The Activated Mesangial Cell: A Glomerular “Myofibroblast”?  

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ABSTRACT
The glomerular mesangial cell may have several important beneficial functions in the normal glomerulus. These include the production of growth factors to allow normal cell turnover, the provision of structural support for the capillaries via the production of mesangial matrix, and the modulation of glomerular hemodynamics via their contractile properties. However, in various types of glomerular injury, the mesangial cell may acquire characteristics of a “myofibroblast”, which may in fact be injurious to the glomerulus. These “activated” mesangial cells can be shown to be proliferating by one or more mechanisms that are mediated by platelets and that also involve the local production of platelet-derived growth factor. Like myofibroblasts in other tissues, the mesangial cell acquires smooth muscle cell-like properties, characterized by the de novo expression of α-smooth muscle actin, and by the development of fibroblast-like properties, characterized by the production of interstitial collagens in addition to normal mesangial matrix constituents. Identifying therapeutic strategies that prevent this phenotypic modulation of the mesangial cell may provide new ways to treat glomerular diseases.

Key Words: Proliferation, platelets, platelet-derived growth factor, α-smooth muscle actin, glomerulonephritis

The glomerular mesangial cell, which was first recognized as a distinct cell less than 60 yr ago (1), is one of the three major cell types present in the normal glomerulus and constitutes approximately 30

to 40% of the total glomerular cell population (2). Spreading out from the hilus of the glomerulus in an arborescent pattern and embedded in their own extracellular matrix, mesangial cells remain in direct contiguity with cells within the juxtaglomerular apparatus (3). Whereas the majority of glomerular mesangial cells are ultrastructurally and morphologically similar to smooth muscle cells (4–6), a small population (<7%) of the cells, at least in the rat, are bone marrow-derived la positive cells with macrophage or dendritic cell characteristics (7).

FUNCTIONS OF THE NORMAL MESANGIAL CELL

The properties and functions of the mesangial cell have been defined largely from cell culture studies and have recently been the subject of several excellent reviews (4–6,8). Mesangial cells in culture can release a variety of growth factors, including platelet-derived growth factor (PDGF), interleukin 1 (IL-1), insulin-like growth factor 1 (IGF-1), and interleukin 6 (IL-6), which may be important in the maintenance of normal glomerular cell proliferation and turnover (reviewed in reference 8) (Table 1). Mesangial cells in vitro also secrete an extracellular matrix that contains types I, III, IV, and V and low-molecular-weight collagens (9) as well as laminin, fibronectin, and heparan sulfate and chondroitin sulfate proteoglycans (6). In vivo, however, the normal mesangial matrix does not appear to contain the interstitial (i.e., types I and III) collagens (6). The mesangial matrix may function to provide a structural support (i.e., scaffolding) for the glomerular capillaries that helps to maintain patency and prevent capillary collapse (3,4,6). Mesangial cells in culture also morphologically resemble smooth muscle cells, express several smooth muscle cell-associated proteins, and contract in response to a variety of agonists (reviewed in references 4–6). This has suggested to Schröndorff (4) that these cells may function like specialized pericytes, potentially modulating glomerular hemodynamics via their contractile properties. As Kriz et al. have demonstrated, mesangial cells have cellular processes that attach to the glomerular basement membrane (GBM) in the paramesangial areas (10). Thus, mesangial cell contraction could potentially cause a retraction of the glomerular capillary, resulting in a decrease in capillary surface area and hence filtration (4,10). However, the effect of mesangial cell
The mesangial cell, a critical component in the glomerular filtration barrier, plays multifaceted roles in maintaining structural integrity and regulatory functions of the renal microcirculation. Its various properties and responses are influenced by a range of stimuli, which can lead to alterations in its phenotype and function. In this context, the table below summarizes the functions of the mesangial cell in normal and diseased states.

