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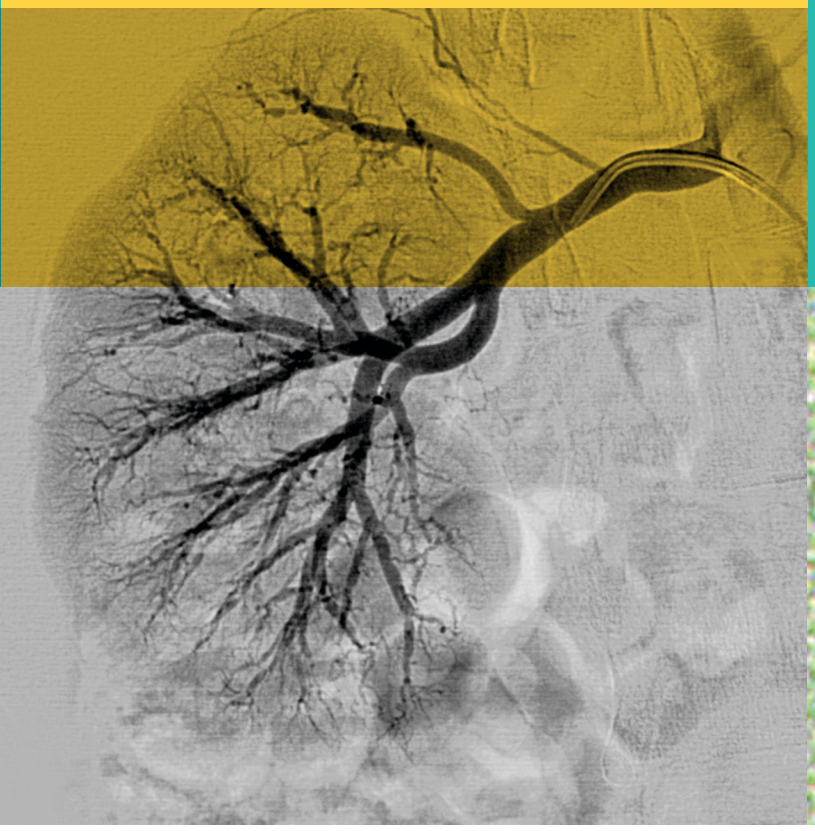

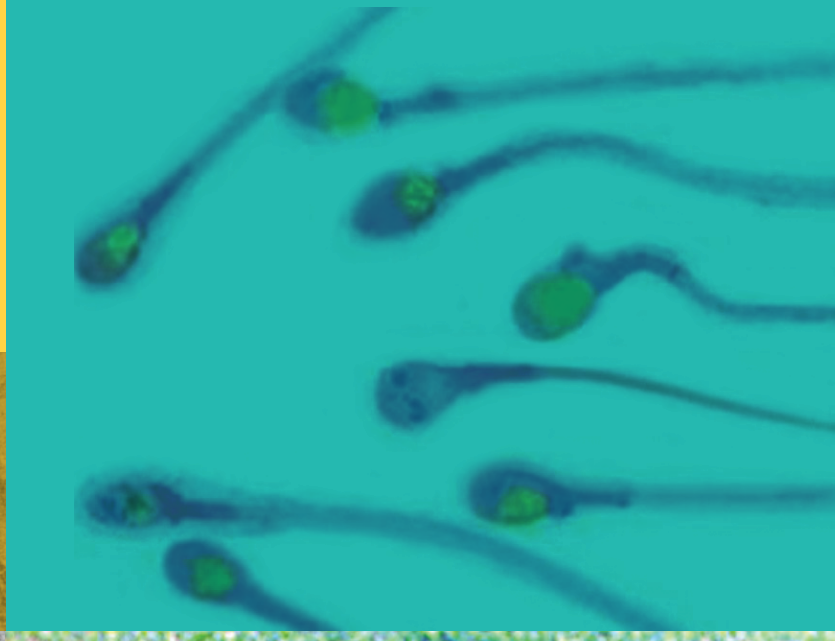

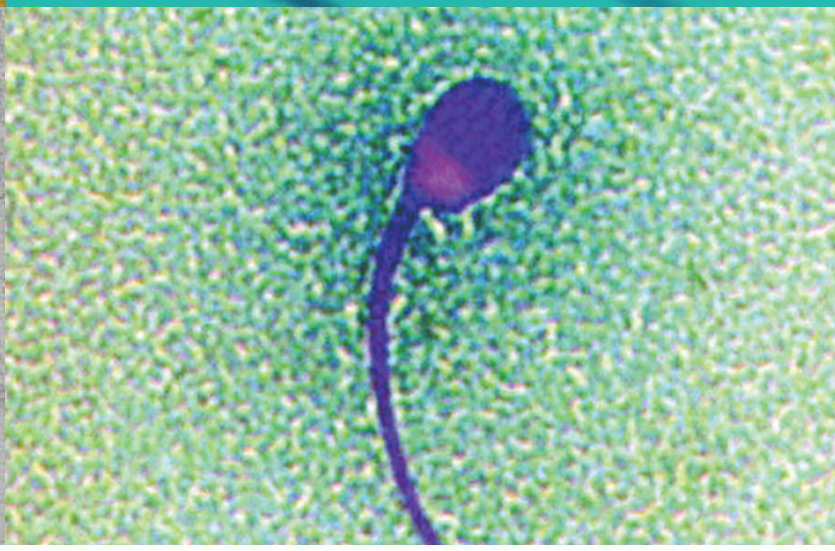

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Review Article

Proteinuria: A Cross Road Where the Complement and the Plasminogen-plasmin Systems Meet

