The Role of Platelet-Activating Factor in Mesangial Pathophysiology

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Platelet-activating factor (PAF) is a powerful proinflammatory mediator that displays an exceedingly diverse spectrum of biological effects. Importantly, PAF is shown to participate in a broad range of pathologic conditions. This review focuses on the role that PAF plays specifically in the pathophysiology of the kidney, the organ that is both a source and a target of PAF. Renal mesangial cells are responsible for glomerular PAF generation and, ultimately, are the victims of its excessive production. Mesangial pathology is widely acknowledged to reflect glomerular damage, which culminates in glomerulosclerosis and proteinuria. Therefore, modulation of mesangial cell responses would offer a pathophysiology-based therapeutic approach to prevent glomerular injury. However, the currently available therapeutic modalities do not allow for targeted intervention into these processes. A more profound understanding of the mechanisms that govern PAF metabolism and signaling in mesangial cells is important, because it could facilitate the quest for improved therapies for renal patients on the basis of PAF as a drug target.

A substantial body of literature is available on the role of platelet-activating factor (PAF) in renal pathophysiology. After the discovery of PAF by Jacques Benveniste,1 a publication burst in the field of PAF in kidney research was observed. We summarize previous findings and point out conflicting reports and information gaps to revive the research interest with this review. Taking into account the recently acknowledged central role of mesangial cells in many forms of glomerular injury and the identification of a gene that, when overexpressed in mesangial cells, leads to an increase in PAF and mesangial matrix expansion, we set the boundaries of this review to focus on the role of PAF in mesangial pathophysiology.

Platelet-Activating Factor

PAF (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a mediator of inflammation. By its chemical nature, PAF belongs to the ether phospholipids.2 Strictly speaking, PAF is not a single entity but rather a generic collective term for a heterogeneous class of molecular species with different saturated or mono-/di-unsaturated alkyl, acyl, or alkenyl chains attached through ether linkage at the sn-1 position of the glycerol backbone3,4 (Figure 1). The structural diversity translates into differences in biological potency, with the predominant and most biologically active species of PAF containing C16:0, C18:0, or C18:1 alkyl groups.3,5

PAF is a proinflammatory autacoid (a local hormone with paracrine effects) with pleiotropic effects. As a matter of fact, the term platelet-activating factor is a misnomer, because the effect of PAF on physiologic processes is not limited to and goes far beyond degranulation of platelets, the first effect that was documented.1,6,7 Diverse biological activities are ascribed to PAF, and it was found to be involved in the pathogenesis of a wide range of diseases.8–10

In addition to the effects of the structural variation, the broad spectrum of PAF effects is achieved through a broad range of downstream mediators (Figure 2), via which PAF can elicit many of the reactions of inflammation and allergy, including enhanced leukocyte adhesion, chemotaxis, leukocyte degranulation, respiratory burst, and increased vascular permeability.11–13

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A number of cell types, such as endothelial, inflammatory, and renal mesangial cells, are shown to produce PAF, which can be synthesized via two distinct enzymatic routes, namely the remodeling pathway and the de novo pathway.\(^2,14,15\) The remodeling pathway involves a structural modification of 1-O-ether–linked membrane phospholipids in which the action of cytoplasmic phospholipase A2 yields a biologically inactive lyso-PAF, which is then acetylated and leads to the formation of PAF.\(^16,17\) (Figure 3A). In the de novo pathway, PAF synthesis occurs from simpler molecules, such as dihydroxyacetonephosphate, in several steps\(^18-20\) (Figure 3B).

PAF is generally regarded as a highly metabolically unstable compound, because it is rapidly converted to biologically inactive lyso-PAF by cytosolic and plasma PAF-acetylhydrolases.\(^21-23\) This is illustrated by the fact that exogenously added PAF at a concentration as low as \(10^{-9}\) mol/L has a half-life of only 5 minutes in the plasma of normal subjects.\(^24\) Similar results were obtained in animal experiments. However, it is debatable whether the rapid disappearance from circulation is indicative of its catabolism or redistribution to peripheral tissues. Furthermore, additional PAF degradation mechanisms exist that involve phospholipases C, D, and A1.\(^4\)

**The Kidney as the Source of PAF**

Although numerous cell types are able to generate PAF, the kidney is an important, if not main, source of PAF production in the body because PAF is virtually undetectable in the blood of anephric patients and experimental animals that have undergone bilateral nephrectomy.\(^25\) This is further supported by the direct experimental evidence of PAF release by the isolated perfused kidney.\(^26\)

All of the elements required for PAF formation are present in the kidney. Considerable activity of PAF biosynthesis key enzymes, such as acetyl-CoA:lyso-PAF acetyltransferase\(^5,27\) of the remodeling pathway and cholinephosphotransferase\(^20\) of the de novo pathway, is found in renal tissue. Production of PAF in the kidney can potentially be attributed to infiltrating inflammatory cells and resident renal cells. Validity of the latter is underscored because increased PAF production is detected in human idiopathic nephrotic syndrome and experimental puromycin aminonucleoside nephrosis,\(^28,29\) forms of renal damage without glomerular leukocyte infiltration. Among the intrinsic renal cell types, glomerular mesangial and medullary interstitial cells can generate PAF.

