Role of the Coagulation/Fibrinolysis System in Fibrin-Associated Glomerular Injury

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Abstract. In the past decade, numerous experimental studies of genetically engineered mice have confirmed the involvement of the coagulation/fibrinolysis system during glomerular inflammation and repair, revealing many unexpected biologic effects far beyond fibrin formation and clearance. Resident glomerular cells and macrophages seem to act in concert to ensure the long-term consolidation of local injury and progression toward glomerulosclerosis. These recent advances will probably pave the way to a new therapeutic approach to renal diseases. However, the balance governing glomerular permeability is very delicate, and many issues will have to be dealt with before targeting this system.

Fibrin deposition is a prominent feature of crescentic glomerulonephritis and of the hemolytic uremic syndrome (HUS). Fibrin clots not only interrupt capillary blood flow, leading to irreversible ischemia and glomerular obsolescence, but also promote the influx of monocyte-macrophages and proliferation of epithelial cells in Bowman’s space (Figure 1). It therefore is important to understand the mechanisms underlying fibrin formation (i.e., activation of intraglomerular coagulation) and fibrin persistence (i.e., deficiency of the fibrinolysis system). Major advances have been made during the past decade, notably by using mice in which key factors in the coagulation/fibrinolysis pathways were genetically altered. Two principal notions have emerged. First, the glomerulus must no longer be seen as a regular capillary ball whose main function is blood ultrafiltration but as an autarchic multicellular structure finely regulating its own vascular permeability. Second, most of the mediators involved have specific, nonredundant properties that extend beyond the vascular compartment and fibrin formation per se, also controlling inflammation and lesion repair. We review the most recent findings in this field and identify the major outstanding questions.

Description of the Coagulation Cascade and of the Fibrinolysis System

Deposition of fibrin monomers in the glomerular capillary follows cleavage of circulating fibrinogen by thrombin, a serine protease (Figure 2). It is classically considered that the intrinsic coagulation pathway does not threaten glomerular permeability, as factor VIII is not detected in the crescents of patients with proliferative glomerulonephritis (1). Coagulation is initiated by the abnormal presence in the glomerular capillary of tissue factor (TF), a transmembrane glycoprotein constitutively expressed by cells that are not physiologically in contact with circulating blood. TF synthesis by endothelial cells and macrophages can be induced by proinflammatory cytokines. TF activates the extrinsic pathway by complexing with factor VIIa. In the presence of calcium, this TF/VIIa complex catalyzes the activation of factor X. Then, factor Xa combines with factor Va to convert prothrombin to active thrombin. This pathway is antagonized by two major inhibitors. The natural TF inhibitor, TF pathway inhibitor (TFPI), binds factor Xa, forming a complex that secondarily binds TF/VIIa and inhibits TF/VIIa activity. TFPI also plays a key role in controlling factor Xa generation at sites of endothelial cell injury. Thrombin formation can be shut down by protein C. Interaction of thrombin with thrombomodulin at the cell surface, together with the endothelial protein C receptor, activates circulating protein C, which exerts potent anticoagulant effects by inactivating factor V.

Fibrin clots can be destroyed by the catalytic action of plasmin, a pivotal broad-spectrum enzyme (Figure 3) (2). Plasmin is generated after activation of circulating plasminogen. Two plasminogen activators (PA) are known: a tissue-type PA (tPA) and a urokinase-type PA (uPA) bound to its specific receptor uPAR. tPA has high affinity for fibrin and mediates intravascular fibrinolysis. uPA converts plasminogen into plasmin at the uPAR-expressing cell surface and mediates the proteolytic action of plasmin. Plasmin directly degrades several components of the extracellular matrix (ECM), such as fibronectin, laminin, and vitronectin, and also activates matrix metalloproteinase 2, a major collagenase. This system is regulated by at least four inhibitors. Type 1 plasminogen activator inhibitor (PAI-1), a serine protease inhibitor ("serpin"), can form covalent complexes with either of the two PA, leading to their elimination. Other PAI have been discovered, but little is known of their role in glomerular pathophysiology. α2-Antiplasmin (α2-AP) is a potent plasmin inhibitor produced mainly in liver and kidney (3). α2-AP is another serpin that directly

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targets plasmin to form a stable, inactive complex; it also has a binding domain that recognizes plasminogen and competes with plasminogen for fibrin binding. α2-AP therefore is a potent antifibrinolytic molecule. When α2-AP is over-whelmed, α2 macroglobulin, another protease inhibitor, may also protect from uncontrolled plasmin activity (4). Finally, fibrin degradation by plasmin exposes C-terminal lysine sites that have the capacity to enhance the rate of plasmin generation, a phenomenon that is efficiently inhibited by thrombin activatable fibrinolysis inhibitor (TAFI) (5).

