primary antibodies: ED1 (Bioproducts for Science, Indianapolis), a murine monoclonal IgG to monocytes–macrophages (9); 19A2 (American Biotech, Plantation, FL), a murine monoclonal IgM to the proliferating cell nuclear antigen (PCNA; ref. 7); and a rabbit polyclonal IgG to the PDGF-R β subunit (see below). The murine monoclonal IgG, PGF-007 (Mochida Pharmaceutical, Tokyo), was used to detect PDGF B chain by an indirect immunoalkaline phosphatase method (11). Negative controls consisted of replacing the primary antibody with either an irrelevant murine monoclonal or rabbit polyclonal antibody.

Tissue was also double immunostained for both PCNA and macrophages by an indirect immunogold procedure with class-specific anti-IgM and anti-IgG antibodies as described (7). Sections were also double immunolabeled for PDGF B chain and smooth muscle α-actin; tissue was incubated with PGF-007 (an IgG1 antibody) followed by a subclass-specific biotinylated rabbit anti-mouse IgG1 antibody (Zymed Laboratories), strepavidin-alkaline phosphatase, and color reagent. Sections were then incubated with anti-αsm-1, a mouse monoclonal IgG2 antibody to smooth muscle α-actin (gift of G. Gabbiani) (10) followed by an anti-mouse IgG conjugated with 5-nm gold particles (Jansenn, Life Sciences, Piscataway, NJ) with silver enhancement. Negative controls included omission of either of the primary antibodies in which no double staining was noted.

Quantitation of Histologic Findings. In each biopsy sample, 20 glomerular cross-sections were sequentially examined and

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Abbreviations: ATS, anti-thymocyte plasma; CVF, cobra venom factor; GN, glomerulonephritis; PCNA, proliferating cell nuclear antigen; PDGF, platelet-derived growth factor; PDGF-R, PDGF receptor; TGF, transforming growth factor.

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the following variables were measured: total cellularity, the mean number of nuclei per glomerular cross-section in periodic acid/Schiff-stained tissue sections; proliferation, the mean number of PCNA-positive cells per glomerular cross-section; monocyte–macrophage infiltration, the mean number of ED1-positive cells per glomerular cross-section; degree of mesangiolysis, graded from 0 to 4+ (9). Glomerular cross-sections containing a minor portion of the glomerular tuft (<20 discrete capillary segments) were excluded from analysis.

RNA Extraction and Northern Blot Analysis. The following cDNA probes were ^{32}P labeled as whole plasmids by random-primer extension: for PDGF A chain, a 1.3-kilobase (kb) *Eco*RI human cDNA fragment (12); for PDGF B chain, a 2.7-kb human cDNA clone (13); for PDGF-R α subunit, a 6.4-kb *Eco*RI rat cDNA fragment (14); and for PDGF-R β subunit, clone "PDGF-R" containing a 5.1-kb mouse cDNA (15). For each hybridization, total RNA from BALB/c mouse 3T3 fibroblasts (which express PDGF-R α and β subunits) and from the human osteosarcoma cell line (U2OS) (which express PDGF A and B chains) were run as positive controls.

Total RNA was extracted from isolated glomeruli of three to six rats from each group and was pooled; Northern blots were prepared (15 μg of RNA per lane) as reported (10). Care was taken to ensure that the isolated glomeruli were pure (>90%) and representative of the lesion (data not shown). At least two Northern blots were analyzed with each probe. Densitometry on autoradiograms was normalized for equivalent 28S ribosomal RNA by hybridization with a specific bovine 280-base-pair cDNA (gift of Luisa Iruela-Arispe, University of Washington, Seattle) as reported (10).

Western Blot Analysis of PDGF-R. Glomeruli from two rats in each group were isolated by differential sieving (7), suspended in 0.25 ml of 0.5% Nonidet P-40/10 mM Hepes, pH 7.4/10% (vol/vol) glycerol for 10 min at room temperature, and centrifuged (11,600 × *g*) for 5 min; the supernatant and pellet were then separated. The pellet was analyzed for DNA content (16). The supernatant, which was void of DNA but contained the solubilized PDGF-R, was loaded onto a 5% stacking/8% resolving SDS/polyacrylamide gel (17) and electrophoresed under reducing conditions. The samples were loaded such that each lane contained the protein from an equivalent number of cells (as determined by DNA content). The proteins were electrophoretically transferred overnight to nitrocellulose filters and immunoblotted with anti-PDGF-R α- and β-subunit antibodies.