PAF is detected in the urine, and its levels are found to be elevated in renal patients and experimental nephrosis models compared with healthy subjects.\(^28,30-33\) This fact has two important implications. First, it supports the notion of the predominantly renal origin of PAF. The strong correlation between kidney and urinary concentration values\(^28\) and the poor recovery of systemically circulating PAF in the urine, even in the case of increased glomerular permeability,\(^31\) confirms its renal origin. Second, it reveals a biomarker capacity of PAF, because its presence in the urine at measurable concentrations that escalate in the renal disease constitutes a potential for a noninvasive diagnostic and/or prognostic test.

**The Kidney as the Target for PAF**

PAF exerts its effects on target cells via a specific G protein–coupled receptor, the PAF receptor (PAFR).\(^34\) In the kidney, PAFR mRNA is ubiquitously expressed. There is a gradient of its expression levels being the richest in the renal
cortex, with a lesser amount in the outer medulla, followed by the inner medulla. Within the nephron, the glomerulus demonstrates the highest PAFR expression, followed by the proximal tubule, with the other tubular segments displaying lower levels. In a unilateral ureter obstruction model, PAFR expression increases almost 70-fold, and the lack of PAFR (by gene knockout) in this model reduces profibrotic signaling, collagen deposition, and albuminuria. To date, PAFR is the only receptor known to bind species of the PAF class of molecules.

Exogenous PAF infusion affects renal hemodynamics and glomerular permeability, resulting in changes in filtration rate and proteinuria. Although the systemic hypotensive effect of PAF is uniform across different experimental species, the renal vascular response may be species specific. In the rat, systemically injected PAF causes renal vasodilation, leading to a dose-dependent fall in glomerular filtration rate (GFR) and renal blood flow. Infusion of PAF directly into the renal artery reproduces these effects independently of systemic hemodynamics; thus, this effect is likely to be direct and not secondary due to PAF-mediated systemic blood pressure decrease. Similar results were observed in the isolated perfused rat kidney model. However, in the dog, intrarenal PAF administration elicits renal vasoconstriction and has no effect on the vasculature of the isolated perfused cat and rabbit kidneys. In addition, in the isolated perfused rat kidney, infusion of PAF results in an approximately sixfold increase in proteinuria over basal values. Consistently, in the experimental puromycin aminonucleoside nephrosis model, PAF production in the kidney increases and peaks before maximal proteinuria, supporting a mechanistic role of PAF in the development of proteinuria.

PAF is a major mediator of antibody- and complement-mediated glomerular injury, as shown in experimental and human studies. PAF is implicated in antithymocyte antibody-induced glomerular damage and other experimental models of immune renal damage and in patients with lupus nephritis and IgA nephropathy. The mechanism underlying PAF involvement in immune-mediated renal pathophysiologic processes includes, among others, stimulation of monocyte chemoattractant protein-1 glomerular expression, thus favoring inflammatory cell influx.

Experimental studies have shown that PAF participates in the development of kidney graft dysfunction, namely transplant rejection, chronic transplant nephropathy, and immunosuppressive drug-mediated nephrotoxicity. The experimental evidence suggests that the plausible mechanisms of PAF effects in the transplantation setting are hemodynamic and immune, such as antibody- and complement-mediated injury. PAF production is significantly enhanced in rejecting renal allografts in experimental animal models, whereas administration of PAF receptor blockers, even with no immunosuppressive therapy, has a beneficial effect on transplant function as measured by GFR and renal blood flow and also decreases thromboxane A2 production by the graft and complement activation. The effect of PAF antagonists on histologic outcome is, however, unclear, because different studies report disparate results about the effect on inflammatory infiltration.