Roles of Resident and Infiltrating Cell Types

The delicate balance between glomerular coagulation and fibrinolysis is maintained by the local regulation, by both circulating and resident cells, of the production of both pro- and anticoagulant agents and of pro- and antifibrinolytic...
agents. Schematically, coagulation is initiated by circulating macrophages or by endothelial injury and is sustained by glomerular resident cells.

**Macrophages**

In crescentic glomerulonephritis, macrophages play a central role in glomerular fibrin deposition and crescent formation. As many as 50 macrophages may be found in the vicinity of a fibrin-positive glomerulus, whereas macrophages are seldom seen in fibrin-negative glomeruli (1). The degree of macrophage infiltration correlates well with the extension of glomerular lesions (1,6), and monocyte depletion from circulating blood markedly attenuates fibrin deposition (7). Macrophages can synthesize TF or induce abnormal TF production by endothelial and mesangial cells by stimulating the release of proinflammatory mediators such as IL-1 (6,8,9).

**Endothelial Cells**

It has proved difficult to isolate and culture glomerular endothelial cells, and most data on their physical and biologic properties come from studies of human umbilical vein cells (10). Endothelial cells maintain a nonthrombogenic surface by producing anticoagulant substances such as thrombomodulin, heparan sulfates, and PA (11). Being located at the interface between blood and solid tissues, endothelial cells can also recruit circulating leukocytes to sites of inflammation. This recruitment is mediated by adhesion molecules such as selectins, integrins, and members of the Ig superfamily. Abnormal expression of adhesion molecules, triggered by inflammatory mediators, promotes the recruitment of monocytes and macrophages. This is a critical step in the activation of coagulation during crescentic glomerulonephritis (12–15). Indeed, under the influence of proinflammatory products secreted by activated macrophages (mainly IL-1 and TNF-α), procoagulant factor production is stimulated (16). IL-1 and TNF augment the TF–like procoagulant activity of human endothelial cells, reduce endothelial surface expression of thrombomodulin (a mediator of the anticoagulant effects of protein S and protein C), and increase the secretion of PAI-1, the main inhibitor of plasminogen activators (17–23). These molecules tip the balance of coagulation/fibrinolysis toward endothelial surface hypercoagulability and fibrin formation, thereby threatening capillary permeability.

Endothelial cells thus have a direct role in sustaining inflammation around the glomerular basement membrane (GBM) and can also be the specific target of external agents. For example, during HUS, shiga toxins produced by enterohemorrhagic *Escherichia coli* attach to globotriaosylceramide molecules (Gb3) on the endothelial cell surface and enter the cell (24). The A subunit of shiga toxin undergoes proteolysis and disulfide-bond reduction. Activated A subunit inhibits protein synthesis, causes cell death, and alters the endothelial surface. TF exposure increases, thrombin is generated, and platelet-bound fibrinogen is cleaved, leading to fibrin formation.

**Mesangial Cells**

Mesangial cells create the ECM supporting the center of the glomerular structure, secrete growth factors controlling normal cell turnover, and modulate the filtration surface by their contraction/relaxation (they are in direct contact with endothelial cells). During glomerular injury, mesangial cells acquire phenotypic characteristics of myofibroblasts. So-called “activated” mesangial cells proliferate, acquire smooth muscle cell–like properties, express α-smooth muscle actin, and secrete abnormal forms of collagen (collagens I and III) and mediators of the coagulation and fibrinolysis systems. Mesangial cells may also participate in local fibrin formation: It has been shown that, when appropriately stimulated (by TNF-α or lipopolysaccharide), rat mesangial cells produce TF-like procoagulant activity; also, TFPI is found in the culture supernatant of human mesangial cells (25,26). More recently, Liu *et al.* (27) detected factor V, the membrane-bound co-factor of factor Xa, in the mesangial areas of kidneys from patients with IgA nephropathy and found that it co-localized with cross-linked fibrin. *In situ* hybridization showed a high level of factor V mRNA in mesangial cells. All of these studies support the concept that mesangial cells participate in coagulation events. Concerning the fibrinolysis system, Lacave *et al.* (28,29) also showed that cultured human mesangial cells synthesize and secrete tPA and PAI-1, the latter being secreted in excess in the ECM, where it co-localizes with vitronectin. PAI-1 secretion has become an activation marker of this cell subtype.

