Kidney α–intercalated cells and lipocalin 2: defending the urinary tract

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A growing body of evidence indicates that the kidneys contribute substantially to immune defense against pathogens in the urinary tract. In this issue, Paragas et al. report that α–intercalated cells (A-ICs) within the nephron collecting duct sense infecting Gram-negative bacteria, resulting in simultaneously secretion of the iron chelating protein lipocalin 2 (LCN2) and protons, which acidify the urine. A-IC–specific LCN2 and proton secretion markedly reduced the ability of infecting uropathogenic *E. coli* (UPEC) to grow and sustain infection. The capacity of A-ICs to sense and actively promote clearance of infecting bacteria in the lower urinary tract represents a novel function for these specialized kidney cells, which are best known for their role in modulating acid-base homeostasis.

The antimicrobial shield in the urinary tract

Due to its close proximity to the gastrointestinal tract, the urinary tract is subject to a constant barrage of bacteria, most of which are enteric in origin. To counter this microbial onslaught, the urinary tract has developed a highly effective antimicrobial "shield" that can rapidly eliminate contaminating bacteria or prevent their growth. Both the flushing action of urine and urinary mucins are mechanical strategies that rapidly eliminate any contaminating bacteria from the urinary tract (1). Additionally, various antimicrobial agents that directly kill pathogens, such as cathelicidin (2) and RNAse7 (3), are constitutively secreted into urine. Periodically, pathogens overcome these antimicrobial defenses and begin to multiply in urine, a relatively rich growth medium, and induce a second wave of responses that involve the secretion of additional antimicrobial factors. Secondary responses to increased levels of bacteria in the urine appear to be triggered by immune sensory machinery in the urinary tract (4). Located at the summit of the urinary tract, the kidneys are a major source of several potent antimicrobial compounds that flow with urine and protect both upper and lower regions of the urinary tract. Kidney-secreted agents exhibit a wide range of antimicrobial actions and include cathelicidin (2), human β-defensin-1 (HBD-1) (5), and RNAse7 (3), which all directly disrupt bacterial membranes, and Tamm-Horsfall protein (THP; also known as uromodulin) (6), which promotes bacterial aggregation and facilitates removal by the urine.

In this issue, Paragas et al. have shown that α–intercalated cells (A-ICs) in the collecting ducts of the kidney serve as both sentinels and defenders of the urinary tract during infection (7). Specifically, they demonstrated that immune sensory TLR4 molecules on A-ICs detect the presence of uropathogenic *E. coli* (UPEC), and TLR4 signaling in A-ICs triggers secretion of the bacteriostatic protein lipocalin 2 (LCN2), as well as secretion of H$^+$ ions into the urine (Figure 1). Together, urine acidification and LCN2 drastically reduced the number of infecting bacteria in the urinary tract.

Protective role of LCN2 against UPEC infections

LCN2 is a member of the large lipocalin protein family, which has a wide range of biological functions (8). LCN2 binds to the secreted siderophore enterochelin (Ent), which UPEC and other pathogens release into the extracellular milieu to acquire essential iron (9), and then delivers Ent/Fe^{3+} complexes to host cells for degradation, effectively abrogating bacterial iron acquisition. Pathogens are unable to grow in the absence of iron, allowing the immune system to eliminate the infection.

Interestingly, Paragas et al. observed a significant elevation of LCN2 in the urine of mice with UTI compared with uninfected animals. The degree of LCN2 upregulation apparently associated with the number of infecting bacteria within the urine, and reduction of bacterial load with antibiotics resulted in decreased LCN2 production. Moreover, a similar correlation between bacterial numbers in the urine and LCN2 levels was also seen in patients with UTIs, providing direct clinical support for LCN2 production in immune defense of the urinary tract. Compared with WT animals, *Lcn2*-deficient mice were more susceptible to UTIs; however, both WT and *Lcn2*$^{-/-}$ animals were infected to similar extents with an *ent* mutant UPEC strain (7), which indicates that the suppressive actions of LCN2 on uropathogens involve iron acquisition.

The observation by Paragas et al. that LCN2 is not constitutively present in the urinary tract, but rather produced in direct proportion to the bacterial numbers in urine (7), is similar to other antimicrobial agents, including pentraxin-3 and HBD-1, that are also secreted in direct proportion to the size of the bacterial threat (10). As urine is a very rich medium and can be retained for many hours in the bladder, the urinary tract can serve as a powerful incubator for bacterial growth. Indeed, bacterial numbers in urine can reach levels in excess of 10^8 bacteria/ml. The ability to produce LCN2 and other antibacterial agents in proportion to bacterial burden in the urinary tract may be adaptation by the kidney to maintain homeostasis in response to overwhelming bacterial infection.