<table>
<thead>
<tr>
<th>Action</th>
<th>Normal Role</th>
<th>Hyperstimulated Disease State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production of growth factors</td>
<td>Normal cell growth and turnover</td>
<td>Cellular proliferation</td>
</tr>
<tr>
<td>Production of mesangial matrix</td>
<td>Structural support of glomerulus</td>
<td>Glomerulosclerosis</td>
</tr>
<tr>
<td>Smooth muscle-like properties</td>
<td>Maintenance of normal glomerular hemodynamics</td>
<td>Response to altered glomerular hemodynamics</td>
</tr>
<tr>
<td>Proinflammatory properties</td>
<td>Clearance and degradation of immune complexes</td>
<td>Local cellular injury and matrix degradation</td>
</tr>
</tbody>
</table>

Contraction on the overall capillary surface area is likely to be small (10) and probably cannot account for the substantial decrease in the ultrafiltration coefficient, *Kf*, that is observed when various agonists that are known to cause mesangial contraction in vitro are administered to animals in vivo (4,10). More likely, the contractile properties of the mesangial cell may be important in maintaining a static counterforce to the distensible pressures within the capillary (10). Finally, in vitro studies have also demonstrated the capability of mesangial cells to function as proinflammatory cells. Mesangial cells in culture can produce numerous vasoactive substances (e.g., prostaglandins, platelet-activating factor), oxidants, proteinases, and proteinase inhibitors (4–6,11,12). Mesangial cells in vitro also express Fc receptors, which enable the cells to endocytose immune complexes (4). Theoretically, these proinflammatory properties may aid the mesangial cell in vivo in the clearance and degradation of immune complexes from glomeruli.

Thus, mesangial cells may have several important functions in the normal glomerulus, in which they produce growth factors that allow normal cell turnover, provide structural support via the secretion of a mesangial matrix, maintain glomerular hemodynamics and counter distending forces via their smooth muscle-like properties as well as by the release of vasoactive substances, and aid in the clearance of immune complexes (Table 1). These functions are presumably tightly regulated and beneficial to the host. However, evidence is accumulating to suggest that mesangial cells, activated during glomerular inflammation, proliferate and undergo a phenotypic modulation in which they markedly up-regulate their expression of smooth muscle-like proteins (i.e., α-smooth muscle actin) as well as develop fibroblast-like characteristics in that they secrete interstitial collagens that are not normally present in the mesangial matrix (Table 1). This phenotypic modulation in which the resting mesangial cell is transformed in vitro to express both smooth muscle-like and fibroblast-like features suggests that the activated mesangial cell may be considered a type of myofibroblast. If limited in response, the result might allow for the restoration of normal glomerular structure. However, the glomerular “wound healing” process often results in an “overcompensatory” reaction, which leads to an abnormal increase in mesangial cells with increased deposition of extracellular matrix, culminating in glomerulosclerosis or “scar” formation. Studies from our laboratories that support this hypothesis will be presented in the following sections.

**CONSEQUENCES OF MESANGIAL CELL ACTIVATION IN GLOMERULONEPHRITIS**

**Mesangial Cell Proliferation**

Numerous glomerular diseases, including immunoglobulin A nephropathy, lupus nephritis, and variants of idiopathic focal glomerulosclerosis are characterized by an increase of cells in mesangial areas. Although it has been frequently assumed that the mesangial hypercellularity represents a proliferation of mesangial cells, the relative contribution of infiltrating mononuclear cells, which can resemble mesangial cells, has often not been excluded. To further complicate matters, infiltrating monocytes and macrophages may proliferate within the glomerulus (13,14), and thus, analyses based on measuring cell proliferation (e.g., by incorporation of [3H]thymidine, etc.) may in fact be including this population of cells.

Our initial studies on mesangioliproliferative nephritis were in the rat model of glomerular injury induced by the injection of antibodies to the Thy-1 antigen present on the mesangial cell membrane (14). This model is characterized by an acute mesangiolysis (i.e., mesangial cell loss with dissolution of matrix) that is maximal at 24 h and is followed by a mesangial hypercellularity from 3 to 7 days after disease induction (15,16). Macrophage infiltration is prominent in this disease, with as many as 10 to 20 cells per glomerular cross-section staining for the monocyte-macrophage marker, ED-1, during the first 5 days of injury (17,18). Thus, much of the mesangial hypercellularity is due to infiltrating leukocytes.