Anti-PDGF-R α- and β-Subunit Antibodies. Antibody to PDGF-R α subunit was raised in rabbits with repeated immunizations with a synthetic peptide [Gln-105–His-115 (14)] of the rat PDGF-R α subunit. Antibody to PDGF-R β subunit was raised in rabbits with repeated immunizations of a mixture of three synthetic peptides [Thr-91–Tyr-117 of the human PDGF-R β subunit (18) and Val-69–Tyr-85 and Tyr-977–Leu-993 from the mouse PDGF-R β subunit (15)]. The collected antiserum was affinity purified over a Sepharose column to which the synthetic peptides had been coupled. By immunoblotting, the anti-PDGF-R α-subunit and the anti-PDGF-R β-subunit antibodies recognized a 170- and a 180-kDa protein, respectively, in normal rat aortic smooth muscle cells; these signals were appropriately reduced by using smooth muscle cells in which the PDGF-R had been down-regulated (19) with exogenous PDGF.

Analysis of Data. Results shown represent individual experiments, but all experiments were repeated multiple times with comparable findings. Values are expressed as mean ± SE. Comparison of groups was by one-way analysis of variance with simultaneous multiple comparisons between groups performed with modified *t* statistics by Bonferroni's method.

RESULTS

Rats with anti-Thy 1 GN developed mesangiolysis followed by cellular proliferation, as reported (7, 8). Mesangiolysis peaked at day 1, at which time there was a 12% decrease in total glomerular cellularity (Table 1). The relative decrease in endogenous glomerular cells was actually greater (30–40%) but was offset by a marked influx of monocytes–macrophages (Table 1). Subsequently, an increase in total cellularity was observed, peaking at day 5 when there was a 40% increase in glomerular cellularity as compared to normal rats (Table 1). Most of the increase in cellularity could be attributed to proliferating cells (i.e., PCNA$^+$ cells) (Table 1). By double immunolabeling, 85% of the PCNA$^+$ cells were negative for the macrophage-specific antibody ED1 at day 3 and 98% were negative at day 5. This was consistent with our prior observation that a majority of proliferating cells were negative for leukocyte common antigen (7).

Complement depletion prevented mesangiolysis, inhibited monocyte–macrophage accumulation, and blocked cell proliferation (Table 1). In contrast, platelet depletion (platelet count, <25,000 per mm^3) did not prevent either the mesangiolysis or macrophage infiltration but did significantly reduce cell proliferation (Table 1). Thus, these results confirm previous studies that demonstrated an important role for both complement (8, 9) and platelets (7) in mediating glomerular cell proliferation in anti-Thy 1 GN.

PDGF Gene Expression in Anti-Thy 1 GN. RNA extracted from isolated glomeruli from normal rats and from rats with anti-Thy 1 GN was examined for the presence of PDGF A- and B-chain mRNA by Northern blot analysis (Fig. 1). PDGF A-chain mRNA was not detectable in normal rats; however, expression of the 2.9-, 2.3-, and 1.7-kb PDGF A-chain transcripts could be detected in glomerular RNA from rats 3 and 5 days after induction of GN (Fig. 1). In contrast to the findings for PDGF A-chain mRNA, PDGF B-chain gene expression could be detected at low levels in normal rat glomerular RNA. Expression of the 3.5-kb transcript for PDGF B chain was markedly increased in rats with anti-Thy 1 GN, with a 1.4-fold (at day 1), 8.0-fold (day 3), and 5.7-fold (day 5) increase in PDGF B-chain mRNA (as measured by densitometry) compared to normal rat glomeruli.

Identification of Cells Producing PDGF B Chain. Tissue sections were immunostained with PGF-007, a monoclonal antibody to the PDGF B chain. Normal glomeruli were negative for PDGF B-chain-positive cells (<1 positive cell per 30 glomeruli). Glomeruli from rats with anti-Thy 1 GN were also negative for PDGF B-chain protein at day 1, despite

Table 1. Total cellularity, proliferation, macrophage infiltration (cells per glomerular cross-section), and mesangiolysis (graded 0–4) in normal rats, in rats with anti-Thy 1 GN, and in complement-depleted (CVF) or platelet-depleted (ATS) rats with anti-Thy 1 GN (n = 6 rats per group)