PAF is implicated in drug-related renal damage of different causes, such as cyclosporin A (CsA), glycerol, gentamicin, and cisplatin. Beneficial effects of PAF antagonists on drug-related nephrotoxicity are demonstrated in experimental studies. Interestingly, in animal models, PAF injection induces similar hemodynamic effects to those observed with CsA. PAF is suggested to be involved in CsA acute nephrotoxicity. However, the relation between PAF and CsA is not straightforward but rather bidirectional. On one hand, CsA induces an increase in PAF synthesis in mesangial cells in vitro. On the other hand, when PAF production is already pathologically elevated as a result of an experimental nephrosis, CsA decreases it.

Fibrosis due to extracellular matrix overproduction is a key feature of progressive renal disease, regardless of the primary cause. PAF may participate ubiquitously in renal fibrosis across all of the kidney structural compartments, including the glomerulus and the tubulointerstitial space.
Acting on renal cells, it triggers a molecular cascade of a large array of proinflammatory and profibrotic agents that induce the sclerotic process. PAF up-regulates matrix proteins in cultured mesangial cells,\textsuperscript{50} tubular epithelial cells, and interstitial fibroblasts.\textsuperscript{51} Among the downstream molecular effectors of PAF, arachidonic acid metabolites, thromboxanes, prostacyclin, and transforming growth factor (TGF)-\(\beta\) up-regulate extracellular matrix production that manifest in fibrosis.

**PAF and Mesangial Cells**

Mesangial cells are believed to be a major source of glomerular PAF generation because in culture they generate almost 10 times as much bioactive PAF per milligram of protein compared with isolated glomeruli.\textsuperscript{5} Generation of PAF by mesangial cells in vivo could have important implications for glomerular function, because PAF exerts a multitude of effects on renal cells as reviewed here. However, physiologic and pathophysiologic stimuli for PAF formation by mesangial cells in vivo remain largely unknown and warrant further investigation.

**Mesangial PAF Metabolism**

Mesangial cells are equipped with both biosynthesis and degradation mechanisms of PAF metabolism.\textsuperscript{52,53} The simultaneous presence of the counterbalancing mechanisms strategically positions mesangial cells in charge of glomerular PAF homeostasis.

Renal mesangial cells possess the complete enzymatic machinery for PAF synthesis through both the remodeling and the de novo pathways. Initially, it was shown that cultured mesangial cells acetylated lyso-PAF to PAF, that is, production to maintain a certain basal level, whereas the shorter remodeling pathway functions to rapidly up-regulate PAF in response to pathologic triggers. Accordingly, cultured mesangial cells are shown to produce PAF both in quiescence and on stimulation with calcium ionophore A23187, IgG, IL-1, tumor necrosis factor (TNF)-\(\alpha\),\textsuperscript{25,56} endotoxin,\textsuperscript{57} lipo polysaccharide,\textsuperscript{58} and other triggers of inflammation. Of note, some studies report no PAF production by mesangial cells under basal conditions,\textsuperscript{59} which might, however, reflect technical limitation of its detection rather than true absence. Evidence that PAF is newly synthesized after stimulation is obtained by using radioactive precursors. Consistent with the induction of PAF production via the remodeling pathway, the enzymatic activity of acetyl-CoA:lyso-PAF acetyltransferase increases multifold on challenging mesangial cells with proinflammatory stimuli.\textsuperscript{55} Interestingly, the increase of acetyltransferase activity does not depend on protein synthesis, suggesting a role for post-transcriptional regulation mechanism.

PAF is a potent bioactive molecule that suggests the existence of regulatory mechanisms to control and restrict the biological actions of this powerful proinflammatory mediator. How negative regulation is achieved under physiologic conditions in largely unknown, but it is shown experimentally that certain biomolecules display inhibiting effect on mesangial PAF production. Plasma \(\alpha_1\)-proteinase inhibitor suppresses induction of the remodeling pathway of PAF synthesis in mesangial cells by preventing activation of its key enzymes, phospholipase A2 and acetyl-CoA:lyso-PAF acetyltransferase.\textsuperscript{55} Experimental hyperinsulinemia reduces PAF levels by decreasing renal synthesis and increasing plasma degradation of PAF through inhibition of acetyltransferase activity and increasing plasma acetylhydrolase activity, respectively.\textsuperscript{60} PAF and nitric oxide have a bidirectional effect on each other’s synthesis in mesangial cells: inhibition of nitric oxide synthase induces synthesis of PAF, which is conversely inhibited by nitric oxide degradation.\textsuperscript{61} Of note, nitric oxide synthase blockade triggers both spontaneous and induced PAF synthesis.