In order to identify cell proliferation within the glomerulus, we immunostained tissue sections with a monoclonal antibody to the proliferating cell nuclear antigen (PCNA) (14). PCNA is a nuclear auxiliary protein for DNA polymerase δ that shows markedly up-regulated expression during the late G1 through
M phase of the cell cycle (19). To address the possibility that some of the proliferating cells were infiltrating leukocytes, we performed double immunolabeling of tissue sections with antibodies to PCNA and a monoclonal antibody to the common leukocyte antigen [also known as CD-45 (14)] or with a monoclonal antibody to monocyte-macrophages (18). In both situations, over 85% of the proliferating (PCNA+) cells were shown to exclude the leukocyte markers (14,18), thereby strongly suggesting that the cell proliferation was of the intrinsic mesangial cell population.

We have used this approach to study human glomerular diseases. An increase in PCNA+ cells was demonstrated in many diseases associated with mesangial injury, including IgA nephropathy, diffuse lupus nephritis, membranoproliferative glomerulonephritis, and focal and segmental glomerulosclerosis (20). Diseases involving the mesangium that have been classically not thought of as proliferative, such as amyloid and diabetic nephropathy, were also associated in some cases with an increase in PCNA+ cells. When tissue sections were double immunolabeled for both PCNA and the common leukocyte antigen, the great majority of the PCNA+ cells were shown to be distinct from those bearing the leukocyte marker. Taken together, these studies provide evidence that, in diseases associated with mesangial injury, some degree of glomerular (and likely mesangial) cell proliferation is ongoing in both acute and more chronic stages of the disease process.

Mechanisms of Mesangial Cell Proliferation: Role of Platelets and Complement

A role for platelets in the in vivo mediation of mesangial cell proliferation has been suggested by several studies in which intraglomerular platelet accumulation can be documented before the development of hypercellularity or proliferation (21,22). The most direct evidence for a role of platelets in mediating mesangial cell proliferation has been provided by studies in which rats were depleted of platelets with an antiplatelet antibody (14,23). In Habu snake venom nephritis, platelet depletion significantly reduced the glomerular hypercellularity (23). Likewise, we demonstrated that the number of proliferating glomerular cells (i.e., PCNA+, CD45− cells) was reduced by platelet depletion in the model of mesangiocapillary glomerulonephritis induced with anti-Thy-1 antibody (14). The mechanism by which platelets mediate cell proliferation remains unknown. However, platelets contain many growth factors and vasoactive substances that mediate mesangial cell proliferation in vitro, including PDGF, IGF-1, transforming growth factor alpha (TGF-α), and serotonin (24–26).

Complement depletion also prevents the mesangial cell proliferation in anti-Thy-1 nephritis (16,27). The mechanism may relate to the effect of complement depletion to prevent the initial mesangiolysis, which then results in a secondary inhibition of the glomerular platelet and macrophage influx (18,27).

Role of PDGF and Other Growth Factors in Mesangiocapillary Glomerulonephritis

Studies were undertaken to examine the role of PDGF in the anti-Thy-1 model. PDGF was selected because it is a known mitogen for mesangial cells in culture and because it can be released by a variety of cells present in the glomerulus during inflammation, including the platelet, macrophage, endothelial cell, and the mesangial cell itself (8,28). An increase in both PDGF A- and B-chain mRNA could be demonstrated in whole glomerular RNA isolated from rats with anti-Thy-1 nephritis at 3 and 5 days, respectively, after disease induction (18). Cells expressing PDGF B-chain mRNA could also be identified in mesangial regions in situ hybridization (29). Cells expressing PDGF B protein were identified by immunostaining with a specific monoclonal antibody, and by double immunolabeling, the majority of these cells were also shown to express α-smooth muscle actin (18). This suggested that most of the PDGF was produced by the activated mesangial cells (see below) (18,29).

In addition to the enhanced PDGF expression, an up-regulation of the PDGF receptor (β-subunit) mRNA and protein was also documented in rats with anti-Thy-1 nephritis (18). By immunocytochemistry, the cells expressing the PDGF receptor localized to mesangial areas (18).