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Abstract

Proteinuria is the hallmark of nephrotic syndrome and a surrogate of progression of renal disease and a risk factor of cardiovascular morbidity. Once proteinuria occurs secondary to glomerular damage, its reabsorption at the proximal tubule causes a constant interstitial inflammation that will eventually lead to a graduate loss of kidney function due to fibrosis, ischemia and tubular atrophy. The plasminogen-plasmin system plays a local critical role in amplifying podocyte damage, deepening the generation of edema, cross-linking inflammatory components at the interstitium and determining the terminal fibrotic processes. Plasmin activity also causes inflammation through the complement system. The interaction between the complement and the plasminogen-plasmin systems is critical in the progression of interstitial inflammation. Plasmin is capable of cleaving C3 and C5 components of the complement system. Moreover, C3a and C5a fractions are chemoattractants of neutrophils and monocytes. The complement system is also involved in microvascular thrombosis contributing to glomerular sclerosis and interstitial fibrosis through ischemic processes. A regulator of plasmin activity is plasminogen activator inhibitor-1, a leading molecule involved in fibrosis and sclerosis, particularly augmented in glomerulopathies. Unraveling the interactions between the plasminogen-plasmin and complement systems will undoubtedly lead to more specific therapies for glomerular diseases.

Key words: Complement system, plasmin, plasminogen, plasminogen activator inhibitor-1, proteinuria

INTRODUCTION

Proteinuria is a hallmark of nephrotic syndrome and a marker of progression of renal disease.[1-3] Moreover, proteinuria is an independent risk factor of cardiovascular disease, the main cause of morbidity and mortality in the renal population.[4-6] Considering the fact that chronic kidney disease is a major worldwide public health concern, and that the three main causes of end-stage kidney disease (i.e., diabetes, hypertension, and glomerulonephritis) are all accompanied by different ranges of proteinuria, unraveling the complex pathophysiology of proteinuria will certainly help to better manage this worrying expanding population.[7] Once proteinuria ensues due to glomerular damage, its reabsorption at the proximal tubule causes a permanent interstitial inflammation that will determine a gradual loss of renal function due to fibrosis, ischemia and atrophy.[8] At the glomerular level, podocyte or endothelial derangements such as genetic mutations, immune-complex depositions, local or systemic autoimmune processes or circulating factors will derive in pathologic situations.[9-11] The plasminogen-plasmin system, already systemically

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activated in proteinuric states, is also playing a critical role locally in amplifying the podocyte damage, in deepening the hemodynamic disturbances and the generation of edema of nephrotic subjects at the tubular level, in taking part in the cross-talk with inflammatory components that respond to protein trafficking mainly at the interstitium and in contributing to the fibrotic processes.[10] When urokinase plasminogen activator (u-PA) is bound to its receptor u-PA receptor (u-PAR) (ubiquitously distributed in the renal parenchyma), plasminogen is converted to plasmin.[10] Besides its well-known actions as a fibrinolytic factor, plasmin is involved in podocyte contraction due to the β_3 moiety of podocyte integrin in conjunction with u-PAR.[10,12] Moreover, after plasminogen activation by u-PA, plasmin stimulates epithelial sodium channels (ENaC) at the distal tubule, causing water and sodium absorption.[10] This is a main pathway by which edema is caused in kidney disease and especially in nephrotic syndrome, and an additional factor in the pathogenesis of hypertension.[13] The local increase in kidney plasmin activity and concentration in association with proteinuria reabsorption also causes matrix-associated growth factors activation enhancing cell migration and regulating inflammatory cells through the production of cytokines, oxidative stress and other mediators as the complement system.[14-16] The interaction between the complement and the plasminogen-plasmin system is critical in the progression of the interstitial inflammation. In this regard, it has been shown that plasmin is effectively capable of cleaving C3 and C5 components of the complement system.[17] Moreover, C3a and C5a fractions exhibit robust chemoattraction of human neutrophils and monocytes.[16,17] It has recently been shown that the local renal complement system compartment rather than the systemic compartment plays a central role in the inflammatory kidney response, in concordance with the local plasmin activity.[18,19]

A regulator of plasmin synthesis is plasminogen activator inhibitor-1 (PAI-1), a molecule involved in fibrotic processes with patent contribution to sclerosis and renal damage.[20] Noteworthy, components of the plasminogen-plasmin system, as plasminogen and plasmin, could be employed as routine, inexpensive practical urinary biomarkers to assess the subclinical activity of proteinuria related inflammatory processes.

The aim of present review is to outline the principal functions and regulations of the plasminogen system and the relationship plasmin and PAI-1 interplay with the complement system with respect to the development of inflammation, fibrosis and proteinuria.