	Total cells	PCNA$^+$ cells	Macrophages	Mesangiolysis
Normal	77 ± 1.8	0.9 ± 0.2	2.7 ± 0.3	0
GN				
Day 1	68 ± 0.4*	1.2 ± 0.1	19 ± 1.2*	2.5 ± 0.3*
Day 3	96 ± 2.4*	22 ± 2.7*	15 ± 0.4*	1.7 ± 0.5*
Day 5	107 ± 2.7*	20 ± 0.8*	10 ± 0.9*	0.7 ± 0.2
GN day 3				
CVF GN	81 ± 2.0†	1.4 ± 0.2†	4.5 ± 0.8†	0†
ATS GN	73 ± 2.4†	7.9 ± 1.7†	13 ± 1.3	2.0 ± 0.2*

Portions of this table have been reported previously in a study investigating the expression of smooth muscle actin in anti-Thy 1 GN (10).

*$P < 0.005$ relative to normal.

†$P < 0.001$ relative to GN day 3.

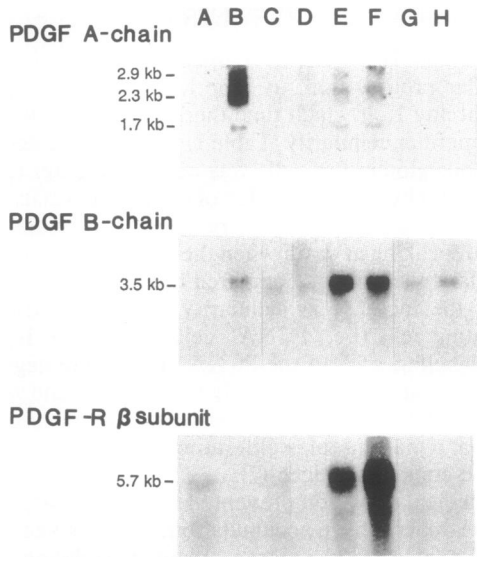

FIG. 1. Northern analysis of glomerular RNA for PDGF A chain, PDGF B chain, and PDGF-R β subunit. Lanes: A, 3T3 cell RNA; B, U2OS cell RNA; C, normal glomerular RNA; D–F, glomerular RNA from rats with anti-Thy 1 GN at days 1, 3, and 5, respectively; G and H, glomerular RNA from complement-depleted and platelet-depleted rats with anti-Thy 1 GN at day 3. Increased expression of PDGF A- and B-chain and PDGF-R β-subunit mRNA is observed in glomerular RNA from rats with anti-Thy 1 GN at days 3 and 5. Expression is reduced in rats with anti-Thy 1 GN that have been complement depleted or platelet depleted.

the presence of 19 macrophages per glomerular cross-section at that time (Table 1). In contrast, glomeruli from rats with anti-Thy 1 GN had between 0 and 10 (averaging $\approx$2) PDGF B-chain positive cells per glomerular cross-section at days 3 and 5 (Fig. 2). Most PDGF B-chain-positive cells were in mesangial locations and some cells were in active mitosis (Fig. 2). Tubules were variably positive for PDGF B chain in both normal and diseased animals. Specificity of the immunostaining was demonstrated by (*i*) absence of tubular and glomerular immunostaining when an irrelevant antibody was substituted for PGF-007, and (*ii*) absorption studies in which preincubation of the PGF-007 monoclonal antibody with a 20-fold excess of a synthetic peptide of PDGF B chain, but not of PDGF A chain, was able to block both glomerular and tubular staining.

The location of the PDGF B-chain-positive cells suggested that they were either mesangial cells or monocytes–macrophages. We have recently demonstrated that, during active mesangial cell proliferation *in vivo*, mesangial cells express the smooth muscle-specific protein, smooth muscle α-actin (10). Isolated peritoneal macrophages are smooth muscle actin negative (data not shown). We therefore performed double immunolabeling of tissue sections for PDGF B chain (with PGF-007) and for smooth muscle α-actin (with anti-αsm-1 antibody). The majority of PDGF B-chain-positive cells were positive for smooth muscle α-actin (88% at day 3 and 92% at day 5) (Fig. 3), demonstrating that the majority of the PDGF B-chain-positive cells were mesangial cells.

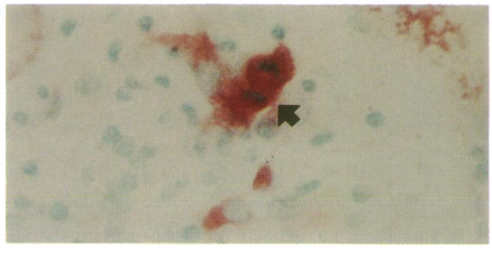

FIG. 2. Portion of a glomerulus from a rat with mesangial proliferative GN (day 3) with several PDGF B-chain-positive cells, one of which is in active mitosis (arrow). (×400.)

