Role of Platelet-Derived Growth Factor in Glomerular Disease

Richard J. Johnson, Jürgen Floege, William G. Couser, and Charles E. Alpers

ABSTRACT
An approach for establishing a role for a growth factor in glomerular disease is presented. Using platelet-derived growth factor (PDGF) as an example, there is strong evidence to support the hypothesis that PDGF is a mediator of mesangial cell proliferation in glomerulonephritis. This includes evidence that (1) PDGF is a mitogen for mesangial cells in culture; (2) PDGF is expressed in both experimental and human glomerulonephritis in which mesangial cell proliferation occurs; (3) infusion of PDGF into rats induces mesangial cell proliferation and a hypercellular lesion; and (4) inhibition of PDGF in a model of experimental nephritis significantly reduces the mesangial cell proliferation. However, these data do not answer the question of whether or not the inhibition of PDGF in human diseases would be beneficial in the long term, because some cell proliferation is likely required for normal healing and repair. Further studies will be necessary to resolve this issue.

Key Words: Glomerulonephritis, cytokine, mesangial cell, cell proliferation

The last several years have witnessed a plethora of reports on the possible roles of various cytokines and growth factors in glomerulonephritis. The observation that a growth factor is expressed in glomerulonephritis, however, does not necessarily mean that it plays a significant role in the pathology or course of the disease. In order to interpret this rapidly expanding literature, it is essential to establish criteria that prove a role for a particular cytokine in glomerular disease. In this article, we suggest such criteria and use platelet-derived growth factor (PDGF) as an example in which these criteria have been satisfied for establishing a role of PDGF in mediating mesangial cell proliferation in glomerular disease.

BIOLOGY OF PDGF AND PDGF-R

After its discovery in 1974 by Ross et al. at our institution (1), it was recognized that PDGF may have an important role in both normal and abnormal cell proliferation (reviewed in reference 2). For example, the proliferation observed with simian sarcoma virus infection is due to the viral oncogene v-sts, which is highly homologous to the gene that encodes the PDGF B-chain (3). PDGF is also expressed in inflammatory conditions, including atherosclerosis, rheumatoid arthritis, idiopathic pulmonary fibrosis, and wound repair (reviewed in reference 2).

PDGF is a 28- to 32-kd basic glycoprotein (pl 9.8 to 10) that contains two peptide chains (a 16-kd A-chain and a 14-kd B-chain) that exist in disulfide linkage either as a heterodimer or a homodimer, resulting in three PDGF isoforms (PDGF-AA, PDGF-AB, and PDGF-BB). Although initially recognized as a component within the alpha granules of platelets, PDGF is also synthesized and released by activated macrophages, activated endothelial cells, and smooth muscle cells (2). Glomerular endothelial cells (4–6), mesangial cells (7–9), and visceral glomerular epithelial cells (10) can also produce PDGF. Thus, numerous cellular sources may produce PDGF in glomerular diseases.

PDGF binds to cells via a cell membrane receptor (PDGF-R). Each PDGF-R consists of a dimer of two subunits (α and β) that are brought together upon PDGF binding to form one of three isoforms (PDGF-R αα, αβ, and ββ). Whereas the α-subunit will bind either the PDGF A-chain or B-chain, the β-subunit only binds the PDGF B-chain. Thus, PDGF-R αα will
bind all three PDGF isoforms, whereas PDGF-R \( \beta \beta \) will only bind PDGF-BB (11).

The binding of PDGF to the PDGF-R results in a rapid activation of a tyrosine kinase, which is present in the intracellular domain of the PDGF-R, leading to an autophosphorylation of the PDGF-R. Other intracellular proteins are also phosphorylated, including phospholipase C, phosphatidyl-inositol kinase, and serine kinases. The activation of membrane phospholipases results in prostaglandin formation, diacylglyceride production, and an increase in intracellular calcium. Several "early response" proto-oncogenes are also expressed, including \( c-my c \) and \( c-f os \). This is followed by a poorly understood sequence of events that ultimately culminates in DNA synthesis (2).