Once synthesized, PAF does not accumulate in the cell, but it is secreted. Mesangial cells secrete PAF via an active transport mechanism that is mediated by multidrug resistance P-glycoproteins.\textsuperscript{62,63} Both spontaneous (basal) and induced (triggered by relevant stimuli) release of PAF occurs via this mechanism. It is unknown whether the regulation of PAF secretion is achieved independently of its production or whether the same mechanisms that orchestrate PAF biosynthesis direct its transport by a certain feedback loop.

Mesangial cells catabolize PAF with acetylhydrolases. Besides the intracellularly present cytosolic enzymes, acetylhydrolase activity is detected on the outer surface of cultured mesangial cells.\textsuperscript{52} Mesangial acetylhydrolases may constitute a defensive mechanism that protects mesangial and other glomerular cells from deleterious effects of locally generated PAF. It is, therefore, important to investigate the regulation of PAF inactivation in mesangial cells.

**PAF Effects on Mesangial Cells**

Mesangial cells express PAFR on their surface, which renders them a target for systemically circulating PAF and autocrine PAF.\textsuperscript{34,44} **PAFR** mRNA expression by mesangial cells is responsive to PAF concentration, although the direction of the effect of such homologous regulation is questionable because different investigators reported disparate trends.\textsuperscript{54,65} The controversial results obtained despite the use of comparable concentrations of PAF can be explained by the nonoverlapping time frames of the experiments, indicating that the regulation of PAFR expression by PAF might be biphasic or multiphasic. In addition, existence of additional regulators cannot be excluded. PAF stimulates multiple signaling pathways in cultured mesangial cells,\textsuperscript{66} leading to arachidonate release and subsequent prostanoid generation,\textsuperscript{67} leukocyte
recruitment, mesangial cell contraction, intracellular lipid accumulation, and matrix production. Although the exact sequence and/or interplay of these molecular events in vivo remains unclear, they all potentially culminate in the development of glomerulosclerosis.

PAF functions as a chemoattractant and increases adhesion of polymorphonuclear leukocytes and monocytes to mesangial cells. The mechanism is likely to involve β2-integrins/intercellular adhesion molecule 1 interaction. Therefore, PAF promotes inflammatory infiltration of the glomerulus.

PAF increases the expression of low-density lipoprotein receptor and scavenger receptors in mesangial cells. This causes an increased uptake of lipids and their accumulation in mesangial cells, leading to the formation of foam cells. Mesangial lipid accumulation is widely recognized as an important stage of glomerulosclerosis and a key factor that participates in the initiation and progression of lipid-mediated renal injury.

Mesangial cells possess a well-developed actin, myosin, and tropomyosin cytoskeleton that renders them contractile. This property is of major importance for mesangial cells in their function of regulating glomerular hemodynamics by changing the geometry of capillary loops. PAF induces mesangial cell contraction through increase in intracellular calcium and cytoskeletal reorganization. In addition, because exogenously added synthetic PAF directly induces mesangial cell contraction, some cytokines recapitulate PAF effects on mesangial cell shape change. Accordingly, endogenously generated PAF serves as a secondary mediator of the effects on mesangial cell shape of IL-12, endothelin, and TNF. A functional consequence of mesangial contraction in vivo is likely to be a change in GFR, because it re-proportions blood volume within a capillary network and therefore affects glomerular hydrostatic pressure, ultrafiltration surface area, and GFR. However, mesangial cell contraction is the first step that leads to matrix overproduction. Sustained cell deformation creates mechanical stress to which mesangial cells respond by generating soluble factors such as TGF-β, vascular endothelial growth factor, and connective tissue growth factor, causing matrix overproduction that results in mesangial expansion and glomerulosclerosis.

Similar to the effect on interstitial fibroblasts and renal tubular epithelial cells, PAF up-regulates extracellular matrix components in cultured rat and human mesangial cells. Specifically, the stimulatory effect is observed on fibronectin and type IV collagen expression. For fibronectin, the induction of the mRNA transcription was accompanied by the increased protein production. Preceding the induction of the matrix components, PAF triggers the production of TGF-β and also stimulates activation of the latent TGF-β. Therefore, the molecular cascade through which PAF might participate in the mesangial matrix accumulation during

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**Figure 4** Schematic of the renal effects of PAF that culminates in glomerulosclerotic kidney damage. GBM, glomerular basement membrane; LDL, low-density lipoprotein; PAF, platelet-activating factor.
glomerular injury is likely to be mediated by autocrine TGF-β. In agreement with these in vitro data, our group has previously found mesangial matrix expansion in mouse inbred strains to be associated with Far2, a gene coding for an enzyme fatty acyl-CoA reductase 2 that catalyzes production of fatty alcohols which may subsequently be used for PAF synthesis. These results not only support the link between PAF and mesangial matrix overproduction but also open potential future research direction that aims to identify additional enzymes and intermediate products of the PAF biosynthetic pathways.