THE PLASMINOGEN-PLASMIN SYSTEM

The plasminogen/plasmin system is formed by an inactive pro-enzyme called plasminogen which is converted to the active enzyme named plasmin[21,22] [Figure 1 and Table 1]. Two physiological plasminogen activators have been described: Tissue plasminogen activator (t-PA) and u-PA[23-26] [Table 1]. The plasminogen/plasmin system inhibition operates at three different levels: One is that where antiplasmin acts, inhibiting plasmin by forming an equimolecular complex; the second level is composed by PAIs, and finally, the third level where fibrinolysis is negatively modulated by thrombin activatable fibrinolysis inhibitor (TAFI), which is in turn activated by the thrombin/thrombomodulin complex.[27] In addition, cellular components modulate the plasminogen-plasmin system, with membrane receptors as the urokinase receptors, plasminogen receptors, or Annexin 2 [Table 1]. It is now well established the multiple roles of the plasminogen-plasmin system. The t-PA mediated pathway is involved in fibrin degradation while the u-PA mediated pathway participates mainly in tissue remodeling.[26,27]

COMPONENTS OF THE FIBRINOLYTIC SYSTEM

Plasminogen

Plasminogen is a proenzyme synthesized in the liver that circulates in plasma at a concentration of 1-5 μmol/L. The molecule is organized in seven structural domains comprising a preactivation peptide, five homologous triple loop structures called Kringles and the proteinase domain. Kringle 1 and Kringle 4 show high and low affinity Lys binding domains of plasminogen respectively. These domains mediate specific interactions with fibrin, cell surface receptors and others proteins such as antiplasmin.[24] Plasminogen is activated by cleavage of a single Arg-Val peptide bond to plasmin by the two main physiological plasminogen activators, t-PA and u-PA. It is noteworthy that t-PA activates plasminogen only in the presence of either soluble or insoluble fibrin isoforms[28] [Figure 2]. Plasminogen and t-PA bind to the lysine residues on the fibrin surface and t-PA, the converts plasminogen to plasmin. Fibrynolysis is amplified by exposition of C-terminal lysine residues generated by plasmin itself.[28,29]

In order to maintain the system in equilibrium, the hemostatic system contains a limb that counterbalances the pro-thrombotic drive: The fibrinolysis system, in charge of removing of the fibrin deposits in thrombi.[30] The main function of plasmin is to cleave insoluble fibrin polymers at specific sites.[16,31] Besides, plasmin has other multiple physiological roles as

Table 1: Main genetic and physiologic characteristics of the plasminogen-plasmin system components

Protein	Size (kDa)	Number of aminoacids	Synthesized	Plasma concentration (mg/L)	Catalytic triad o reactive site	Gene name	Gene location/ length (kb)	Function
PLG	92	791	Liver	100-200		PLG	Chrom 6/52.5	Zymogen (plasmin precursor)
Plasmin	88	715		—	HisS602, Asp645, Ser740			Proteolysis of fibrin, extracellular matrix, and others proteins
t-PA	59	527	Endothelial cells	0.0005-0.01	His322, Asp371, Ser478	PLAT	Chrom 8/32.4	Main PLG activator on fibrin surface
u-PA	46	411	Endotelial cells Macrophages Renal epitelial cells Some tumor cells	0.000-0.01	His204, Asp371, Ser478	PLAU	Chrom 11/6.4	Main PLG activator on cellular surface
APL	50	452	Liver	70	Arg 364, Met365	SERPINF2	Chrom 17/16	Main plasmin inhibitor
PAI-1	47	379	Endothelial cells, vascular smooth muscle, platelet, adipoce tissue	0.024 (variable)	Arg346, Met347	SERPINE1	Chrom 7/12	t-PA and u-PA inhibitor
PAI-2	46	413	Human placent	<0.01		SERPINE2	16	t-PA and u-PA inhibitor in pregnancy
TAFI	56	423	Liver megakaryocytes	4-15		CPB2	Chrom 13/48	Inhibition of fibrinolysis through removal of lysine residues from fibrin

PLG: Plasminogen, t-PA: Tissue type plasminogen activator, u-PA: Urokinase-type plasminogen activator, PAI-1: Plasminogen activator inhibitor-1, PAI-2: Plasminogen activator inhibitor-2, TAFI: Thrombin activatable fibrinlolysis inhibitor, APL: α2-antiplasmin

has been shown in different experiments with plasminogen deficient mice. These plasminogen -/- knock-out mice suffered from spontaneous thrombosis, fibrin deposits in lungs, liver and stomach, impaired monocytes recruitment, impaired neointima formation after electrical injury and premature death.[32] Thus, apart from their direct role in activating downstream components of the enzymatic cascade, the coagulation and fibrinolytic systems can be viewed as intermediaries that convert mechanical information (fibrin deposits, blood clots) from a damaged tissue or leaky vessel into biochemical signals that trigger cell responses. In this regard, novel proteinase-triggered inflammatory mechanisms have been discovered for plasmin: In addition to regulating u-PAR activity,[33] plasmin can cleave the annexin A2 heterotetramer, triggering chemotactic responses by monocytes and macrophages.[14,15]

PLASMINOGEN ACTIVATORS

Tissue plasminogen activator

t-PA is a multi-domain serine protease containing five structural domains: An epidermal growth factor-like cassette, fibronectin-like finger, two Kringle structures similar to plasminogen Kringles and a serine protease domain.[24,25] The t-PA molecule itself has a circulating half-life of approximately 5 min and is synthesized and secreted by endothelial cells in different ways according to the location of the endothelial cell itself. There exist a vast number of stimuli that induce the release of t-PA from endothelial cell such as thrombin, histamine, bradykinin, adrenaline, acethylcoline, vasopressin, physical exercise, venous occlusion and shear stress. t-PA is the main intravascular activator of plasminogen to dissolve the fibrin clot and to maintain vascular hemostasis[30] [Figure 2].