**PROPOSED ROLES FOR PDGF IN GLOMERULAR DISEASE**

Numerous studies have suggested various functions of PDGF that might be important in glomerular disease (Table 1). Although most evidence supports a role for PDGF in mesangial cell proliferation (see below) (8,9,12), there are also reports that PDGF may function as a mesangial cell chemoattractant (13), induce mesangial cell contraction (14), and stimulate mesangial cell synthesis of cytokines such as interleukin-1 (15) or transforming growth factor beta (16), or PDGF itself (9). PDGF has also been reported to mediate type IV collagen synthesis by mouse mesangial cells in response to advanced glycosylation end products (17) and to be synergistic with other cytokines in the stimulation of mesangial cell prostaglandin production (18). PDGF may mediate the mitogenic effects of other cytokines on mesangial cells (9,19). PDGF has also been reported to be a chemotactic factor for leukocytes (20,21), which may be relevant to inflammatory glomerular diseases associated with an influx of neutrophils or monocytes. PDGF is also a powerful vasoconstrictor (22) and has been reported to adversely affect RBF and GFR (23).

**TABLE 1. Proposed roles for PDGF in glomerular disease**

<table>
<thead>
<tr>
<th>Actions on Mesangial Cell (Ref. No.)</th>
<th>Proposed role</th>
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<tbody>
<tr>
<td>Mesangial cell mitogen (8, 9, 12)</td>
<td>Stimulate mesangial cell migration (13)</td>
</tr>
<tr>
<td>Mesangial cell contraction (14)</td>
<td>Stimulate mesangial cell synthesis of cytokines (e.g., transforming growth factor beta and interleukin-1) (15, 16)</td>
</tr>
<tr>
<td>Extracellular matrix production in response to advanced glycosylation end products (17)</td>
<td>Mediate extracellular matrix production in response to advanced glycosylation end products (17)</td>
</tr>
<tr>
<td>Synergistic with other cytokines in prostanoid production (18)</td>
<td>Synergistic with other cytokines in prostanoid production (18)</td>
</tr>
<tr>
<td>Mediates the mitogenic effects of other cytokines on mesangial cells (e.g., epidermal growth factor, bFGF) (9, 19)</td>
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<tr>
<th>Actions on glomerular epithelial cells</th>
<th>Proposed role</th>
</tr>
</thead>
<tbody>
<tr>
<td>? Role in epithelial cell proliferation or disease (10)</td>
<td>Chemotaxis and activation of neutrophils and monocytes (20, 21)</td>
</tr>
<tr>
<td>Modulate GFR and RBF (22)</td>
<td>Modulate GFR and RBF (22)</td>
</tr>
</tbody>
</table>

**TABLE 2. Criteria to establish a role for a cytokine in mediating a specific biologic effect in glomerular disease**

<table>
<thead>
<tr>
<th>Cytokine in vivo</th>
<th>Cytokine in vitro</th>
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</thead>
<tbody>
<tr>
<td>Inhibition of the Cytokine in Disease</td>
<td>Blocks the Postulated Effect of the Cytokine in Disease</td>
</tr>
<tr>
<td>Corollary issues: Identify functional receptors for the cytokine on the target cells</td>
<td>Corollary issues: Identify functional receptors for the cytokine on the target cells</td>
</tr>
<tr>
<td>The Cytokine Has a Specific Effect on Target Cells</td>
<td>The Cytokine Has a Specific Effect on Target Cells</td>
</tr>
<tr>
<td>The Cytokine Is Expressed in Diseases and Correlates With the Proposed Biologic Effect</td>
<td>The Cytokine Is Expressed in Diseases and Correlates With the Proposed Biologic Effect</td>
</tr>
<tr>
<td>Administration of the Cytokine In Vivo (or Overexpression in Transgenic Animals) Reproduces the Effect</td>
<td>Administration of the Cytokine In Vivo (or Overexpression in Transgenic Animals) Reproduces the Effect</td>
</tr>
<tr>
<td>Inhibition of the Cytokine In Vivo Blocks the Postulated Effect of the Cytokine in Disease</td>
<td>Inhibition of the Cytokine In Vivo Blocks the Postulated Effect of the Cytokine in Disease</td>
</tr>
</tbody>
</table>

*Modified from reference 24.*
PDGF IS AN IMPORTANT MEDIATOR OF MESANGIAL CELL PROLIFERATION IN GLOMERULAR NEPHRITIS