Effect of Complement Depletion on PDGF Expression. Complement-depleted rats with anti-Thy 1 GN (day 3) had no detectable PDGF A-chain mRNA and only a 1.1-fold increase in PDGF B-chain mRNA in glomerular RNA compared to normal rats; these levels were substantially lower than that observed in unmanipulated rats with anti-Thy 1 GN (Fig. 1). Glomeruli from complement-depleted rats were also negative for PDGF B-chain-positive cells by immunostaining (<1 cell per 30 glomeruli). Thus, complement depletion prevents the induction and up-regulation of PDGF expression.

Effect of Platelet Depletion on PDGF Expression. Platelet-depleted rats with anti-Thy 1 GN (day 3) had undetectable levels of PDGF A-chain mRNA and a 1.2-fold increase in PDGF B-chain mRNA (Fig. 1). The increase in PDGF B-chain mRNA was substantially lower than that observed in glomerular RNA from unmanipulated rats with anti-Thy 1 GN. Immunostaining demonstrated a slight increase in PDGF B-chain-positive cells (1–3 cells per 30 glomeruli) relative to normal glomeruli and was compatible with the mild proliferation observed (Table 1).

PDGF-R Gene Expression in Anti-Thy 1 GN. Glomerular RNA was examined for both PDGF-R α- and β-subunit mRNA by Northern blot analysis. No PDGF-R α-subunit mRNA was detected in glomerular RNA from normal or diseased rats, although the 6.7-kb transcript could be identified in mouse 3T3 cell RNA (i.e., a positive control). In contrast to the findings for PDGF-R α-subunit mRNA, PDGF-R β-subunit mRNA was expressed in normal rat glomerular RNA (Fig. 1). PDGF-R β-subunit mRNA was not detected 1 day after induction of GN, corresponding to the time of maximal mesangial cell loss. With the onset of mesangial cell proliferation, the levels of the 5.7-kb PDGF-R β-subunit transcript were markedly increased, being 8.4-fold greater at day 3 and 25-fold higher at day 5 relative to normal glomerular RNA (Fig. 1).

PDGF-R Protein in Anti-Thy 1 GN. Immunoblotting with anti-PDGF-R α-subunit antibody revealed the 170-kDa PDGF-R α-subunit protein in detergent extracts of normal rat smooth muscle cells (positive control), but no PDGF-R α-subunit protein was detected in normal or diseased glomerular detergent extracts, consistent with the results from Northern blotting (data not shown). Unlike the findings with the PDGF-R α-subunit protein, the 180-kDa PDGF-R β-subunit protein was detected in normal glomeruli by Western analysis and was markedly increased in glomeruli from rats with anti-Thy 1 GN 5 days after induction of disease (Fig. 4).

To identify the cells expressing PDGF-R β subunit, kidney tissue from normal and diseased rats was immunostained with the anti-PDGF-R β-subunit antibody. In normal glo-

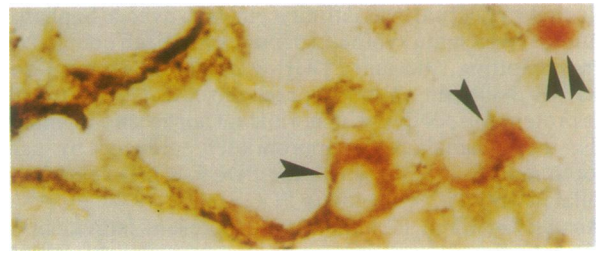

FIG. 3. Glomerulus from a rat with mesangial proliferative GN (day 3) immunostained for PDGF B chain (red) and for smooth muscle α-actin (light brown). Two PDGF B-chain-positive cells can be identified that are also positive for smooth muscle α-actin (arrowheads). (×400.)