Urokinase–type plasminogen activator

Human u-PA is synthesized mainly in the lung and the kidney (renal epithelial cells) but also in endothelial cells, keratinocytes and by some tumor cell lines. It consists of a single chain multi-domain glycoprotein that contains an epidermal growth factor-like domain, a single plasminogen like Kringle and a serine protease catalytic triad.[24] Plasmin, kallikrein and coagulation FXII are capable of cleaving single u-PA chains generating two high molecular weight u-PA and also low molecular weight u-PA, the major form of which is present in the urine. Both forms of u-PA can activate plasminogen but only the high molecular weight form binds to u-PAR maintaining u-PA activity. u-PA is an effective plasminogen activator both with or without the presence of fibrin. Mainly, this activator is primarily involved in cell migration and tissue remodeling.[21,24,34,35] Finally, the main physiological inhibitors of u-PA are PAI-1 and PAI-2.[30,34,35]

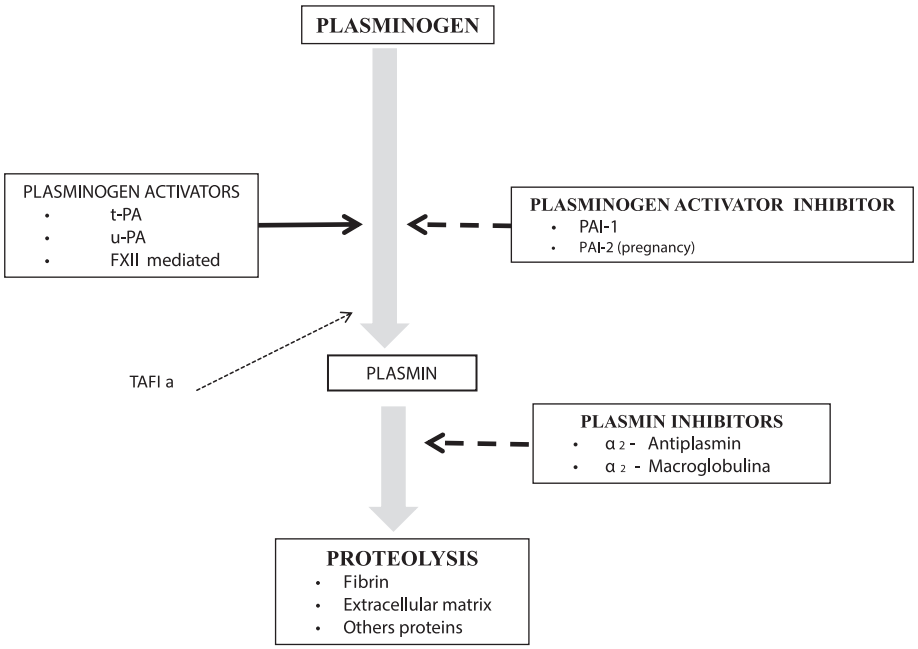

Figure 1: Traditional view of Plasminogen system. t-PA: Tissue-type plasminogen activator, u-PA: Urokinase-type plasminogen activator, PAI-1: Plasminogen Activator Inhibitor-1, TAFI: Activated thrombin activatable firbinolysis inhibitor

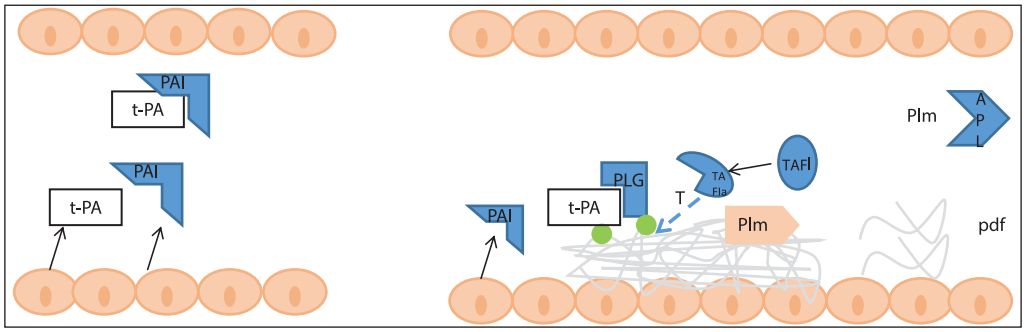

Figure 2: Intravascular Fibrinolysis: A In fibrin absence, tissue plasminogen activator release by endothelial cell is inactivated by plasminogen activator inhibitor. Tissue plasminogen activator/plasminogen activator inhibitor complexes are removed from the circulation via hepatic clearance. Only small amounts of plasmin is generated B-When fibrin is present, tissue plasminogen activator and plasminogen are bound to fibrin via lysine residues. Thus, plasminogen is quickly converted to plasmin which digests fibrin and exposes additional lysine residues on partially degrade fibrin. Thrombin activatable fibrinolysis inhibitor, activated by thrombin, removes Lysine binding site type attenuates fibrinolysis. When the ends fibrinolysis free plasmin is inactivated by antiplasmin

INHIBITORS AND MODULATORS OF THE PASMINOGEN/PLASMIN SYSTEM

Plasmin inhibitors

Plasmin is inhibited by a family of serine protease inhibitors called serpins or suicide inhibitors. The plasmin inhibitors are alpha 2 antiplasmin, alpha 2 macroglobulin and alpha protease inhibitor. Antiplasmin, is a serpin molecule synthesized in hepatocytes, circulates in plasma at high concentrations and is present in alpha-granules of platelets. Anti-plasmin is the main physiological inhibitor of plasmin but it also inhibits chymotrypsine and trypsin.[21-25]