A-ICs: the cellular source of LCN2

Notably, Paragas and colleagues determined that LCN2 is produced by highly specialized A-ICs located in the collecting duct of the kidney medulla. The renal collecting ducts are responsible for regulating electrolyte and fluid balance through reabsorption and excretion processes. The collecting ducts are lined by both A-ICs
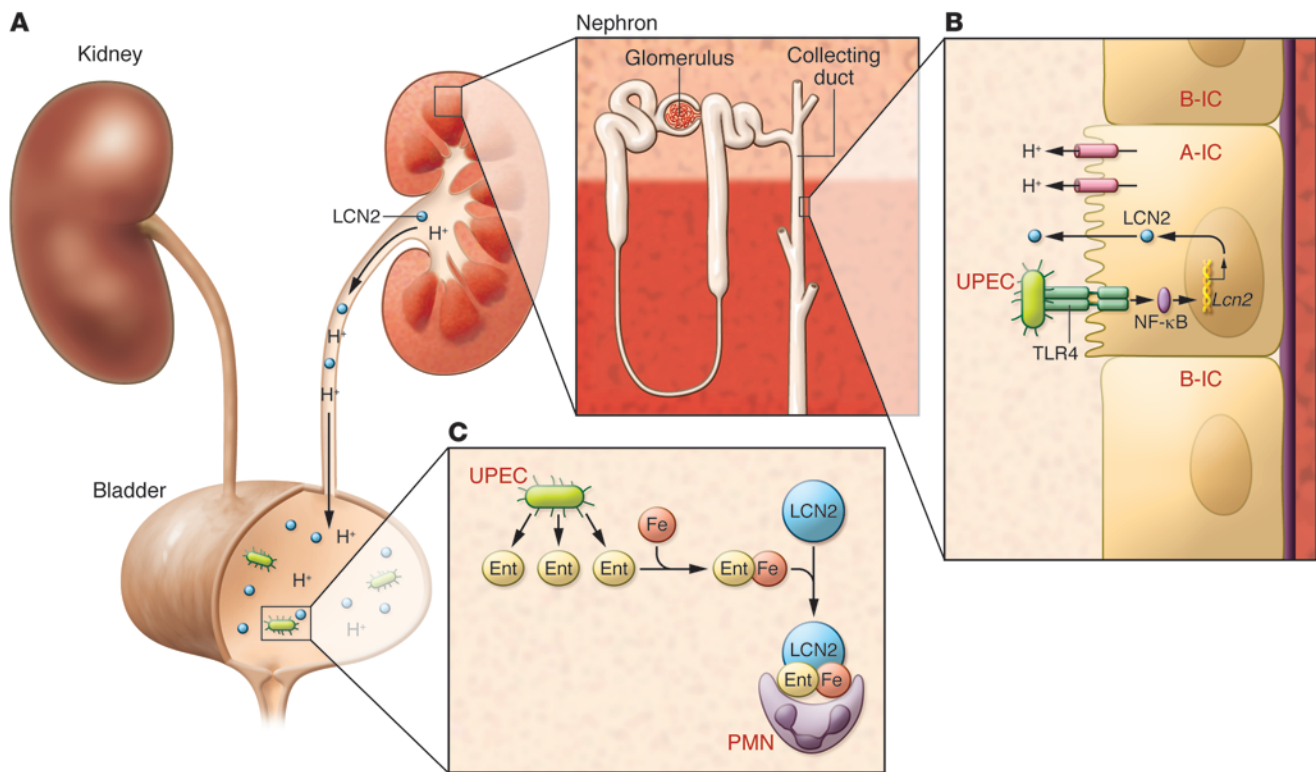
Figure 1
A-ICs defend against urinary pathogens. (**A**) Overview of the urinary system. A-ICs within the collecting duct of the kidney secrete both the bacterial static protein LCN2 and protons (H+) in response to bacterial infection. LCN2 from the kidney travels along the ureters to the bladder, where it inhibits bacterial growth. (**B**) Interaction between UPEC and TLR4 on A-ICs activates NF-κB, which translocates to the nucleus to induce *Lcn2* transcription. LCN2 is then secreted into the urinary space, from where it travels to the bladder. (**C**) In the bladder, UPEC secrete siderophores (Ent) into the urine to bind Fe. LCN2 binds Ent/Fe complexes and delivers them to host cells, such as polymorphonuclear neutrophils (PMNs), for degradation. LCN2-dependant removal of Ent/Fe complexes from urine effectively prevents bacterial iron acquisition, limiting growth.