**Epithelial cells**

The pathophysiology of crescents is controversial, but it is generally believed that proliferating glomerular parietal epithelial cells are the dominant cellular component (30). Visceral epithelial cells may be an important trigger in this proliferation (31). *In vitro*, they can express molecules involved both in the coagulation cascade and in the fibrinolysis pathway (uPA, tPA, and PAI-1) (28–30,32–35). PAI-1 production has been shown to be potentiated by thrombin (33,36).

**Key Players in Fibrin Formation and Destruction**

The individual roles of specific mediators of the coagulation/fibrinolysis systems have been studied in genetically engineered mice with experimental fibrinous nephropathies. However, as gene knockout of some components of the coagulation pathway induces 100% lethality during embryonic and/or neonatal life, their role in glomerular injury cannot be studied in this way (Table 1).

**Coagulation System**

**Role of TF and of Its Inhibitor TFPI.** The role of TF in crescentic glomerulonephritis was first demonstrated by Erlich *et al.* (37). In rabbits, concurrent injection of antibodies directed against the GBM and against TF halves the severity of glomerular lesions (fibrin deposits and the proportion of crescents), along with the level of proteinuria, and the degree of renal failure, without affecting macrophage infiltration. An-
ti-TF antibodies can also modify macrophage activation. The cellular source of glomerular TF is controversial. There is a discrepancy between animal models, in which TF seems to be produced by macrophages, and the human disease, in which few CD68-positive cells contain TF mRNA, contrary to glomerular resident cells (6). This discrepancy could be related to the timing of renal tissue examination.

TFPI synthesis has been demonstrated in the glomerular tuft, in both physiologic and pathologic conditions. In human glomerulonephritis, TFPI is detected in glomeruli showing extracapillary proliferation (38,39). Immunoperoxidase staining suggests that endothelial cells are the main physiologic source of TFPI and/or the main binding site. During crescentic glomerulonephritis in rabbits, TFPI synthesis is initially downregulated, potentially enhancing TF activity and thereby facilitating fibrin deposition (38). Early infusion of recombinant human TFPI attenuates both glomerular fibrin deposition and renal impairment, suggesting that this recombinant protein could have therapeutic potential in fibrinous glomerulopathies.

**Role of Thrombin.** Thrombin directly activates platelets, cleaves fibrinogen to fibrin monomers, and interacts with specific receptors (PAR) belonging to the G protein–coupled receptor family. PAR-1 is strongly expressed on the surface of endothelial, mesangial, and epithelial cells (Figure 4). Thrombin binding leads to internal cleavage of the receptor, releasing a small N-terminal peptide and unmasking a new amino-terminal domain that activates the receptor through intramolecular interactions (40). Thrombin receptor is then internalized (41). The biologic effects of PAR-1 activation include cytoskeleton rearrangement and proliferation of fibroblasts and glomerular epithelial cells (36,42). Moreover, thrombin upregulates the expression of uPA and tPA and of their inhibitor PAI-1, whereas uPAR expression is downregulated. The net effect is fibrin formation and a blunting of the fibrinolysis system (36).