Plasminogen activators inhibitors

PAI-1 is the main physiological inhibitor of t-PA and u-PA but it also inhibits thrombin, plasmin and activated protein C.[35] PAI-1 is a negative regulator of plasmin driven proteolysis, not only in its fibrinolytic role but also in other biological processes in which plasmin is involved. PAI-1 is mainly secreted by endothelial cells, and its expression is regulated by several cytokines, hormones, endotoxins, and growth factors. Agents that haven shown to enhance PAI-1 expression and/or secretion include inflammatory cytokines, lipopolysaccharides, tumor necrosis alpha, basic fibroblast growth factor, low-density lipoprotein, and angiotensin II. PAI-1 exists in two different pools: In plasma and in platelets. The plasmatic concentration of PAI-1 active form is low while the one in platelets is higher but 90% of it corresponds to the inactive form. Finally, there exists an inactive form of PAI-1 which is bound to vitronectin in the extracellular matrix.[34,35]

In humans, numerous clinical and epidemiologic studies have demonstrated that PAI-1 plays a central role in many pathophysiologic processes such as obesity, apoptosis, cell adhesion, cell migration, inflammation, and fibrosis. PAI-1 is the main responsible for the fibrinolytic shutdown during sepsis.[36] Only trace amounts of PAI-1 are produced by healthy kidneys, whereas it is synthesized in higher levels in acutely or chronically injured kidneys.[20,37] Finally, in normal individuals higher PAI-1 levels are associated with a polymorphic variance in the number of guanine bases (4G rather than 5G) at position - 675 upstream of the transcription start box. Some studies have found that the homozygosity for the 4G allele could be an independent risk factor for the development of atherosclerosis, thrombosis and cardiovascular disease, highly frequent in chronic kidney disease patients while other studies did not confirm these results.[38-40]

Thrombin Activatable Fibrinolysis Inhibitor

TAFI is an unstable carboxypeptidase formed by the action of thrombin on its procarboxypeptidase proenzyme. The TAFI proenzyme is synthesized in the liver and is present in platelets.[25,27] The activation of TAFI accomplished by thrombin is drastically accelerated by thrombomodulin and has a half-life of 8-15 min. TAFI is a potent attenuator of fibrinolysis, and its anti-fibrinolytic effect is due its capacity to remove C-terminal lysine residues from the surface of the fibrin clot. These lysine residues are necessary for the binding of plasminogen and t-PA to the fibrin net. Other substrates for TAFI include bradykinin, complement factors C3a y C5a, and cellular plasminogen receptors.[9,41]

PLASMINOGEN CELLULAR RECEPTORS

Two types of cell surface receptors exist for plasminogen: Activation receptors, which localize on cellular membranes and potentiate plasminogen activation, and clearance receptors that eliminate plasmin and plasminogen activators from the circulation. Plasminogen receptors are a group of proteins expressed on different cell types, and as a group it comprises anexin 2, enolase and the glycoprotein IIb/IIIa complex among others.[42,43] Anexin 2 is produced by endothelial cells, monocytes, macrophages, dendritic cells, epithelial cells and tumor cells. Anexin 2 accelerates t-PA mediated plasminogen activation by 60-fold, and presents binding activity for both plasminogen and t-PA but not for u-PA.[44] Anexin 2 promotes fibrinolysis, angiogenesis and cell migration, and mice with total deficiency of anexin 2 have shown impaired clearance of arterial thrombin, fibrin deposition in microvasculature and angiogenic defects. Although several investigations have showed a multifaceted role for anexin 2 in human health and disease, there are still many questions about the regulation of this system.[44]

UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR

The u-PAR is a cell surface receptor involved in a large number of physiological and pathologic processes such as extracellular proteolysis, cell migration, adhesion, signaling and proliferation.[45,46] u-PAR is a glycosylphosphatidylinositol (GPI) molecule anchored to the cell membrane and has proteolytic and nonproteolytic functions[10] [Figure 3]. u-PAR has three domains (DI, DII, DIII) and a tail that binds the receptor to the cell membrane; the loss of some of these domains originates different soluble and anchored u-PA forms with different molecular weights and functions. Soluble u-PAR (su-PAR), the most common soluble form, only contains DII and DIII domains and is present in plasma, urine and cerebrospinal fluid. This receptor is present in endothelial cells, keratinocytes, fibroblasts, megakariocytes, some tumor cell lines, podocytes, renal tubular cells and also in monocytes, macrophages and activated T cells.[45,46]

The main proteolytic function is to hasten plasminogen activation by u-PA. u-PAR modulates pericellular proteolysis by regulating the activity of the plasminogen/plasmin system.[45] The generated plasmin would thereafter cleave several components of the extracellular matrix. The nonproteolytic function of u-PAR is in direct relationship to vitronectin, a multi-functional component of the extracellular matrix. u-PAR is an adhesion receptor for vitronectin, a molecule basically involved in cellular adhesion. Besides vitronectin binding to u-PA, it presents affinity for PAI-1, suggesting that vitronectin may play a key role in the enhanced activation of plasminogen by u-PA and u-PAR. Moreover, u-PAR regulates intracellular signaling pathways involved in cell migration and extracellular proteolysis.[10,46] The signaling and the adhesive function of this receptor are closely interconnected and reciprocally regulated.