The up-regulation of glomerular PDGF and PDGF receptor was also correlated with the mesangial cell proliferation. When mesangial cell proliferation was prevented or reduced by maneuvers such as complement depletion or platelet depletion, respectively, the up-regulation of PDGF and the PDGF receptor in glomeruli was largely prevented (18).

Thus, these studies demonstrate that mesangiocapillary nephritis induced by anti-Thy-1 antibody is associated with a marked up-regulation of PDGF (A and B chain) and of the PDGF receptor (β-subunit). Gesualdo et al. have also observed that PDGF is expressed in a murine model of mesangiocapillary nephritis (30). These authors have also shown that PDGF can be immunolocalized to glomeruli of patients with mesangiocapillary nephritis (30), and others (31) have reported that the PDGF receptor is up-regulated in this group of patients.

However, these studies implicate but do not establish PDGF as an essential mediator of mesangial cell proliferation. Recently, we have performed experi-
ments that provide direct evidence that PDGF mediates mesangial cell proliferation in glomerulonephritis (32). Rats with anti-Thy-1 nephritis were administered a polyclonal neutralizing antibody to PDGF. The inhibition of PDGF did not prevent the mesangiolysis nor the initial cell proliferation observed 2 days after disease induction. However, the cell proliferation observed at day 4 was reduced by approximately 60%. The anti-PDGF antibody treatment had no discernible effect on circulating leukocyte or platelet counts, serum complement levels, or glomerular macrophage infiltration.

This suggests that there may be different phases in the mesangial cell proliferative response. The initial phase may likely involve growth factors other than or in addition to PDGF. One likely source, which is suggested by our studies, would be degranulating platelets. Another source of growth factors could be the mesangial cells themselves, which could release these factors during mesangiolysis. In addition to IGF-1, IL-1, and IL-6, an attractive candidate is basic fibroblast growth factor. Recent studies have demonstrated a critical role for basic fibroblast growth factor in the initial proliferation of smooth muscle cells that occurs in rats after carotid angioplasty (33). This growth factor is also a mesangial cell mitogen and induces mesangial cells to express PDGF mRNA (34).

After the initial cell proliferation, a secondary phase occurs that is PDGF dependent and that would appear to be autocrine driven as originally hypothesized by Abboud (8,34). It is possible that up-regulation of the PDGF receptor during this phase may increase the responsiveness of the mesangial cell to PDGF. Finally, a resolution phase occurs in which cell proliferation ceases. The mechanism responsible for terminating the proliferation is unknown. One possibility would be the local release of TGF-β by mesangial cells or macrophages, because this factor is known to be expressed in the anti-Thy-1 model (35) and inhibits mesangial cell proliferation in vitro (36). Other possibilities include the potential antiproliferative effects of heparan sulfate proteoglycan or other heparin-like substances (37,38). Finally, recent data suggest that the extracellular glycoprotein SPARC/osteonectin, which is also present in glomeruli (unpublished observations), may be a specific endogenous inhibitor of the PDGF isoforms, PDGF-AB and PDGF-BB (39).

Matrix Expansion and Glomerulosclerosis

Numerous glomerular diseases are associated with an expansion of the mesangial matrix, which may ultimately result in focal glomerulosclerosis. The composition of the sclerotic areas has been an area of intensive study. To investigate the extracellular matrix components involved and in order to determine if there was a relationship to mesangial cell proliferation, we again studied the anti-Thy-1 model of mesangioliproliferative nephritis (40). Previous studies had reported that there was a transient expansion of the mesangial matrix and that this was associated with an increased deposition of fibronectin and chondroitin sulfate/dermatan sulfate proteoglycans (35). With specific antibodies, we were able to demonstrate an increase in mesangial staining for other extracellular matrix components as well, including type IV collagen, laminin, heparan sulfate proteoglycan, and entactin/nidogen. Northern analysis of whole glomerular RNA showed a corresponding 7- to 10-fold increase in type IV collagen and laminin B2 mRNA at days 3 and 5 after disease induction. There was also de novo induction of type I collagen mRNA and protein, which is an interstitial collagen not normally expressed in glomeruli. The increase in the mesangial matrix was coincident with the mesangial cell proliferation (as determined by immunostaining for PCNA) and was prevented if proliferation was inhibited by complement depletion (40). Thus, these studies suggest that the mesangial matrix expansion that accompanies mesangial cell proliferation involves an increased synthesis of matrix components normally found in the mesangium, as well as interstitial collagens.