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<th>Table 1. Effect of the invalidation of the components of the coagulation and fibrinolysis system on the course of crescentic, anti-GBM glomerulonephritis in mice</th>
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<td><strong>Coagulation pathway</strong></td>
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a PAR-1, Plasminogen activator receptor 1; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor; PAI-1, plasminogen activator inhibitor 1.

b Gene suppression of the following components of the coagulation system produce a drastic lethality in mice during embryonic development and/or during the neonatal period: tissue factor, factor V, factor VII, factor X, prothrombin, thrombomodulin, endothelial C protein receptor, and protein C.
In human glomerulonephritis and HUS, we found that PAR-1 protein expression on the surface of glomerular cells was downregulated, whereas the expression of its specific messenger RNA was upregulated (43). This suggested that receptor internalization was activated by thrombin (44). We also found that, during experimental anti-GBM glomerulonephritis, the cellular (proinflammatory) effects of thrombin mediated by PAR-1 activation were at least as important as its extracellular (procoagulant) effects (45). Thus, whereas hirudin, a potent serine protease inhibitor, significantly reduces the glomerular lesions (i.e., macrophage infiltration, fibrin deposition, and crescent formation), PAR-1 knockout mice are protected to a similar extent. Administration of TRAP peptide, which can directly activate PAR-1 but lacks the enzymatic properties of thrombin, also accentuates glomerular lesions. It should be noted that ligands other than thrombin could bind PAR-1 and have deleterious effects in this model.

Immunohistochemical studies have shown that thrombomodulin expression is strongly enhanced on the plasma membrane of glomerular endothelial cells in human proliferative glomerulonephritis (46). In experimental acute glomerulonephritis induced in rats by concomitant injection of lipopolysaccharide and antibodies directed against the GBM, recombinant human soluble thrombomodulin improved animal survival and had both antiinflammatory and anti-inflammatory properties (47).

**Role of Fibrin.** Fibrin is the end product of the coagulation cascade and, as such, can be considered a key actor in the pathogenesis of acute glomerulonephritis. Fibrin clots compromise glomerular capillary flow (leading to focal ischemia and necrosis) and attract both macrophages and leukocytes. Defibrination by ancrud reduces the severity of glomerulonephritis in rabbits (48), and suppression of the fibrinogen A-α chain gene protects mice (49). These knockout mice have no circulating fibrinogen, and fibrin clots do not form in the injured glomeruli, yet diffuse glomerulonephritis with crescents develops despite the absence of fibrin, and the level of proteinuria is significantly lower in Fib−/− mice than in Fib+/+ mice.

The importance of fibrin degradation products released after plasminogen activation is unclear. Like fibrin, fibrin degradation products have chemoattractant properties and could play a role in glomerular inflammation (50). However, as plasminogen deficiency considerably enhances the severity of anti-GBM glomerulonephritis, fibrinolysis is thought to have an overall protective effect.

Taken together, these data underline the double-edged action of the coagulation cascade: Fibrin formation and capillary obstruction is clearly a threat, but “glomerular inflammation,” whether it is due to procoagulatory mediators or not, could be even more critical.

**Fibrinolysis System**

**Role of PA and Plasminogen.** In the anti-GBM model, Malliaros et al. (51) observed a marked decrease (40-fold) in net intraglomerular fibrinolytic activity, as a result of a 10-fold decrease in tPA activity in the glomerular supernatant (uPA activity was unchanged) and to a marked increase (5-fold) in the activity of PAI-1. It is interesting that this fall in fibrinolytic activity occurred despite the upregulation (at the mRNA and protein level) of PA, uPA, and, to a lesser extent, tPA, in cells composing glomerular crescents in human renal biopsy specimens (52). Together, these data suggest that acute glomerular injury increases both coagulation and fibrinolysis but that fibrinolysis is blunted by a marked increase in antifibrinolytic agents such as PAI and/or α2-AP.

However, anti-GBM glomerulonephritis is much more severe in tPA and plasminogen knockout mice, with more crescents, more fibrin deposition, and more severe renal failure than in wild-type mice (53). By contrast, gene suppression of uPA or its receptor uPAR has little impact on the outcome of the disease, even though uPA−/− mice have less severe inflammatory infiltrates inside glomeruli. This confirms that uPA and tPA play distinct, nonredundant pathologic roles. Thus, tPA, locally produced by endothelial cells in mice and humans, is probably the main PA involved in the clearance of glomerular fibrin.