HYPOFIBRINOLYSIS AND THROMBOSIS

A hypofibrinolytic state is generated when plasminogen deficiency and/or elevated PAI levels exist.[23,25] Due to decreased synthesis or increased consumption, acquired plasminogen deficiency is present in liver disease, in sepsis, in Argentinian hemorrhagic fever and in diseminated intravascular coagulopathy. PAI-1 expression and secretion by endothelial

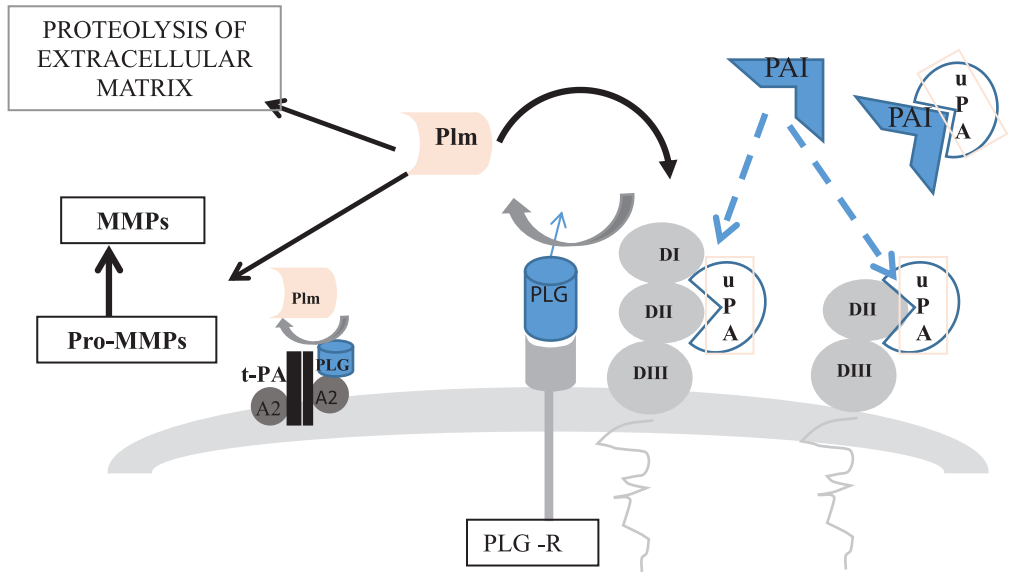

Figure 3: Plasminogen system on cell surface. u-PA: Urokinase-plasminogen activator, u-PAR: u-PA receptor, PLG: Plasminogen, Plm: Plasmin, PAI: Plasminogen activator inhibitor, MMPs: matrix metalloproteinase, Pro-MMPs: p rometaloproteinase, A2: Anexin 2

cells is strongly induced by a number of pro-inflammatory cytokines.[34,35] An excessive amount of PAI-1 levels is the most commonly abnormality found in the fibrinolytic system in inflammatory states. Several studies in humans have shown that high PAI-1 levels are generally associated with increased risk for thrombosis, atherosclerosis, or cardiovascular disease. In addition, PAI-1 levels play a critical role in fibrosis.[20,38-40]

THE NONFIBRINOLYTIC ACTIONS OF PLASMINOGEN SYSTEM

When components of the plasminogen/plasmin system such as u-PA, u-PAR, plasminogen and plasminogen receptors assemble on the cell surface, they modulate cell migration and tissue remodeling by interactions with the extracellular matrix and by signal transduction. Plasmin generated in cellular surfaces converts pro-metalloproteinases to metalloproteinases triggering extracellular matrix degradation. *In vitro*, plasmin can degrade thrombospondin, laminin, fibronectin and fibrinogen. Impaired wound healing is observed in knockout plasminogen -/- mice. Finally, plasmin promotes cell proliferation by activating latent growth factors and in the blood vessel it exerts proliferative responses to injury by converting latent transfroming growth factor-beta (TGF-ß) to the active form.[16,33]

THE PASMINOGEN/PLASMIN SYSTEM AND THE COMPLEMENT SYSTEM

The complement system acts as a key sentinel of innate immunity and the coagulation system as a main factor in hemostasis, demonstrating that they both belong to the first line of defense against injurious stimuli and invaders.[47] There is increasing evidence that the rapid activation of the coagulation cascade is accompanied by a very early onset of an uncontrolled, progressive inflammatory response.[48] Obviously, acute blood loss and tissue trauma activate the complement cascade in humans.[49] Especially generation of the powerful anaphylatoxin C3a and C5a, and consumption of complement may play a detrimental role.[50] Generally, the complement enzymes contain a single serine protease with an extremely restricted substrate specificity. The classical pathway proceeds through the sequential cleavage of C4 and C2 by active C1s and formation of the C3 convertase (C4b2a-complex). Additional binding of C3b leads to generation of the C5 convertase (C4b3b2a-complex). Similarly, the active center of the C3- and C5-convertase of the alternative pathway (C3bBb- and (C3b)2Bb(P)-complex, respectively) resides in the serine protease domain of factor B. Activation of the lectin pathway results in subsequent activation of mannose associated serine proteases (MASP), which in turn activate C4 and C2 to assemble C4b2a. Activated MASP-1 also reveals serine protease specificity for a direct C3 cleavage.[51] Plasmin as the strongest serine protease of the fibrinolytic system is capable of cleaving both C5 and C3, respectively.[52]