Criteria 1. PDGF Mediates Mesangial Cell Proliferation in Vitro

Early studies investigating the mitogenic properties of serum (25) and platelet contents (26, 27) for cultured mesangial cells implicated PDGF as an important mesangial cell mitogen. This was later confirmed with purified PDGF (8,9,12). However, a difference in the stimulatory properties of the various PDGF isoforms has been noted, with PDGF-BB and PDGF-AB being much more mitogenic than PDGF-AA (12,28). Some controversy also exists because some investigators have found PDGF to be a "complete" mitogen (8,12), whereas others have reported that PDGF acts as a "competence factor," priming the mesangial cell so that it can complete the cell cycle after being exposed to "progression factors" such as interleukin-1 or insulin-like growth factor 1 (25,29).

Corollary. Mesangial Cells Express Functional PDGF-R. The observation that both rat and human mesangial cells proliferate in vitro in response to PDGF-AB and PDGF-BB (12,28) suggests that these cells express both α and β PDGF-R subunits. Indeed, both the α and β PDGF-R subunits are expressed by cultured rat and human mesangial cells, although the β-subunit markedly predominates (12,28). As with other cell types, the binding of PDGF to mesangial cells is followed by phospholipase activation, inositol phosphate release, and a rise in intracellular calcium before DNA synthesis (14,30).

An interesting observation is that rat mesangial cells grown in a three-dimensional gel matrix are unresponsive to PDGF, and this is associated with a down-regulation of the cell surface PDGF-R (28). This

Criteria 2. PDGF Is Expressed in Experimental and Human Glomerulonephritis and Correlates With Cell Proliferation

A critical requirement to demonstrating that a growth factor or cytokine has a biologic action in glomerular injury is to demonstrate its expression in diseased glomeruli and to correlate expression with its postulated effect. Thus, in relation to the role of PDGF in mesangial cell proliferation, one would like to demonstrate PDGF expression in models of nephritis and demonstrate that the expression correlates with mesangial cell proliferation. Corollary issues would be to determine whether there is modulation of the PDGF-R, to identify the types of cells expressing PDGF, and to determine the stimuli for PDGF expression.

After an initial report that PDGF was localized by immunostaining to the glomeruli of patients with diffuse proliferative lupus nephritis (34), both Gessler, Abboud, and colleagues (35) and our laboratory (31,36) reported that PDGF expression is increased in human and experimental models of mesangial proliferative nephritis. Subsequently, there have been numerous studies that have confirmed

<table>
<thead>
<tr>
<th>Disease</th>
<th>Ref. No.</th>
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<tbody>
<tr>
<td>Murine</td>
<td>35,37</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>35,37</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>37</td>
</tr>
<tr>
<td>BSA nephritis</td>
<td>38</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
</tr>
<tr>
<td>Anti-Thy 1 nephritis</td>
<td>31,36</td>
</tr>
<tr>
<td>Remnant kidney model</td>
<td>39</td>
</tr>
<tr>
<td>Aminonucleoside nephrosis</td>
<td>40</td>
</tr>
<tr>
<td>Antiglomerular basement membra ne-nephritis</td>
<td>41,42</td>
</tr>
<tr>
<td>DOCA-SALT hypertension</td>
<td>43</td>
</tr>
<tr>
<td>Habu snake venom</td>
<td>44</td>
</tr>
<tr>
<td>Passive Heymann nephritis</td>
<td>10</td>
</tr>
<tr>
<td>Streptozotocin-induced diabetes</td>
<td>45</td>
</tr>
<tr>
<td>Human Glomerulonephritis</td>
<td></td>
</tr>
<tr>
<td>Diffuse proliferative lupus nephritis</td>
<td>34</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>35,46,47</td>
</tr>
<tr>
<td>Mesangial proliferative nephritis</td>
<td>35,46</td>
</tr>
<tr>
<td>Anti-antineutrophil cytoplasmic antibody + Crescentic nephritis</td>
<td>48</td>
</tr>
</tbody>
</table>

TABLE 3. PDGF is expressed in experimental and human glomerulonephritis
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That PDGF is expressed in a variety of models of glomerular injury and in humans (Table 3) (31,34–48).