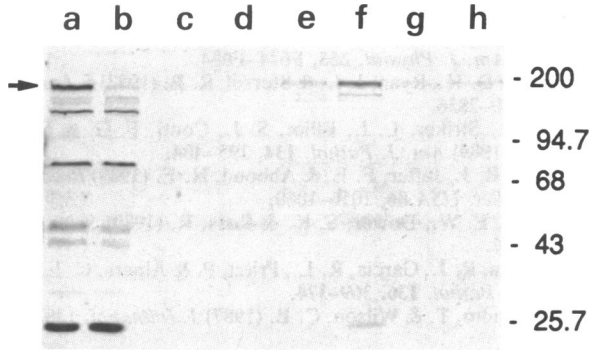

FIG. 4. Western analysis of glomerular proteins for PDGF-R β subunit. Lanes: A and B, normal rat aortic smooth muscle cells (lane A) and smooth muscle cells in which the PDGF-R has been down-regulated by incubation with exogenous PDGF (lane B); C, normal rat glomerular extract; D–F, glomeruli from rats with anti-Thy 1 GN at days 1, 3, and 5, respectively; G and H, glomerular extracts from complement-depleted (lane G) and platelet-depleted (lane H) rats with anti-Thy 1 GN at day 3. The 180-kDa PDGF-R β subunit (arrow) is present in normal (lane A) and reduced in down-regulated (lane B) rat aortic smooth muscle cells. The PDGF-R β subunit is expressed in normal glomeruli and is markedly increased in glomeruli of rats with anti-Thy 1 GN at day 5.

meruli, faint staining of the mesangial regions was present (Fig. 5A). In rats with anti-Thy 1 GN, a focal increase in staining was noted at day 3 in areas of segmental or diffuse mesangial cell proliferation. By day 5, a marked increase in mesangial staining was present and was due to both an increase in the number of positive cells and the intensity of staining per cell (Fig. 5B). However, some caution is necessary in the interpretation of the immunostaining, as in Western blots the anti-PDGF-R β-subunit antibody also recognizes additional antigens in extracts from smooth muscle cells and glomeruli (Fig. 4).

Effect of Complement Depletion and Platelet Depletion on PDGF-R β Subunit. The 8.4-fold increase in PDGF-R β-subunit mRNA that was observed in glomerular RNA from rats with anti-Thy 1 GN at day 3 was reduced in rats that were complement depleted (1.7-fold increase) or platelet depleted (1.2-fold increase) (Fig. 1). Immunostaining with the anti-PDGF-R β-subunit antibody in complement-depleted or platelet-depleted rats also revealed only trace staining of mesangial regions, similar to that observed in normal rats and different from diseased rats in which focal increases in staining were observed in mesangial areas. By Western analysis, no definite increase in PDGF-R β-subunit protein per μg of DNA could be demonstrated in anti-Thy 1 GN until day 5 (Fig. 4), and therefore an effect of complement depletion or platelet depletion on PDGF-R β-subunit protein per μg of DNA could not be shown.

DISCUSSION

Mesangial proliferative GN was induced in rats with polyclonal antibody to the Thy 1 antigen present on mesangial cells. Acutely there was severe mesangial cell injury with a 30–40% reduction in endogenous glomerular cells at 24 hr. Morphometric studies in normal Wistar rats suggest that 30% of the total glomerular cells are mesangial in origin (20). Thus, the vast majority of mesangial cells were injured acutely. The mesangiolysis is complement dependent but does not require the presence of platelets or leukocytes (refs. 7–10 and this study).

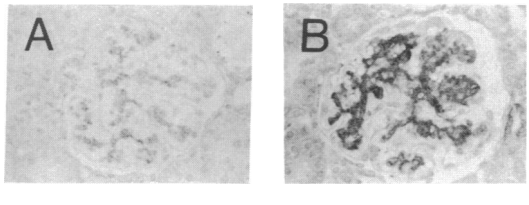

FIG. 5. PDGF-R β subunit can be detected by immunostaining in a mesangial pattern in a normal glomerulus (A) and is markedly increased in a glomerulus of a rat with anti-Thy 1 GN at day 5 (B). (×400.)

Three and 5 days after disease induction, a marked proliferation of cells was documented in glomeruli with a >20-fold increase in the number of cells positive for PCNA. PCNA is an auxiliary protein to DNA polymerase δ that is expressed from late G_1 to the M phase of the cell cycle (7). A great majority of the $PCNA^+$ cells were in mesangial locations, and >85% were negative for the common leukocyte antigen (7) and for the monocyte–macrophage antigen detected by the antibody ED1 (this study). This strongly suggests that the proliferating cells are of mesangial origin.

Involvement of PDGF. Northern analysis of glomerular RNA demonstrated a marked increase in PDGF A- and B-chain mRNA in rats with mesangial proliferative GN coincident with cell proliferation (i.e., at days 3 and 5). The increase in PDGF B-chain mRNA was 8-fold and coincided with a 1.4-fold increase in total glomerular cellularity with a maximum 2-fold increase in mesangial cells. Thus, the increase in PDGF mRNA is not simply due to an increase in mesangial cell numbers. These observations are consistent with a recent report by Gesualdo *et al.* (21) that an increase in PDGF A- and B-chain mRNA in whole kidney RNA occurs in mice with mesangial proliferative GN.