Plasmin effectively cleaves C3 and C5 to C3a and C5a fibrinolysis proteases may act as natural C3 and C5 convertases, generating biologically active anaphylatoxins.[52] Traditionally, the complement and coagulation systems have been described as separate cascades. However, both proteolytic cascades are composed of serine proteases with common structural characteristics, such as highly conserved catalytic

sites of serine, histidine, and aspartate.[53,54] Clark *et al*. suggested that thrombin and plasmin may contribute to nontraditional complement activation.[55] In the setting of systemic inflammation as glomerulopathies and proteinuria, activation of the coagulation cascade is accompanied by a profound activation of the complement system, resulting in the generation of the anaphylatoxins C3a and C5a.[56] C5a induces tissue factor (TF) activity in human endothelial cells and may therefore be involved in the activation of the extrinsic coagulation pathway.[57] Furthermore, C5a has been shown to stimulate the expression of TF on neutrophils via the C5aR, which was associated with a higher procoagulant activity.[58] C5a has been described as having fibrinolytic effects by downregulating the PAI-1 expression in humans.[59] Recently, a C5a-induced "switch" in mast cells from a pro-fibrinolytic (t-PA release) to a pro-thrombotic phenotype mediated by an increase in PAI-1 release has been reported.[52,60] When plasmin is only moderately triggered, activation of the complement cascade may be mainly achieved through the cleavage of C3, whereas, when coagulation/fibrinolysis is massively activated, C5 may represent the primary target for cleavage.[17] As mentioned above, coagulation and complement are two distinct systems with unique pathophysiological roles. Nevertheless, these networks have several common functional attributes, which are often overlooked. (a) Both systems serve as innate defenses against external threats (microbial invasion). (b) The presence of foreign or altered cellular surfaces is required for initiation of both pathways. This requirement ensures tight orchestration of a rapid but controlled initiation of the cascade in terms of its spatiotemporal localization.[16] Early observations have pointed to the existence of significantly higher levels of complement activation products in human serum than in anti-coagulated blood, strongly suggesting the development of complement activation during blood clotting.[61] Plasmin can, therefore, serve as another common bridge between innate immunity and hemostasis. Farther downstream in the coagulation cascade, platelet activation and subsequent expression of P-selectin have been associated with complement activation, supporting a novel mechanism for local inflammation at the site of vascular injury.[62] In addition to platelets and endothelial cells, mast cells play an important role in the regulation of coagulation. Mast cells are often present at sites of inflammation and can prevent thrombosis through the expression of t-PA, which generates the fibrinolytic proteinase plasmin.[63] Treating mast cells and basophils *in vitro* with C5a causes an upregulation in PAI-1, a vital regulatory component of the fibrinolysis cascade that is able to neutralize the enzymatic activity of t-PA.[59] By inducing the expression of PAI-1, C5a can abolish the fibrinolytic activity of mast cells, in favor of a procoagulant phenotype.[60] TAFI, (also known as carboxypeptidase R or plasma carboxypeptidase B), generated by a thrombomodulin–thrombin complex, can play a dual role in the inhibition of plasmin-mediated fibrinolysis and the inactivation of C3a and C5a.[64,65]

PLASMINOGEN-COMPLEMENT AND PROTEINURIA

Proteinuria can be viewed as the result of primary or secondary podocyte damage. Filtered proteins-mainly albumin-are reabsorbed at the proximal tubule, and the kidney interstitial trafficking causes an inflammatory milieu in which complement plays a major role. Components of the complement system are locally synthesized are actively participate as chemo-attractants for neutrophils and lymphocytes, contributing to scarring and fibrosis, both at the glomerular and interstitial compartments. This phenomenoncontributes to the amplification of proteinuric pathways.[7,10,11] Moreover, plasmin levels which are elevated at the glomerular and luminal compartments of the nephron, causes proteinuria by stimulating podocyte contraction and indirectly by an increase of C3a and C5a components of complement, which act as inflammatory triggers of neutrophils and lymphocytes.[10,16-19]

Recently, an interesting relationship between a nephritogenic antigen and poststreptococcal glomerulonephrtis has been discovered. Namely, the isolation of a nephritogenic isotope of group A stretoccocus appears to be identical to a plasmin (ogen) receptor, termed antigen nephritis-associated plasmin receptor (NAPlr) by the authors.[66] *In vitro* experimental data indicate that the pathogenic role of NAPlr occurs through its ability to bind to plasmin and maintain its proteolytic activity.[67] Renal biopsies revealed the presence of the antigen NAPlr in the glomeruli in all patients with early-phase acute poststretococcal glomerulonephrtis. These data may suggest that the plasmin receptor plays a direct, nonimmunologic function as a plasmin receptor and may contribute to the pathogenesis of acute poststretococcal glomerulonephrtis by maintaining plasmin activity.[66] The plasmin receptor and plasmin activity could also be observed in a similar fashion in other glomerular diseases, such as dense deposits disease, IgA nephropathy, and immunecomplex-mediated membranoproliferative glomerulopnephrtis.[66,67]

In addition, plasmin stimulates ENaC at the distal tubule, causing water and sodium absorption.[10] This is a main pathway by which edema is caused in kidney disease and especially in nephrotic syndrome, and an adding factor for the generation of hypertension.[13] The increase in local kidney plasmin activity and concentration in association with proteinuria reabsorption also causes matrix-associated growth factors activation enhancing cell migration and regulating inflammatory cells through the production of cytokines. In

its nonproteolytic function, u-PAR also can initiate signal transductions that could promote tumor proliferation and angiogenesis.