In many of these studies, the evidence for PDGF involvement is based on the demonstration of increased glomerular PDGF A-chain and/or B-chain mRNA as assessed by Northern analysis, by the reverse transcription-polymerase chain reaction of isolated glomerular RNA, or by in situ hybridization. Although this type of evidence is supportive, important caveats include considerations that mRNA may be increased but not be associated with an increase in protein if there is a translational block and, conversely, that the absence of an increase in mRNA does not rule out the possibility that PDGF protein may be present (e.g., released from α granules in platelets). Other studies have resolved some of these concerns by localizing PDGF by immunostaining (31,34–36,48) and immunoelectron microscopy (47) in both experimental and human proliferative glomerulonephritis.

It is also important to realize that the demonstration of increased expression of a cytokine/growth factor such as PDGF in a disease may not be of biologic significance unless its expression can be correlated to its postulated effect (e.g., mesangial cell proliferation). Furthermore, it would be ideal to show that measures that inhibit the effect (e.g., mesangial cell proliferation) are associated with a relative inhibition of the expression of that particular growth factor (e.g., PDGF).

We have addressed these issues in two experimental models of nephritis in rats, i.e., the anti-Thy 1 model and the remnant kidney model (31,36,39). The anti-Thy 1 model was studied because it is an excellent model for acute mesangial proliferative nephritis; in contrast, the remnant kidney model results in a low-grade, chronic mesangial cell proliferation that culminates in focal and segmental glomerulosclerosis and renal failure, such as is seen in many progressive sclerosing diseases in humans.

The anti-Thy 1 model is induced by the injection of complement-fixing antibody to the Thy 1 antigen that is present on the mesangial cell membrane (49). The initial phase is characterized by an acute complement-dependent loss of mesangial cells with disruption of matrix (i.e., "mesangiolysis"), which peaks at 24 h (31). Accompanying the mesangiolysis is a significant infiltration of platelets and monocyte-macrophages into the glomeruli (31,50). The second phase occurs between Days 2 and 6 and is characterized by a massive proliferation of mesangial cells, which have an altered phenotype in that they express the vascular smooth muscle-associated protein α-smooth muscle actin (51). A third phase follows (Days 3 to 9) in which increased glomerular synthesis and deposition of extracellular matrix can be documented (52), as well as expression of matrix-degrading pro-
teases such as type IV collagenase (53). This is followed by a resolution phase (Days 8 to 21) in which mesangial cell and macrophage numbers return to normal, excess matrix is removed, and glomerular architecture is largely restored (52). Although there is no direct counterpart in human disease, this model does have similarities to immunoglobulin (Ig)A nephropathy, in which the proliferative lesions are similar and in which IgG antibodies to mesangial cells are present in some patients (54).

Studies performed by Dr. H. Iida and Dr. A. Yoshimura and colleagues in our laboratory have documented that PDGF is expressed in this disease model (31,36). In normal rats, very little glomerular PDGF B-chain protein (Figure 1) or PDGF A-chain or B-chain mRNA can be detected. However, a marked (8- to 10-fold) increase in glomerular PDGF A-chain and B-chain mRNA can be shown in isolated glomerular RNA by Northern analysis at Days 3 and 5 (31). Cells expressing the PDGF B-chain protein and mRNA could also be shown in mesangial locations by immunostaining and in situ hybridization, respectively (31,36) (Figure 1).

PDGF expression was correlated with cell proliferation by immunostaining tissue sections for the proliferating cell nuclear antigen (PCNA), a nuclear protein that is expressed from late G1 to the M phase of the cell cycle (peaking in S) (55). Cell proliferation was maximal at Days 3 and 5, at which time there was a 20-fold increase in PCNA-positive cells. The majority (>85%) of these proliferating cells were shown to be intrinsic mesangial cells, because by double immunolabeling, they did not express leucocyte (i.e., common leukocyte antigen) and macrophage (ED-1) markers but did express Thy 1 (a general mesangial cell marker) and α-smooth muscle actin (which is expressed by activated mesangial cells) (31,51,56).

The observation that PDGF expression correlated with the mesangial cell proliferation was further strengthened by showing that measures that inhibited the cell proliferation, such as complement or platelet depletion, also inhibited PDGF mRNA and protein expression (31,36).