Studies of the anti-Thy-1 model are informative, but the model is atypical in that the mesangial cell proliferation is massive but transient, and although accompanied by mesangial matrix expansion, development of glomerulosclerosis is uncommon unless the animal receives repeated injections of anti-Thy-1 antibody (H. Iida et al., manuscript in preparation). We have therefore studied the remnant kidney model induced by uninephrectomy with ligation of two of the three arterial branches of the remaining kidney (i.e., the % nephrectomy or remnant kidney model) (41). Within 1 wk, a low-grade mesangial cell proliferation was documented (by double immunostaining with anti-PCNA and anti-Thy-1 antibodies) and was accompanied by a platelet influx and enhanced glomerular PDGF B-chain mRNA and protein expression (41). After the initial proliferation, there was an infiltration of glomeruli with macrophages and a progressive development of glomerulosclerosis (41). As with the anti-Thy-1 model, the increase in the mesangial matrix was in part due to extracellular matrix components normally associated with the mesangial matrix (i.e., type IV collagen, laminin, fibronectin, and heparan sulfate proteoglycan) as well as the de novo expression of interstitial type I collagen (42). It is also likely that the sclerotic areas contain other as yet unidentified proteins. Nevertheless, these studies demonstrate the important association between mesangial cell proliferation and the development of glo-
merulonosclerosis, a finding that has been previously emphasized (38) and that has been clearly demonstrated in mice transgenic for growth hormone or growth hormone releasing factor (43).

Acquisition of Smooth Muscle-Like Phenotype by the Mesangial Cell in Glomerulonephritis

In culture, mesangial cells resemble smooth muscle cells, express a variety of smooth muscle cell-associated proteins, and are contractile in response to a variety of agonists (4–6). In contrast to in vitro studies, the principal actin isofrom associated with vascular smooth muscle, i.e., α-smooth muscle actin, is not normally expressed by rat mesangial cells (in vivo (44). However, we have noted that there is a marked induction of α-smooth muscle actin mRNA and protein in the anti-Thy-1 model of mesangiproliferative nephritis (Figure 1) (44). The cells expressing the α-smooth muscle actin were in mesangial regions and were also actively proliferating (as determined by double immunolabeling with anti-PCNA and anti-α-smooth muscle actin antibodies). α-Smooth muscle actin expression by mesangial cells was also induced in other experimental models of mesangiproliferative nephritis but not in models of glomerular epithelial cell injury or in anti-GBM disease (44). Recently, we have also demonstrated that mesangial expression of α-smooth muscle actin is up-regulated in human glomerular diseases and that, in general, expression is greatest in lesions showing the highest levels of cell proliferation (Figure 1) (20).

These studies demonstrate that mesangial cell proliferation in glomerulonephritis is associated with a change in phenotype in which the mesangial cell expresses the actin isofrom associated with vascular smooth muscle. Moreover, our recent studies suggest that α-smooth muscle actin may also be expressed when mesangial cells are activated in the absence of frank proliferation. In rats chronically infused with anti-Thy-1 antibody, a marked expression of α-smooth muscle actin in the glomerular mesangium can be documented coincident with the mesangial cell proliferation (b; day 5 after disease induction). In humans, glomeruli from normal kidneys are also usually negative or trace positive for α-smooth muscle actin (c), but mesangial expression of this actin isofrom is marked in many glomerular diseases, including IgA nephropathy (d). In humans, peritubular interstitial cells, including those adjacent to Bowman's capsule, frequently express α-smooth muscle actin in both normal and diseased kidneys (c and d). The extraglomerular structures with strong staining for α-smooth muscle actin are arterioles. Immunoperoxidase staining with anti-α-sm-1 antibody, a monoclonal antibody to α-smooth muscle actin (gift of G. Gabbiani) (41).
angiotensin II, there is a dramatic up-regulation of α-smooth muscle actin in the mesangium but only a minimal increase in glomerular cell proliferation (45). In the remnant kidney model as well, α-smooth muscle actin is expressed before the onset of proliferation (41). Although the functional consequences of these findings remain unknown, these studies suggest that α-smooth muscle actin expression may serve as a useful marker of mesangial cell activation in glomerular diseases.