THE ROLE OF UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR ON THE PODOCYTES AND THE SIGNALING PATHWAYS

The u-PA and t-PA are very similar serine proteases with the same physiological action, the activation of plasminogen. One of the receptors that converts plasminogen to plasmin, u-PAR, is an extensively N-glycosylated membrane receptor tethered to the cellular plasma membrane. u-PAR orchestrates a wide variety of cellular processes, including extracellular proteolysis, cell migration, adhesion, signaling, and proliferation, both under physiologic and pathologic conditions. On the plasma membrane, u-PAR acts as the high-affinity binding site for u-PA, promoting plasmin generation at the cell surface. The activation of proteolytic cascades after u-PA–u-PAR interaction is widely believed to be responsible for the biologic activity of u-PAR. This interaction is blocked by amiloride, but t-PA action is not modified by amiloride.[68] As reviewed by Ferraris and Sidenius, additional studies have documented the existence of a variety of biologic activities induced solely by overexpression of u-PAR or by the binding catalytically inactive u-PA derivatives. These effects, referred to as the "nonproteolytic" functions of u-PAR, rely on direct and functional interaction with other proteins on the plasma membrane or in the pericellular environment. The absence of a cytoplasmic tail makes u-PAR unable to directly contact signaling molecules in the cytoplasm, and the signaling activity of u-PAR has therefore been ascribed to interactions with a growing number of signaling receptors. It has indeed been shown that u-PAR regulates signaling downstream of tyrosine kinase receptors, integrins, and G protein-coupled receptors. In addition, nonproteolytic u-PAR functions include the direct effect played by u-PAR on cell adhesion through its specific interaction with the extracellular matrix protein vitronectin. The importance of the u-PAR-vitronectin interaction in the modulation of cell adhesion is becoming more and more compelling because it seems to be intimately connected to most of u-PAR's nonproteolytic functions. u-PAR modulates pericellular proteolysis by regulating the activity of the plasminogen system.[26] Urokinase receptors, located on cell surface of certain cells, are bound to the cell by a GPI molecule, which can be split by immunological factors. Once detached from the cell, u-PAR is converted into a soluble circulating molecule, su-PAR.[69] In a recent publication, Wei *et al*. provide ample evidence that elevated su-PAR levels may play a role as a circulating factor with permeability properties on the glomerular membrane, leading to podocyte contraction and proteinuria. Once described in primary focal and segmental glomerulosclerosis, su-PAR has thereafter been shown to be nonspecific to this entity, having been encountered in other glomerulopathies with or without nephrotic syndrome, and even in subjects without kidney disease.[70-72] Interestingly, it has been recently shown that both su-PAR and interleukin-2 levels are elevated in patients with primary focal and segmental glomerulosclerosis and are associated with the response to treatment.

Moreover, Zhang *et al*. showed that the calcineurin–nuclear factor of activated T cells (NFAT) axis stimulates u-PAR expression,[73] while Ranjan *et al*. demonstrated that NFAT also binds to interleukin-2 gene promoter and increases interleukin-2 synthesis and secretion.[74] Thus, the mechanism of interleukin-2 and su-PAR elevation and their interaction in primary focal and segmental glomerulosclerosis both need further investigations, but may represent another component of the inflammatory milieu in which the nephrotic syndrome (NS) expresses its constituents and complications. As a circulating permeability factor, su-PAR is regulated by several cytokines and chemokines.[69] In the pathogenesis of primary focal and segmental glomerulosclerosis, Huang *et al*. found that elevated urinary su-PAR could activate β3 integrin on cultured human podocytes *in vitro*, suggesting that su-PAR may have a direct role in mediating podocyte injury and disease development.[75] Finally, Alfano *et al*. have recently demonstrated that su-PAR down-regulates nephrin expression nephrin expression in podocytes.[76]

Podocyte u-PAR expression can be reduced using amiloride. Amiloride plays a significant role in reducing podocyte cell motility *in vitro* and proteinuria in mice.[77] Amiloride inhibits the synthesis of u-PAR and u-PAR mRNA and consequently the αvβ3 integrin activation mediated by u-PAR on αvβ3 integrin. Amiloride capacity to inhibit u-PAR synthesis by T lymphocytes should be of particular interest in different causes of nephrotic syndrome, because blocking their activation would inhibit αvβ3 integrin activation and the development of proteinuria with final renal dysfunction.[69,77]

Through these mechanisms plasminogen system components would participate in the pathogenesis of a wide range of diseases. The involvement of all three complement limbs in the pathophysiology of glomerulonephritis is well-known.[11,16,17] Complement itself or immunocomplexes can damage the glomerular basement membrane at the subendothelial, intramembranous or subepithelial levels, at the mesangium or in the interstitium.[11,18] Moreover, when proteinuria occurs, the tubular reabsorption of proteins triggers a local immune response characterized by the interstitial infiltration mainly of

mononuclear cells, the secretion of cytokines and chemokines and the release of complement and fibrinolytic components.[8] Zhang *et al*. demonstrated in mice models that plasminogen and consequently plasmin deficiency significantly attenuated tubular epithelial to mesenchymal transition and renal fibrosis. This results appeared to be related to TGF-β.[78] PAI-1, t-PA and plasmin have been shown to participate in the development of renal interstitial fibrogenesis. While PAI-1 is secreted by inflmmatory cells and already transformed tubular epitethial to myofibrobalsts, t-PA and plasmin intervene in the conversion of fibroblasts to myofiboblasts, which eventually lead to the final consequences of the fibrotic process: Ischemia and organ failure.[79] In accordance with this findings, PAI-1 has been shown not only to be related to fibrotic processes in kidney diseases as mentioned above, but it has been implicated in the progression of crescentic glomerulonephritis.[80-83] All these events caused by proteinuria lead to chronic inflammation and hypoxia, being fibrosis the main final result.

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Conflicts of interest
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