Mesangioproliferation is widely recognized as a process underlying many types of glomerular injury, such as mesangial expansion, sclerosis, and glomerulonephritis. Reports are controversial on the relation between PAF and mesangial cell hyperplasia. PAF does not induce proliferation in cultured mesangial cells, however, some other studies report otherwise. Our own unpublished data support the notion of PAF having no effect on proliferation of mesangial cells. However, in renal carcinomas, PAF may mediate the mitogenic effect of CD154.

Cross-Talk between Mesangial Cells and Podocytes

It is unclear whether podocytes are also capable of PAF synthesis. Cultured podocytes do not produce PAF even on stimulation with calcium ionophore, IgG, IL-1β, or TNF-α; however, some studies report PAF production by TNF-α-stimulated podocytes. However, there is a consensus that podocytes are a target of PAF because they express PAFR. PAF effects on podocytes include loss of nephrin, cytoskeletal rearrangements, and decreased proteoglycan production, leading to a decrease in anionic charge of the glomerular basement membrane and, consequently, loss of its charge selectivity. A cross-talk between mesangial cells and podocytes is postulated as a mechanism responsible for the development of proteinuria. PAF might be one of the cytokines released by mesangial cells that mediate their communication with podocytes.

Therapeutic Approaches

PAF Receptor Antagonists

The targeting of the PAF pathway and the exploration of therapeutic possibilities are mostly focused on the PAF-PAFR interaction. Many antagonists are described that can competitively or noncompetitively displace PAF from its binding sites. These antagonists are mostly studied in the context of asthma and allergy, but also in models for cardiovascular disease, psoriasis, and kidney injury. Treatment with the antagonist WEB2170 in a rat model of mesangial cell injury ameliorates the loss in GFR and reduces structural damage in nephritic glomeruli. The same antagonist was recently used in a unilateral ureter obstruction model. Treatment of mice with WEB2170 at the first 3 days after unilateral ureter obstruction leads to a decrease in proteinuria, lowers levels of urinary NGAL, and reduces expression of the profibrotic markers TGF-β and type 1 collagen.

PAF Acetylhydrolase Activation

Another possible target for therapy that has been explored is an increase in PAF acetylhydrolase, the enzyme that catalyzes the degradation of active PAF. Rats pretreated with recombinant PAF acetylhydrolase have decreased footpad edema and vascular leakage after PAF injection. Additional studies have shown beneficial effects in mouse models for sepsis, pancreatitis, atherosclerosis, and diabetes, but no effects on the kidney are reported yet.

PAF Production

Our recent findings that increased Far2 expression is associated with mesangial matrix expansion, likely through increased PAF production, open the possibility for targeting this pathway. Intervention studies in which FAR2 activity is down-regulated are under way.

Concluding Remarks and Future Directions

Numerous studies have implicated PAF in the pathogenesis of renal diseases because of its detrimental effects on virtually all aspects of kidney functions (Figure 4). However, a plausible beneficial role of PAF in modulating normal physiologic processes should not be overlooked and commands separate investigation. A number of questions on the basic biology of PAF in the context of renal pathophysiology remain to be answered. The information gaps and inconsistencies identified in this review include i) which additional enzymes and potential intermediate products of the PAF biosynthetic pathways operate in mesangial cells?, ii) how are PAF secretion and degradation regulated under normal and pathophysiologic conditions?, iii) what are the effects of PAF on the other components of mesangial matrix?, and iv) does PAF induce mesangioproliferation? Answering these questions is of importance, because it would navigate prospective intervention efforts to develop PAF-targeting therapies.

Recent works suggest that biological activity of PAF may not be confined to an exclusively proinflammatory function, but it might be involved in a large variety of processes. Renal phenotyping of the available PAFR transgenic and knockout mouse models is warranted because it might provide insights into the role that PAF plays in the kidney. Despite the structural variety of PAF molecular species, most studies were limited to characterizing only 16:0- and 18:0-alkyl–PAF forms. These species are also the mainstay in the anti-PAF drug development. Consequently, little is known about other PAF variants. The biological and pathophysiologic
significance of the full range of molecular composition of the biosynthesized PAF, such as the balance in constitutive and inducible production of PAF species in health and disease states and its potential cell-type specificity, need to be addressed in future studies, because the nature of the PAF molecular composition may well orchestrate the character, severity, course, and outcome of PAF-mediated renal disease.

References