Recombinant tPA (rtPA) has been available since 1983 and has been tested as a therapeutic agent in animal models of acute crescentic glomerulonephritis (54,55). Because the enzymatic activity of tPA is increased in the presence of fibrin (no significant plasminogen activation occurs in plasma), it is potentially less dangerous than other fibrinolytic drugs, such as urokinase and streptokinase (56). In 1990, the group of Remuzzi studied the effect of rtPA administered to rabbits from day 7 to day 13 after anti-GBM serum injection. Despite a longer bleeding time, rtPA-treated animals did not experience hemorrhagic episodes. The number of glomerular crescents was significantly lower in rtPA-treated animals, and the crescents were segmental rather than circumferential. Fibrin deposition was also less pronounced, and renal function was significantly improved. These results confirm that fibrin removal is protective in the early phase of crescentic glomerulonephritis.

More recently, Haraguchi et al. (57) studied the action of tPA in a rat model of anti-Thy-1 nephritis, with a special focus on glomerular matrix proteins. The authors’ approach was original. They claimed that the enhancement of plasmin generation by rtPA, as a result of the local presence of fibrin, would create local proteolytic activity, as plasmin is involved not only in the fibrinolysis system but also in the remodeling of ECM proteins. Using indirect immunoperoxidase staining, they observed less fibrin deposition and also less mesangial matrix expansion. Another interesting finding was that glomerular active TGF-β1 content was not affected by the increase in plasmin activity. Thus, so far, plasmin should be regarded as a profibrinolytic and proproteolytic agent in kidney. However, the property of plasmin to activate the latent form of TGF-β was recently shown to be experimentally relevant in a model of vascular fibrosis (58).

In addition to plasmin, matrix metalloproteinase 9 (MMP9) could have a fibrinolytic action in the glomerulus: It was recently reported that MMP9 gene knockout mice developed more severe renal failure and glomerular thrombosis than wild-type mice after injection of anti-GBM antibodies (59).
Role of PAI. The main PAI, PAI-1, is a glycoprotein belonging to the serpin family. It limits plasmin generation by binding to tPA and uPA. PAI-1 is also involved in cell adhesion and migration: By blocking the interaction among uPAR, integrin αVβ3, and vitronectin, PAI-1 prevents cell adhesion to the ECM. Recent data on the role of PAI-1 in tumorigenesis further extended the duality of this key molecule, as PAI-1 was found to play a pivotal role in angiogenesis (60). Three other PAI are known, namely PAI-2, PAI-3 (a protein C inhibitor), and protease-nexin I. Little is known of their involvement in glomerular pathology. Protease nexin I has been shown to be upregulated in a murine model of proliferative glomerulonephritis, but the significance of this finding is not known (61).

PAI-1 can form a complex with uPA and uPAR at the cell surface. The complex is rapidly internalized, along with uPAR, and is degraded in a process mediated by the LDL receptor-related protein, after which uPAR is regenerated; uPAR receptor may be a clearance receptor for PAI-1 (62).

In rat models of crescentic glomerulonephritis induced by anti-GBM antibodies, early (6 h) and long-lasting (17 d) upregulation of PAI-1 gene transcription and PAI-1 biologic activity is observed (63). The strong temporal relationship between the level of PAI-1 synthesis and the extension of fibrin deposition points to a major pathophysiologic role in this model. PAI-1 could be deleterious in the intravascular compartment by limiting plasminogen activation by tPA (and subsequent fibrin clearance) and also in the extravascular compartment by limiting the plasmin activation by uPA (and the subsequent ECM degradation). Recent findings obtained during the accelerated model of anti-GBM glomerulonephritis suggest this scenario: Compared with wild-type mice, PAI-1 knockout mice show fewer glomerular crescents, less fibrin deposition, and less collagen accumulation. By contrast, PAI-1-overexpressing mice exhibit more severe glomerular disease (64). However, this study did not examine the impact of PAI-1 suppression on renal fibrinolysis, and it therefore is not known whether the mechanism by which PAI-1 exacerbates the disease is related to inhibition of plasmin formation or to other (e.g., proinflammatory) properties of PAI-1. We recently obtained conflicting results in the passive model of anti-GBM glomerulonephritis (65). In mice with a genetic background similar to those used by Kitching et al. (64), PAI-1 gene deficiency led to more severe renal injury. We found that disease exacerbation was related to uncontrolled, PA-dependent activation of TGF-β, a cytokine with a major role in the CD4 T cell response to heterologous serum (66). Moreover, the absence of PAI-1 did not enhance renal plasmin activity, thus challenging the importance of PAI-1 in glomerular fibrinolysis (67). These conflicting results underline the paramount importance of the genetic background and the experimental model.