Source of PDGF. Cells expressing PDGF B-chain protein were identified in mesangial regions by immunostaining with the specific monoclonal antibody PGF-007. The majority (>85%) of the PDGF B-chain-positive cells were also positive for smooth muscle α-actin by double immunolabeling. Smooth muscle α-actin is expressed by activated and proliferating mesangial cells but not by macrophages or endothelial cells (10). This suggests that the major source of PDGF is the smooth muscle-like mesangial cell. The observation that only a small subpopulation of mesangial cells have detectable PDGF B-chain protein by immunostaining may reflect the fact that PDGF is only expressed strongly during a portion of the cell cycle; this is consistent with the observation that cells in mesangial regions that were undergoing mitoses were invariably positive for PDGF. Alternatively, it may be that only a subpopulation of mesangial cells express PDGF B chain. Nevertheless, these results provide an *in vivo* correlate of *in vitro* observations that PDGF may serve as a mesangial cell autocrine growth factor (5).

It is likely that some PDGF originates from other cells within the glomerulus, including the endothelial cell, Ia^+ mesangial cell, and the monocyte–macrophage. However, at day 1, which corresponds to the maximal macrophage infiltration, only a slight increase (1.4-fold) in glomerular PDGF mRNA and no increase in PDGF-positive cells were noted. Similarly, in platelet-depleted rats with anti-Thy 1 GN, there was a minimal increase in PDGF mRNA and protein expression despite the presence of numerous macrophages within glomeruli (Table 1). Nevertheless, the observation that 8–12% of the PDGF B-chain protein-positive cells were smooth muscle actin negative suggests that other cells besides the mesangial cell may be expressing PDGF or that some PDGF is also produced by a subpopulation of smooth muscle actin-negative mesangial cells.

Involvement of PDGF-R. Neither PDGF-R α-subunit mRNA nor PDGF-R α-subunit protein was detected in normal or diseased glomeruli. In contrast, both PDGF-R β-subunit protein and mRNA were demonstrated in glomeruli from normal rats. After the induction of GN, there was an increase in both PDGF-R β-subunit mRNA and product, with a

modest elevation at day 3 (8-fold) and a marked elevation at day 5 (25-fold). This pattern was temporally different from that observed with PDGF A and B chain, in which the mRNA levels were roughly equivalent at both days 3 and 5 (i.e., closely correlating with the cell proliferation).

The cells expressing the PDGF-R β-subunit protein were most likely mesangial cells, as demonstrated by the strongly positive cells in mesangial locations noted by immunostaining with the anti-PDGF-R β-subunit antibody. An increase in PDGF-R β subunit in mesangial regions has also been noted in human biopsy samples from patients with mesangial proliferative GN (22).

Effect of Depletion of Complement or Platelets on PDGF and PDGF-R. Measures that inhibited glomerular cell proliferation, such as complement depletion and platelet depletion, also inhibited PDGF and PDGF-R gene expression. This demonstrates that PDGF and PDGF-R expression is tightly coupled to glomerular cell proliferation.

Potential mechanisms by which platelets could stimulate PDGF mRNA in glomeruli *in vivo* would include the release by the platelet of PDGF or epidermal growth factor/transforming growth factor α (TGF-α), both of which are able to induce PDGF A- and B-chain mRNA expression in cultured mesangial cells (5). Platelets also contain TGF-β, which induces PDGF A- and B-chain mRNA production in mesangial cells (5, 23), but which may either stimulate or inhibit mesangial cell proliferation in culture depending on the culture conditions and concentrations of TGF-β (23, 24). The potential role of TGF-β in PDGF-R regulation should also be considered, as TGF-β can up-regulate PDGF-R β subunit on mouse 3T3 fibroblasts (25).

In conclusion, PDGF and PDGF-R β-subunit gene expression and protein synthesis are up-regulated in rats with mesangial proliferative GN coincident with glomerular cell proliferation. The primary cells expressing PDGF and PDGF-R appear to be mesangial cells. The inability to detect the PDGF-R α subunit suggests that rat mesangial cells may interact primarily with the PDGF-BB isoform. Further studies are necessary to determine whether the increased expression of PDGF is responsible for the marked proliferation observed.

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