*In Vivo* Evidence for the Mesangial Cell as an Inflammatory Effector Cell

Mesangial cells in culture produce a 68- to 72-kd neutral protease capable of degrading the type IV collagen present in GBM (11,12). Using an antibody specific for this protease, Lovett *et al.*, in collaboration with our group, have demonstrated that there is an increased expression of this protease in the anti-Thy-1 model that occurs during the mesangial cell proliferation (46). Immunoelectron microscopy was able to demonstrate both intracellular and extracellular localization of the protease. One may speculate that the mesangial cell secretion of the neutral protease may help in the degradation of the increased mesangial matrix and in the general restoration of the normal glomerular architecture that occurs in this model.

The Activated Mesangial Cell as a "Glomerular Myofibroblast"

There is now accumulating evidence, as has been suggested by Menè *et al.* (6), that the activated mesangial cell develops myofibroblast-like features, i.e., characteristics of both smooth muscle cells and fibroblasts (47), in *in vivo* glomerular injury. The fibroblast-like characteristics are manifested by the production by the activated mesangial cell of interstitial collagens, in addition to the normal mesangial matrix proteins. The importance of this phenotypic modulation is that the normal proteinases produced by the mesangial cell are gelatinases/type IV collagenases (11,12), which are unable to degrade interstitial as opposed to basement membrane collagens (11,12). The mesangial cell, when activated during glomerular inflammation, also develops smooth muscle-like characteristics. In its normal resting state *in vivo*, it does have certain smooth muscle-like features, including its expression of the intermediate filament protein, desmin (44). However, unlike the smooth muscle cell, it normally does not express α-smooth muscle actin, which is the principal actin isoform of vascular smooth muscle (44). With mesangial cell activation or proliferation *in vivo*, this actin isoform is expressed (44). Transient proliferation with expression of α-smooth muscle actin is also characteristic of myofibroblasts in dermal wounds (48) and in pulmonary fibrosis (49,50) and is to be distinguished from true smooth muscle cells, which generally lose α-smooth muscle actin during cell proliferation (51). Whereas in wounds, the expression of the actin isoform by myofibroblasts is thought to be important in wound contraction, one may speculate that in the glomerulus the expression of α-smooth muscle actin by mesangial cells may help to maintain capillary structure in response to the altered glomerular hemodynamics and pressures that occur with glomerular injury, especially in the setting of an abnormal or partially degraded mesangial matrix.

In conclusion, these studies suggest that injury to the mesangial cell results in a "hyperstimulated" response by the mesangial cell that triggers proliferative and synthetic activities, which may eventually lead to glomerulosclerosis. A partial interruption of the process, which allows normal glomerular architecture to be restored but which prevents excess mesangial cell proliferation and matrix expansion, should be the goal of future therapies in glomerular diseases. Given the important role for platelets in mediating mesangial cell proliferation, antplatelet agents might appear to be attractive therapeutic agents. Unfortunately, antplatelet agents such as aspirin are ineffective in blocking platelet adherence to collagen or the subsequent release reaction (52) and are therefore unlikely to have a major effect on inhibiting platelet-mediated cell proliferation. The recent studies by Border *et al.* have demonstrated that the inhibition of TGF-β by a specific antibody can prevent the mesangial matrix expansion in the anti-Thy-1 model (53). However, pharmacologic agents that can be used long term to block TGF-β or other growth factors, such as PDGF, are still in the very early stages of development. When available, these new agents may become useful therapies for the treatment of a variety of glomerular diseases in humans.

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