Recently, an elegant study supported the use of PAI-1 inhibitors in glomerular diseases (68). By competing with endogenous PAI-1, a human mutant PAI-1 that still binds to vitronectin but no longer inhibits PA, was found to attenuate glomerular fibrosis induced by anti-Thy-1 antibodies in rats. It is not clear whether this is related to the absence of endogenous PAI-1 in the matrix or to long-term occupancy of vitronectin sites. Vitronectin-bound PAI-1 may be required to prevent the adhesion of inflammatory cells or may inhibit plasmin activity, thereby accelerating progression to renal fibrosis. In this second hypothesis, the stimulating effect of aldosterone and angiotensin II on PAI-1 synthesis provides another putative mechanism by which the renin-angiotensin system could be deleterious, by promoting fibrin persistence and glomerulosclerosis (69,70). The recent confirmation that angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists decrease PAI-1 plasma concentrations in humans could partially explain the nephroprotection conferred by these agents (71). Although the balance between wound repair and fibrogenesis is very tight, an experimental study has shown that PAI-1 deficiency can facilitate the onset of vascular fibrosis (58). Conversely, total tPA deficiency was recently shown to protect from renal interstitial fibrosis in mice after ureteral ligation (72). Together, these findings indicate that targeting PAI-1 with the aim of slowing organ fibrogenesis might have contrary effects.

In human pathologic conditions such as acute crescentic glomerulonephritis with fibrin deposition and thrombotic microangiopathy, we detected PAI-1 in glomeruli by means of immunofluorescence and in situ hybridization (73,74). PAI-1 seems to be synthesized by cells inside the crescents and by mesangial cells (6). This might of course have implications for glomerular fibrin deposition. Of note, the 4G/4G PAI-1 genotype, which is associated with higher plasma PAI-1 concentrations and activity, is linked to higher nephritic activity and more extensive necrotizing lesions in patients with systemic lupus erythematosus (75). The influence of the PAI-1 genotype is somewhat controversial, however. More recently, the plasma activity of PAI-1 was reported to be predictive of the onset of a HUS in the early phase of enteric infection by Escherichia coli O157:H7 (76).

Role of α2-AP. Downstream of plasminogen activation, plasmin activity can be inhibited by α2-AP. By contrast to PAI-1, α2-AP is constantly expressed by the kidney, ensuring permanent and efficient control of plasmin activity in the renal cortex. However, at least in mouse kidney, α2-AP expression is restricted to proximal tubular epithelial cells (3). Thus, its main role might be in controlling the fibrinolytic and/or proteolytic activity of plasmin in the tubular compartment, rather than the control of glomerular fibrinolysis, a function that could be exerted by the circulating pool of α2-AP. This important point is controversial: So far, α2-AP deficiency has been found to confer protection against fibrin deposition in a model of extensive tubular injury induced by intraperitoneal endotoxin injection (77) but not in a model of glomerular injury (passive anti-GBM glomerulonephritis), despite a significant enhancement of “total kidney” (mostly tubular?) fibrinolytic activity, as shown by in situ zymography (78). In mice, the absence of PAI-1 does not enhance renal plasmin activity after either glomerular or interstitial injury (65,79), suggesting that increased plasmin generation, facilitated by the absence of PA inhibition, remains efficiently countered in the kidney by a local or systemic plasmin inhibitor (in the mouse, other inhibitors, e.g., α2-macroglobulin or TAFI, might play
such a role). Patients with glomerulopathies in whom plasmin–
\(\text{\(\alpha_2\)-AP} \) complexes are detected in the glomerular tuft exhibit
more endothelial damage and more crescents and have more
severe hematuria (80). Whether the presence of plasmin–
\(\text{\(\alpha_2\)-AP} \) complexes is a secondary phenomenon or accounts for
the greater disease severity is unknown.