The relationship of PDGF with glomerular cell proliferation has also been examined in the remnant kidney model (39). This model is induced by unilateral nephrectomy with the ligation of two of three branches of the renal artery on the contralateral side and results in a progressive and irreversible glomerulosclerosis within 10 to 12 wk (39,57). Mesangial cell proliferation (assessed by immunostaining for PCNA) and α-smooth muscle actin expression can be documented within the first week of the model and are correlated with an increased glomerular expression of PDGF B-chain mRNA and protein (39).

PDGF expression has also been correlated with cell proliferation in several other glomerular diseases. In
rats with aminonucleoside nephrosis, glomerular PDGF mRNA expression is increased and correlates with the increased glomerular PCNA mRNA expression (40). Furthermore, rats placed on a low-protein diet had a reduction in PCNA and PDGF expression and glomerulosclerosis (40). In the glomerular injury induced by Habu snake venom, a preliminary report by Barnes and Abboud suggests that PDGF B-chain expression precedes, whereas PDGF A-chain mRNA coincides, with the mesangial cell proliferation (44). In human diseases, no direct correlation of PDGF expression with mesangial cell proliferation has been reported. However, diseases in which PDGF has been demonstrated, such as IgA nephropathy and lupus nephritis, are associated with an increase in PCNA-positive, common leukocyte antigen-negative cells (58), which are likely to be mesangial in origin. Taken together, these studies provide strong evidence that PDGF expression is increased in diseases characterized by mesangial cell proliferation. Finally, three related issues that naturally follow from demonstrating the expression of a cytokine in disease are (1) to determine if this is associated with modulation of its receptor; (2) to identify the cells expressing PDGF; and (3) to determine the factors responsible for inducing the cytokine expression. Each of these questions will be briefly discussed.

**PDGF-R Are Up-Regulated in Mesangial Proliferative Nephritis.** Studies in both the anti-Thy 1 model and the remnant kidney model have demonstrated that PDGF-R β-subunit mRNA and protein are upregulated in association with the increase in PDGF expression (31) (Figure 1). In the anti-Thy 1 model, the increase in glomerular PDGF-Rβ mRNA was 25-fold at Day 5 compared with normal glomeruli and was associated with a corresponding increase in PDGF-Rβ protein by Western blot of isolated glomerular extracts (31). Immunostaining with a specific polyclonal antibody to PDGF-Rβ confirmed that the increased expression was in a mesangial cell pattern (Figure 1). An increase in PDGF-Rβ immunostaining has also been observed in mesangial proliferative nephritis in humans (33).

In contrast to the PDGF-Rβ subunit, no PDGF-Rα
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subunit mRNA or protein could be identified by Northern or Western analysis of glomeruli in normal rats or rats with anti-Thy 1 nephritis (31). This suggested that any biologic effect of PDGF in this rat model involved PDGF-BB and PDGF-Rβ receptors.

Identity of Cells Expressing PDGF. As discussed earlier, PDGF could be released by infiltrating (e.g., platelets and macrophages) or intrinsic (endothelial, mesangial, or visceral epithelial) cells within the injured glomerulus. The platelet is likely to be one source, because platelets can be demonstrated in many types of experimental and human glomerulonephritis and often precede the development of mesangial cell proliferation and hypercellularity (reviewed in reference 59). We have studied four experimental models of glomerular injury in rats in which an influx of platelets into glomeruli can be demonstrated within minutes to hours after disease induction; this is then followed by prominent glomerular cell proliferation (39,50,60,61). In one model (the anti-Thy 1 model), rats that were depleted of platelets with an antiplatelet antibody before the induction of disease developed significantly less mesangial cell proliferation (56). Recently, PDGF has also been localized within platelets in capillary loops and within mesangial immune deposits in IgA nephropathy (47).

Another source for PDGF is the activated macrophage. Studies in experimental and human atherosclerosis suggest that much of the PDGF B-chain present in the lesions is produced by macrophages (62). In IgA nephropathy, circulating monocytes can also be shown to be activated and to be expressing PDGF B-chain mRNA (63). A preliminary report by Barnes et al. has also correlated the presence of the PDGF B-chain in glomeruli of rats with Habu snake venom-induced injury with the early infiltration of monocyte-macrophages and platelets (44).