and β–intercalated cells (B-ICs), which regulate acid-base homeostasis (11). A-ICs excrete acid into urine, while B-ICs excrete bicarbonates to make the urine more basic (12). Paragas et al. used a bioluminescent reporter mouse system to identify A-ICs as the primary source of LCN2 production. In parallel, mice infected with GFP-labeled UPEC revealed that the bacteria specifically associated with a group of cells in the collecting ducts in the kidneys that expressed markers characteristic of A-ICs. Furthermore, *Lcn2* transcription was induced upon activation of TLR4 receptors on A-ICs (7). It is somewhat surprising that A-ICs undertake antimicrobial activities, as these cells are primarily known for maintaining acid-base homeostasis. To further confirm an antimicrobial function of A-ICs, Paragas et al. generated mice lacking the IC lineage. Infection of IC-deficient animals revealed that not only do these mice not produce LCN2 in response to infection, but they are unable to control bacterial burden after

infection (7). Interestingly, urine from IC-deficient mice exhibited limited acidity, consistent with the well-known acid-secreting role of A-ICs. Together, these results indicated that the two A-IC secretory products work in concert to impair bacterial growth in the urinary tract.

Some unanswered questions

While the results of Paragas and colleagues comprehensively reveal a previously overlooked role for A-ICs and their secreted products in limiting bacterial growth in urine, some aspects of this study are less clear-cut. For example, the authors inexplicably report that renal A-ICs were capable of detecting and responding to bacterial pathogens, even when the infection was limited to the bladder. This conclusion was inferred on the inability to culture bacteria from the kidney following bladder infection; however, it is possible that some bacteria from the bladder may have transiently refluxed into the kidneys via

the ureter and activated A-ICs. Indeed, bacteria from the bladder would routinely reflux into the kidneys during the initial intravesicular instillation of bacteria in the model used (13), especially if the procedure was hastily undertaken. Another confounding issue is the location of these putative sentinel A-ICs, which are located deep in the kidneys and not at the interface between the host and the external environment, where immune sentinels are typically located. Perhaps A-ICs also monitor the blood for signs of infection, and their location in the renal intermedullary collecting duct allows simultaneous monitoring of the urinary and vascular systems. If LCN2 in the urinary tract primarily functions to control bacterial infections, as implied by Paragas and colleagues, why does urinary secretion of LCN2 increase so dramatically in response to acute and aseptic kidney injury, as previously reported (14)? Could enhanced LCN2 secretion during kidney injury serve to preempt secondary bacte-

rial infections? In view of the critical protective role played by A-ICs, do their sister cells, B-ICs, play a complementary role in combating infection? Despite any lingering questions, the results of this study are important and reveal how a specialized kidney cell, previously implicated in acid-base homeostasis, combats bacterial infections of the urinary tract.

Highly specialized epithelial cells are dispersed on various mucous membranes and are involved in maintaining the integrity of the mucosal barrier, mediating secretion, selective absorption, or transcellular transport. The revelation by Paragas and collaborators that, in addition to maintaining acid-base homeostasis, kidney A-ICs play a key role in abrogating bacterial infection in the urinary tract (7), has implications for human renal diseases. For example, human diseases that involve A-IC dysfunction, such as chronic distal renal tubular acidosis, are characterized by recurrent UTIs and pyelonephritis (15). As the aged population has dramatically grown in recent years, recurrent UTIs have become a substantial clinical problem in hospitals and nursing homes (16). Antibiotics are increasingly ineffective for combating UTIs; therefore, harnessing and boosting the innate anti-

microbial properties of cells in the urinary tract, such as A-ICs, may become viable therapeutic alternatives.

Acknowledgments

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Loss of P2Y$_{14}$ results in an arresting response to hematological stress

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The regenerative capacity of tissues to recover from injury or stress is dependent on stem cell competence, yet the underlying mechanisms that govern how stem cells detect stress and initiate appropriate responses are poorly understood. In this issue of the *JCI*, Cho and Yusuf et al. demonstrate that the purinergic receptor P2Y$_{14}$ may mediate the hematopoietic stem and progenitor cell regenerative response.

Senescence and stem cell decline

Cellular senescence, a state of permanent irreversible growth arrest, was initially described over half a century ago by Leonard Hayflick and Paul Moorhead, who observed that normal human fibroblasts cease to replicate after 50 to 60 cellular divisions (1). This barrier to everlasting cellular proliferation later became termed the "Hayflick limit," denoting the loss of proliferative potential even though the cell remains viable and metabolically active. While this phenomenon was originally connected to long-term in vitro cell propagation, cellular senescence is now understood to be a complex mechanism that may limit cell growth as well as prevent cancer in vivo and that can be initiated in response to a variety of cellular stresses, including oxidative damage, telomere shortening, DNA damage, and gene deregulation (2–4).

As with the majority of tissues, the hematopoietic system exhibits signs of age-related decline, including immune dysfunction, decreased red blood cell production, increased incidence of malignancies, and impaired recovery from injury, much of which appears to arise through cell autonomous changes in the HSC compartment (5–8). These age-related changes in the HSC compartment appear to be