**Conclusion**

Most of the data presented here come from animal models of
acute crescentic glomerulonephritis induced by injection of
heterologous antibodies directed against the autologous GBM.
This model is highly valuable for studying the different steps of
the inflammatory response and the key molecules involved in
fibrin deposition and cell proliferation. However, it is a highly
acute model, especially in its accelerated form, and more subtle
regulatory events thus may be missed. Human data are more
descriptive and have so far failed to unveil the mechanisms
underlying glomerular lesions.

It is highly probable that the inflammatory reaction, espe-
cially macrophage infiltration, is necessary for TF production,
initiating the coagulation cascade in the glomerulus. Which
cell type subsequently produces which coagulation factor re-
mains to be determined, but inflammatory cells and glomerular
resident cells clearly cooperate to produce components in-
volved in coagulation and fibrinolysis. This questions the rel-
evance of a cell-targeted approach: The goal is rather to iden-
tify target molecules for preventing fibrin formation and/or
persistance, and glomerular obsolescence.

A so-called prothrombotic state is observed in children with
HUS, before renal abnormalities develop: Evidence was re-
cently obtained of early thrombin generation and enhanced
PAI-1 activity in peripheral blood some days before the onset
of renal impairment (76). In addition, during human HUS and
crescentic glomerulonephritis, thrombin receptor and PAI-1
mRNA synthesis is locally upregulated (43,73,74). Together,
these findings indicate that procoagulant and antifibrinolytic
systems, whether systemic or local, are critically involved in
human renal disease.

The complexity of the system lies in the fact that most of
the proteins so far studied, from TF to PAI-1, are multifunctional
and have effects independent of the coagulation/fibrinolysis
pathways. This suggests that nonselective inhibition of these
proteins might have profound—and possibly deleterious—
consequences for cellular behavior, not only for fibrin deposi-
tion or clearance. These coagulation-independent effects target
inflammatory responses, cell proliferation and migration, ma-
trix remodeling, angiogenesis, and other phenomena. It is
reasonable to envisage that all of these effects participate in
wound repair and that the coagulation/fibrinolysis system is
closely involved in tissue scarring. Members of this system
probably act in a cascade, which seems to be much more
durable and economic than previously suspected.

The final step in the coagulation cascade, namely thrombin
activation leading to fibrin deposition, plays a pivotal role, and
major efforts are being made to blunt the action of thrombin.
Thrombin not only cleaves fibrinogen but also has multiple
cellular effects mediated by its receptors (PAR-1 is the most
abundant thrombin receptor in the kidney). Inhibition of throm-
bin activity (enzymatic or PAR-1-mediated actions) is a very
promising therapeutic approach. However, glomerular ne-
phropathies are often diagnosed late, meaning that treatment
can be only curative rather than preventive, by contrast to most
animal models. One noteworthy exception is *Escherichia coli-
related HUS*, in which renal endothelial damage follows
marked clinical intestinal manifestations. The generally late
diagnosis of fibrin-associated glomerular diseases, together
with the poor efficacy of classical anticoagulant therapies for
human glomerulonephritis (81), makes the fibrinolysis path-
way a more promising therapeutic target. Although plasmino-
gen activators are involved in clearing fibrin and in remodeling
the ECM, they make poor candidate drugs because of the
increased bleeding risk. Much hope is being placed in the main
physiologic inhibitor of plasminogen activators, namely PAI-1,
for which antagonists are currently being developed. Recent
reports that key molecules involved in fibrosis, such as aldo-
sterone and angiotensin II, also regulate the synthesis of PAI-1
by renal cells are generating much interest. Indeed, this re-
ciles the coagulation/fibrinolysis pathways with the mecha-
nisms of durable wound repair and also provides invaluable
clues to the mechanisms by which antagonists of the renin-
angiotensin system promote the regression of fibrosis, which is
the major goal in nephrology (82). However, current data do
not yet show that inhibition of PAI-1 will promote the clear-
ance of fibrin and limit ECM accumulation. On the contrary,
two independent studies show that total PAI-1 deficiency leads
to pathogenic overactivation of TGF-\(\beta\) (58,65).

In summary, glomerular injury triggers inflammation, which
in turn triggers coagulation. Fibrinolysis is then placed under
the control of molecules that carry on tissue repair by consol-
idating, in a profibrotic way, the lesion. Measured inhibition of
antifibrinolytic molecules might be the key to preventing or
reversing fibrosis.

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