Although platelets and macrophages are likely to be providing some of the PDGF in glomerular diseases, recent studies suggest that most of the PDGF may be produced by the mesangial cell itself. Mesangial cells in culture can synthesize both PDGF A-chain and B-chain (7–9). In both the remnant kidney model and the Thy 1 model, PDGF B-chain mRNA and protein localized by in situ hybridization and immunostaining to mesangial locations (31,36,39). Although these cells could theoretically be macrophages, double immunostaining in the anti-Thy 1 model demonstrated that the majority (i.e., >85%) of PDGF B-chain–positive cells also expressed α-smooth muscle actin (31). This actin isomorph is exclusively expressed by proliferating mesangial cells in this model (51). Mesangial cells are also likely to be the source of the PDGF B-chain expression in the remnant kidney because the peak in PDGF expression preceded the influx of monocytes (i.e., ED-1 positive cells) (39). The observation that mesangial cells may be producing PDGF in disease suggests that the PDGF is acting as an autocrine growth factor and is consistent with studies performed by Silver and his colleagues on mesangial cells in vitro (9).

Although glomerular endothelial cells are known to produce PDGF in vitro (5,6), there are currently no data that they express PDGF in disease. However, we have recently documented in an experimental model of membranous nephropathy induced by antibody to the glomerular epithelial cell (i.e., passive Heymann nephritis) that visceral glomerular epithelial cells will transiently proliferate and express PDGF B-chain mRNA and protein (10). The consequences of the PDGF B-chain expression are currently unknown, however, because the visceral epithelial cell does not express PDGF-R (10). Thus, although there is good correlation between the cell proliferation and PDGF expression in this model, it is unlikely in this case that the PDGF is mediating the proliferation.

Stimuli for PDGF Expression in Glomerulonephritis. There are probably many stimuli that induce PDGF expression in glomerular disease. For example, a large number of growth factors stimulate the mesangial cell production of PDGF in vitro, including basic fibroblast growth factor (bFGF), epidermal growth factor, endothelin, thrombin, and PDGF itself (9,64,65). The presence of shear forces can also induce PDGF expression in glomerular endothelial cells (5). In the Thy 1 model, many of these stimuli are present. Thus, in the glomeruli of rats with anti-Thy 1 nephritis, there are platelets (which contain numerous growth factors in addition to PDGF) (59), bFGF that has been released by mesangial cells during the initial mesangiolysis (66), local fibrin (and hence thrombin) formation (67), and increased endothelin (68). It is likely that shear forces may also be present, resulting from the initial disruption of the mesangium. Some of these factors appear to be important in stimulating the PDGF expression in this model, as evidenced by the reduction of PDGF expression by the depletion of platelets (31,36), by the administration of anti-PDGF antibody (69), and by the administration of heparin (which prevents fibrin formation) (67).

Criteria 3. Administration of Exogenous PDGF Can Induce Mesangial Cell Proliferation In Vivo

One might hypothesize that if a growth factor is an important mediator of cell proliferation in glomerulonephritis, then the exogenous administration of that growth factor to normal animals should reproduce the proliferative lesion. This, however, does not have to be true. For example, it is possible that the growth factor mediates cell proliferation in disease but not when it is administered to normal animals. This could happen if there is a difference between diseased and normal glomeruli with regard to the
presence of synergistic cytokine(s) and/or endogenous inhibitor(s) or the degree of receptor expression. Likewise, it is possible that a significant proliferative response to pharmacologic doses of a growth factor may not equate to the type and magnitude of cell proliferation that occurs in a diseased glomerulus in response to physiologic amounts of that particular growth factor. This may also be a concern in interpreting studies with animals transgenic for a particular cytokine.

With these caveats in mind, we have recently conducted studies to determine if the exogenous administration of human recombinant PDGF-BB can induce mesangial cell proliferation in rats. Whereas the continuous infusion of 40 μg over 24 h resulted in mild mesangial cell proliferation in normal rats, the same dose in rats pretreated with subnephrotogenic doses of anti-Thy 1 sera caused a massive mesangial cell proliferation with a histologic lesion resembling acute proliferative nephritis (unpublished data). The observation that the infusion of a growth factor results in greater cell proliferation in rats that had been "primed" with subnephrotogenic doses of anti-Thy 1 sera as compared with normals has also been shown by our group with bFGF (66). Further studies are ongoing to address the mechanism(s) responsible for this difference.

