Is PKC-δ a New Killer Molecule in Kidney?

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The prevalence of chronic kidney diseases is increasing worldwide, and many of these nephropathies progress to renal failure. Our understanding of the pathophysiology of deteriorating renal function is still limited, and the choices for therapy are confined to a few general interventions. The degree of proteinuria correlates with the rate of progression to ESRD, suggesting that proteinuria itself could be one of the mechanisms for progression. Finding that upon therapeutic intervention the rate of decline of GFR correlates negatively with reduction in proteinuria and positively with persistent proteinuria provides further evidence for a pathogenic role of proteinuria in renal progression.1

Albumin is the major protein present in nephrotic urine, and a variety of studies have examined the toxic effect of filtered albumin in the kidney. Special attention has been paid to proximal tubule cells (PTCs), because the majority of the albumin in the glomerular filtrate is absorbed by phosphatidylinositol-3-kinase–mediated endocytosis after the binding to the multiligand receptors megalin and cubulin2 for tubular degradation by lysosomes. Gene expression profiling of PTCs from mice overloaded with BSA identified 2000 genes that are regulated differentially.

In vitro and in vivo models of albumin overload also describe increased production of proinflammatory molecules, matrix genes, and profibrogenic cytokines such as monocyte chemoattractant protein 1, TGF-β, IL-8, endothelin 1, transcriptional factor NF-κB, and RANTES. Most of these mediators are responsible for tubulointerstitial inflammation and fibrosis through recruitment and activation of lymphocytes, macrophages, and fibroblasts. In addition, numerous studies have reported that high concentrations of albumin in vitro and in vivo induce apoptosis in PTCs, although some issues on the effect of albumin on tubular cell apoptosis are still misunderstood or controversial. First, it has been argued that tubular cell apoptosis is induced by high molecular weight proteins (100 to 440 kD) rather than albumin.2 Second, the concentration of albumin required for tubule apoptosis is not defined yet. Albumin at low concentrations seems to exert a mitogenic effect on PTCs, whereas high concentrations induce apoptosis.3 Third, the fatty acid moiety of albumin, rather than albumin itself, stimulates inflammation and increases extracellular matrix production1 and also triggers apoptosis, but the latter results are variable according to the PTC line used for study.4,5

Although apoptosis is a physiologic mechanism to eliminate unwanted cells, it also is active in pathologic conditions such as ischemic and nephrotic injury, obstructive nephropathy, and polycystic kidney disease.6 Activation of caspases, a family of cysteine proteases, is a central mechanism in apoptotic cell death. Caspases are either initiators or effectors of the different apoptotic pathways and are induced by various intrinsic and extrinsic signals. The extrinsic pathway of caspase activation is stimulated when integral membrane death receptor Fas (CD95) or TNF receptor 1 is activated by apoptotic stimuli. This interaction leads to the cleavage of procaspase 8 to caspase 8. The intrinsic pathway of apoptosis is mediated by mitochondria, and this pathway is activated by various stimuli, such as Ca2+ or increased free radical production. Mitochondrial apoptosis is initiated mainly by translocation of Bax, a proapoptotic protein from the Bcl-2 family, to the mitochondrial membrane followed by release of cytochrome c and activation of caspase 9.9,6 A third, unrelated pathway involves stress in the endoplasmic reticulum (ER) that induces the activation of caspase 12, which then directly cleaves and activates caspase 9 in the cytosol, in a cytochrome c–independent manner, different from the mechanism described for the intrinsic pathway.7

The upstream intracellular molecular signals that initiate apoptosis upon albumin overload are not fully identified, and the signaling pathways of apoptosis are still controversial. It seems that albumin overload activates all of the pathways of apoptosis, including ER stress and the intrinsic and extrinsic pathways. The contribution of the extrinsic pathway was first described by Devarajan’s group,5 who showed a dosage- and time-dependent up-regulation of the extrinsic Fas–FADD–caspase 8 pathway in PTCs in culture. A few years later, the same group demonstrated that the intrinsic mitochondrial pathway also contributes to the apoptosis observed in PTCs after albumin overload.8 Finally, two studies reported that albumin induces ER stress–associated apoptosis in PTCs.9,10 Although it is unclear which pathway transduces the ER stress signal, the very recent results obtained by Zhang’s group10 strongly suggest that albumin induces apoptosis by activating RNA-dependent protein kinase (PKR)-like ER kinase (PERK), one of the ER transmembrane receptors. PERK activation occurs before increases in CCAAT/enhancer-binding protein-homologous protein (CHOP/GADD153)
and cellular apoptosis.\textsuperscript{10} CHOP is an ER protein that plays an important role in the signaling of ER stress--induced apoptosis, but it also is involved in the intrinsic mitochondrial pathway by inhibiting Bcl-2 and upregulating Bax and Bak.\textsuperscript{11} CHOP thus could be one of the key proteins in albumin-induced PTC apoptosis at the interface of these different apoptotic pathways.

