Kloths are essential for maintaining renal function, and a loss of klotho in rodent models leads to end-stage renal disease. However, the role of klotho in human kidney disease is less clear. In this issue, the authors discuss the potential therapeutic role of klotho in improving cardiovascular health in patients with CKD. They propose that klotho may be a potential target for new therapeutic approaches in the treatment of cardiovascular disease in patients with CKD.
phosphate adsorption. In concert, these actions lead to a decrease in overall body phosphate load.

Other than the membrane-bound αKlotho acting as a coreceptor for FGF23, a shorter-length protein is generated through alternative splicing of the Klotho gene and released from cells. In addition, αKlotho is subjected to proteolytic cleaving, and therefore, the entire extracellular domain is released into blood and urine.² Currently, it is not clear how far circulating αKlotho levels reflect (renal) tissue αKlotho expression. It is also unknown whether this extracellular αKlotho protein can act as a coreceptor for FGF23 or whether it functions as a soluble factor independent of FGF23. Nonetheless, given that αKlotho expression is largely confined to the kidney and parathyroid glands, most extrarenal effects would be expected to be mediated by soluble αKlotho. On the basis of experimental data in various animal models, the hypothesis has been put forward that CKD is a state of general αKlotho deficiency, because significant downregulation of αKlotho mRNA and protein in renal tissue was documented together with low levels of soluble αKlotho in blood and/or urine.⁷,⁸ Vice versa, maintenance of higher αKlotho levels by genetic manipulation in a CKD animal model protected kidney function and reduced soft tissue calcification.⁸

Clinical data supporting the above concept of CKD being a state of Klotho deficiency are scarce. In early diabetic kidney damage, renal expression of the Klotho gene decreased markedly.⁹ Recently published clinical studies using a commercially available ELISA for the measurement of soluble αKlotho in blood mostly confirmed low αKlotho levels in CKD but yielded conflicting results with respect to the predictive power for cardiovascular and renal events. Sakan et al.¹⁰ evaluated αKlotho expression in kidney biopsy samples and also measured levels of soluble αKlotho in serum and urine from 239 patients with CKD. Sakan et al.¹⁰ found reduced αKlotho and elevated FGF23 serum levels in early CKD stages. Renal αKlotho expression was an independent determinant of soluble αKlotho. In children with CKD stages 1–5D and after kidney transplantation, only a weak association between soluble αKlotho and eGFR was detected,¹¹ whereas the correlation was stronger in adults with CKD.¹² In the latter study, the decrease in soluble αKlotho preceded the increase in FGF23 levels, but the correlation between eGFR and FGF23 was much stronger than that of eGFR and αKlotho in both studies. In a post hoc analysis of a prospective study, lower serum αKlotho levels were associated with more severe CKD stage, and baseline αKlotho independently predicted the renal outcome after adjustment for several variables, including eGFR and FGF23.¹³ In contrast, in a larger study comprising 444 well characterized patients with CKD stages 2–4, we failed to observe a strong relationship between eGFR and soluble αKlotho.¹⁴,¹⁵ Of note, although we confirmed a weak association between age and soluble αKlotho, we did not find any correlation of soluble αKlotho with parameters of calcium–phosphate metabolism, progression,¹⁶ or cardiovascular end points.¹⁴ Instead, FGF23 levels turned out to be the best parameter of calcium–phosphate metabolism for predicting adverse outcomes. Presuming that the above ELISA test indeed reliably measures soluble αKlotho,¹⁵ these variable clinical data obviously must be reassessed in large epidemiologic studies. This point is particularly pertinent if one considers the use of recombinant soluble αKlotho protein for the prevention of progression and/or cardiovascular morbidity related to CKD to avoid overtreatment and potentially, deleterious circulating αKlotho levels.¹⁶ Until then, we are left with the usual: give an angiotensin-converting enzyme inhibitor and thereby, increase renal Klotho expression.¹⁷

DISCLOSURES

REFERENCES
Endotoxin and AKI: Macrophages Protect after Preconditioning

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Sepsis remains a major cause of morbidity and mortality in hospitalized patients, with AKI serving as an ominous prognostic factor. According to the current literature, the pathogenesis of AKI-induced sepsis, albeit being poorly understood, is thought to be multifactorial. Consequently, therapeutic interventions to curtail deterioration in kidney function in the setting of sepsis have been mostly supportive and, at best, modestly successful. In this issue of JASN, Hato et al. present data that demonstrate a renoprotective effect of macrophages in a model of endotoxin preconditioning and sepsis-induced AKI. The concept of disease tolerance as a possible host defense strategy against infection was recently highlighted in animal immunity. New data suggest that tissue protection by being “tolerant” to an insult may play an important role in sepsis.

Endotoxin preconditioning, or “tolerance,” is achieved by pretreatment with a low dose of endotoxin and has been shown in various models to alleviate the adverse effects of a large dose of endotoxin, as would be present in Gram-negative sepsis. Endotoxin preconditioning not only protects the host from damage caused by secondary exposure to endotoxin, but also mediates protection from other insults (cross-tolerance) such as ischemia-reperfusion injury and other Toll-like receptor (TLR) agonists. Endotoxin preconditioning has shown promise in improving outcomes of sepsis in various organs, including the kidney. A more profound understanding of the underlying mechanisms of protection by endotoxin preconditioning is key to developing therapeutic interventions in sepsis.

In previous work, the authors demonstrated that S1 proximal tubular epithelial cells acquire systemically administered endotoxin via a TLR4-dependent mechanism, subsequently initiating oxidative stress in S2 and S3 segments downstream. These findings established that endotoxin damage to renal tubular epithelial cells is a local renal event. Furthermore, the application of 2-photon (2P) intravital microscopy allowed the authors not only to interrogate the temporal and spatial location of endotoxin uptake in the kidney but also to assess the outcome of endotoxin uptake. Oxidative stress, directly visualized in vivo by fluorescent markers, was shown to occur in the S2 and S3 tubules.

In this issue of JASN, Hato et al. continue this work by investigating whether endotoxin preconditioning protects the kidney in vivo against future sepsis-induced AKI and elucidate the mechanism of endotoxin preconditioning in the mouse kidney. They first show in bone marrow chimeric mice that are reconstituted with TLR4/−/− bone marrow that endotoxin-mediated renal injury is independent of hematopoietic cells and their produced cytokines. Furthermore, they demonstrate that exposure to low-dose endotoxin is protective not only against high-dose endotoxin but also in cecal ligation and puncture and live Escherichia coli injection models of sepsis. This was evident by decreased kidney injury markers (kidney injury molecule-1, neutrophil gelatinase-associated lipocalin), preserved kidney function, and decreased oxidative stress detected by intravital imaging of renal proximal tubules. Notably, protection occurred despite the increased uptake of endotoxin by S1 tubular cells in preconditioned mice. On the basis of the evidence established by the authors, it was thus likely that endotoxin preconditioning was also a local renal event independent of hematopoietic cells. However, experiments using bone marrow chimeric mice revealed that TLR4−/− expressing hematopoietic cells are required for the renoprotective effects of endotoxin preconditioning. Likewise, bone marrow chimeric mice demonstrated that the LPS coreceptor CD14 on hematopoietic...