Criteria 4. Inhibition of PDGF in vivo Reduces Mesangial Cell Proliferation in Glomerulonephritis

In order to test the hypothesis that PDGF mediated mesangial cell proliferation in the Thy 1 model, an experiment was performed in collaboration with E. Raines and R. Ross in which PDGF was inhibited in rats with anti-Thy 1 nephritis by the administration of a neutralizing anti-PDGF antibody (69). The inhibition of PDGF was associated with a 57% reduction in mesangial cell proliferation and a less but still significant reduction in mesangial matrix expansion at Day 4 (69). No effect of anti-PDGF treatment was seen on initial mesangiolysis, serum complement levels, circulating platelet counts, or the number of infiltrating macrophages (69). In addition, no effect was seen on the initial mesangial cell proliferation observed at Day 2. This suggests that the initial proliferation may have been due to other growth factors (e.g., bFGF) in addition to PDGF or that insufficient levels of anti-PDGF antibody were present to inhibit PDGF completely.

Recent studies also suggest that trapidil, an antiplatelet agent, may function as a PDGF-R antagonist and inhibit mesangial cell proliferation in vitro to either PDGF or to fetal bovine serum (70,71). In a preliminary report, Futamura et al. have also reported that trapidil treatment of anti-Thy 1 nephritis results in less glomerular hypercellularity (71). In contrast, in antiglomerular basement membrane nephritis, trapidil treatment did not improve the histologic lesion, although glomerular cell proliferation was not directly assessed (72).

Thus, PDGF is one of the factors that mediate mesangial cell proliferation in glomerulonephritis in the rat. Additional studies will be needed to confirm that this is also true in humans. However, we would like to complete the review by discussing two additional and relevant issues—the parallels between PDGF expression in glomerular diseases with that during development and the question of whether the inhibition of PDGF should be a goal of future therapies.

PDGF in Glomerular Development—Parallels with Glomerular Injury

We recently reported the developmental patterns of PDGF B-chain expression in human glomerulogenesis (73). In early glomerular development, the PDGF B-chain is localized to the epithelium of the glomerular vesicle, whereas the undifferentiated metanephrine blastema is negative for the PDGF B-chain but does express the PDGF-R β-subunit. However, with maturation, the PDGF B-chain, PDGF-Rβ, and α-smooth muscle actin are all expressed in mesangial patterns in the fetal glomerulus (Fig. 1) (73). This pattern is reminiscent of the up-regulation of the PDGF B-chain, PDGF-Rβ, and α-smooth muscle actin expression that occurs in diseases associated with mesangial cell proliferation (Fig. 1).

SHOULD INHIBITION OF PDGF BE A GOAL OF FUTURE THERAPIES OF PROLIFERATIVE GLOMERULONEPHRITIS?

The observation that PDGF mediates mesangial cell proliferation in disease does not necessarily mean that it should be a target for intervention. Mesangial cells have an important role in maintaining normal glomerular structure and function (reviewed in reference 74). In the anti-Thy 1 model, the initial mesangiolysis results in an almost complete decimation of the mesangial cell population (31,51), so that some mesangial cell proliferation is necessary to reconstitute this population of cells. Mesangiolysis can also be documented in many other glomerular diseases in humans (75). The fact that PDGF is also expressed in glomerular development suggests that PDGF expression in diseases may represent a normal component of the proliferative response that accompanies tissue remodeling and repair.

However, there is evidence that glomerular cell proliferation is often excessive in disease and that the increase in cell proliferation is intricately linked to an expansion of the mesangial matrix and the development of focal and segmental glomeruloscle-
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in the normal recovery of glomerular structure ating

that occurs in many types of glomerulonephritis. Other growth factors are likely to be involved in this proliferative response as well. However, further studies are needed to determine the conditions in which these growth factors have a beneficial role in mediating the normal recovery of glomerular structure and function as opposed to situations in which these cytokines mediate progressive and irreversible glomerular sclerosis.

ACKNOWLEDGMENTS

We acknowledge all of our collaborators who have contributed to these studies, especially Hiroyuki Iida, Ashio Yoshimura, Katherine Gordon, Pamela Pritzl, Kelly Hudkins, Daniel Bowen-Pope, Ron Seifert, Elaine Raines, and Russell Ross. Support for these studies was provided by U.S. Public Health Service grants DK 43422 and DK 02142.

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