Another important protein involved in early upstream events that induce the morphologic features of cell apoptosis is PKC-\(\delta\).\textsuperscript{12,13} It is known that PKC-\(\delta\) undergoes phosphorylation on specific tyrosine residues in response to many apoptotic stimuli; it then translocates from the cytoplasm to the nucleus, where it is cleaved by the effector caspase 3 to generate a constitutively activated, proapoptotic PKC-\(\delta\) catalytic fragment. Nuclear accumulation of PKC-\(\delta\) commits a cell to undergo apoptosis, whereas retention of PKC-\(\delta\) in the cytoplasm is compatible with survival.\textsuperscript{12,13} Because PKC-\(\delta\) is a substrate for caspase 3, it was first believed that PKC-\(\delta\) acts downstream; however, the bulk of evidence strongly suggests now that PKC-\(\delta\) may be involved upstream from the apoptotic pathway, because there is a reciprocal PKC-\(\delta\) and caspase-3 proteolytic activation, PKC-\(\delta\) activity is required for maximal activation of caspase 3, and studies of mice deficient in PKC-\(\delta\) provide a defective intrinsic mitochondrial--dependent pathway of apoptosis.\textsuperscript{12}

In this issue of JASN, Li et al.\textsuperscript{14} confirm these results and demonstrate a critical role for PKC-\(\delta\) in kidney tubular cells. They show that albumin overload in PTCs induces phosphorylation of PKC-\(\delta\). By using a combination of pharmacologic approaches \textit{in vitro}, with an inhibitor of PKC-\(\delta\) in culture medium and transfection of cells with a dominant negative mutant of PKC-\(\delta\), and a genetic approach \textit{in vivo} with PKC-\(\delta\)-deficient mice, they further demonstrate that blockade of PKC-\(\delta\) activation inhibits distal events, including caspase activation and DNA fragmentation as well as proximal apoptotic events such as Bax translocation and cytochrome c release. These results suggest that PKC-\(\delta\) in kidney is activated after exposure to albumin, and this promotes the intrinsic mitochondrial pathway of apoptosis; therefore, PKC-\(\delta\) could be an important proapoptotic molecule because its activation occurs before cells display any apoptotic features.

The exact mode of action of PKC-\(\delta\) in renal apoptosis is still unclear because PKC-\(\delta\) seems to be involved in the different signaling pathways. First, PKC-\(\delta\) could be a target of apoptotic stimuli that affect either intrinsic or extrinsic cell death pathways, because it is cleaved by the effector caspase 3, which is then activated by apical caspsases 8 and 9.\textsuperscript{12} Second, studies show that rottlerin, a PKC-\(\delta\) inhibitor, exerts an opposite effect on the mitochondrial versus receptor-mediated cell death pathway and stimulates cell death by the receptor-mediated pathway.\textsuperscript{12} Because the role of the extrinsic pathway of apoptosis is active in the albumin-overload model of PTC apoptosis,\textsuperscript{5} it would be very interesting to determine whether PKC-\(\delta\) itself could inhibit this pathway and thereby modify caspase 8 activity. Interesting enough, the inhibition of PKC-\(\delta\) activity by either pharmacologic or genetic approaches results in only 50% reduction of the number of apoptotic nuclei. This finding opens up many questions: Does this reflect the percentage of apoptosis that is not mediated by the intrinsic mitochondrial pathway? Is the extrinsic pathway of apoptosis stimulated by PKC-\(\delta\) inhibition? Does the ER stress--induced pathway of apoptosis that most likely is not regulated by PKC-\(\delta\) account for 50% of apoptosis in this model? Is the incomplete inhibition of apoptosis due to disruption of cross-talk between PKC-\(\delta\) and other signaling pathways such as mitogen-activated protein kinase, c-Jun N-terminal kinase, Akt/protein kinase B, and NF-\(\kappa\)B?

Recent studies also demonstrated a novel role for PKC-\(\delta\) in the regulation of hypoxia-induced autophagy in fibroblasts.\textsuperscript{15} Autophagy is a parallel process to apoptosis, but there is a cross-talk between the two pathways through Bcl-2. Bcl-2 interacts with Beclin-1 to inhibit autophagy. It would be interesting to determine the contribution of PKC-\(\delta\) to autophagy in PCTs after an albumin overload. It will also be important to understand the contribution of PKC-\(\delta\) to kidney apoptosis in acute kidney injury. These remaining issues are a prerequisite for the use of PKC-\(\delta\) inhibitors as therapeutic agents.

**DISCLOSURES**
None.

**REFERENCES**

in renal tubular epithelial cells through a CHOP-dependent pathway. OMICS 14: 61–73, 2010