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Kidney α-Intercalated Cells, NGAL and Urinary Tract Infection

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Abstract

It is well known that kidney α-intercalated cells can acidify the urine and acidified urine can inhibit bacterial growth and other urinary organisms. However, regulation of acid-base balance rather than a dedicated function in preventing urinary tract infection has been assigned to α-intercalated cells. A series of studies, culminated by the publication of a paper (J Clin Invest. 2014 Jul 1;124(7):2963–76) from Dr. Barasch's lab unearthed a novel mechanism by which α-intercalated cells function in the innate immune defense of urinary tract infection. This mechanism involves production and release of neutrophil gelatinase-associated lipocalin by α-intercalated cells to chelate the siderophore containing host iron to achieve bacteriostasis.

Introduction

The urinary tract composed of the kidneys, ureters, bladder, and urethra. These are the structures that urine passes through sequentially before being removed from the body. A Urinary Tract Infection (UTI) is an infection involving any part of this system. In the United States, UTIs account for more than 8 million office visits and 1.7 million emergency room visits annually. According to the most recent available data, UTIs costs over 3.4 billion dollars in 2000 in the USA alone [1].

UTI has become one of the most formidable challenges in clinical practice due to its high prevalence, frequent recurrence and increasing antibiotic resistance. Therefore, identification of the underlying cellular and molecular mechanism is an essential step to develop alternative strategies with the potential to reduce UTIs.

There are specialized cells in the organs that access the outside environment. These specialized cells, including Paneth cells in the gastrointestinal tract, intercalated cells in the frog skin, and clear cells in the epididymis resist bacterial invasion. They not only acidify or alkalinize the media, but also release antimicrobial peptides as part of their bacteriostatic activities. The counterpart of these specialized cells in kidney is the α-Intercalated Cells (α-IC), which acidify the urine to regulate pH in the range of 4.5 to 6.3 by secreting H^+. Although acidified urine can inhibit bacterial growth and other urinary organisms [2],

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regulation of acid-base balance rather than a dedicated function in defending UTI has been assigned to α-IC.

Most recently, Dr. Barasch's group reported that α-ICs function as both sentries and effectors in the defense against UTIs [3]. Using a mouse UTI model, the authors found that α-ICs can sense and bind uropathogenic E. coli and inhibit bacterial growth, primarily through two different mechanisms. In addition to acidification of urine, production and secretion of Neutrophil Gelatinase-Associated Lipocalin (NGAL) to chelate the siderophore containing host iron constitute a novel mechanism by which α-ICs achieve bacteriostasis. More specifically, the paper presented convincing data showing that 1) NGAL is significantly up regulated in human and mouse UTI models; 2) NGAL is necessary and sufficient to suppress uropathogenic E. colis; 3) The α-ICs in the kidney medulla express NGAL and are required for bacteriostasis; and 4) NGAL is activated by the LPS receptor TLR4.

NGAL is a new type of antimicrobial protein with iron chelating activity

Previous studies from Dr. Barasch's lab have demonstrated that NGAL is a new type of antimicrobial molecule and may be considered as a new marker of kidney disease. First, unlike the well-known antimicrobial proteins [4,5], the cathelicidins (hCAMP, mCRAMP), the defensins [6,7], Tamm–Horsfall [8], and Lactoferrin [7], which are produced constitutively, urinary NGAL is expressed only at very low levels and thus unlikely to play a large role at steady state. Secondly, while infections cause only mild upregulation of these proteins, NGAL is dramatically induced by significant infections and aseptic stimuli [9–11]. Urinary NGAL is also intensely increased in patients by tubular and tubulointerstifial damage, particularly when associated with infection, sepsis or urosepsis.

As a member of lipocalin family, NGAL can interact with a bacterial molecule Enterochelin (Ent) [12], a siderophore produced by Gram^{-} bacteria. Ent can take Fe away from transferrin with high affinity to form Ent: $Fe3^{+}$ complex. The complex can be converted into NGAL: Ent: $Fe3^{+}$ when NGAL is available, allowing Ent: $Fe3^{+}$ for degradation and inhibiting Fe transfer to bacteria [12,13]. The high stability of the complex even at pH 4.0 suggests that NGAL can sequester Ent: $Fe3^{+}$ in most biological fluids, even acidified urine [14]. Therefore, NGAL interferes with extracellular bacterial growth by binding Ent: $Fe3^{+}$, restricting its availability in biological fluids [15]. The significance of NGAL in innate defense is demonstrated by the sensitivity of *NGAL*-deficient mice to infection. These animals yielded to systemic infection with Ent-expressing laboratory strains, because of uncontrolled growth [16].

α-ICs are the primary source of urinary NGAL

Generation and characterization of NGAL-Luc2-mCherry bioluminescent reporter mice revealed the source of urinary NGAL [17]. The reporter was constructed by placing Luc2 at the start codon of the Lcn2 gene, which encodes NGAL, so that expression of Luc2 faithfully and quantitatively paralleled the expression of NGAL and mRNA, and between in and different types of kidney damage and can be continuously monitored in real-time in

whole-body scans. When reporter mice were exposed to ischemia-Reperfusion (MR), systemic sepsis, or a UTI, NGAL bioluminescence originated from the kidney medulla. I/R rapidly induced reporter expression. NGAL bioluminescence and urinary NGAL were tightly correlated with the intensity of the stimulus and identical in timing. In situ hybridization unearthed that TALH and collecting ducts expressed NGAL, most prominently the α-IC's [17].

Ablation of ICs results in defects in bacterial clearance

Deletion of the α-ICs is the most direct approach to address their contribution to UTIs. Hence, mice lacking these cells were generated by disrupting Tcfcp2l1, a transcription factor critical for α-IC development, using Cre-LoxP technology with Ksp-Cretransgene as the driver for Cre-mediated recombination [3]. As expected, the resulting mice apparently lacked both α-IC and β-IC, but otherwise appeared normal. The defect in IC was demonstrated by V-ATPase staining, a marker recognizing all ICs including α-IC, β-IC and non-A, non-B type IC. The otherwise normality was evidenced by the normal serum creatinine and urinary protein profiles and normal distribution and density of segment-specific markers including principal cells (Aqp2) and TALH (Tamm-Horsfall) [3]. Nevertheless, urinary NGAL and urinary acidification were decreased, and consequently bacterial clearance was impaired [3]. Based on all these findings, the authors concluded that α-ICs are critical not only for regulating acid-base homeostasis, but also for urinary defense against pathogenic bacteria [3]. The notion that α-ICs function as an overlooked member of the innate immune response is also supported by the fact that defects in these cells have been identified as the origin of the alkaline urine of distal renal tubular acidosis (dRTA), which is associated with urinary infections.

Future directions

It should be stressed that attribution of the phenotype of the Tcfcp2l1 conditional knockout mice to α-IC is heavily dependent on the specificity of the Cre expression. Unfortunately, Tcfcp2l1 was deleted using the Ksp-Cre driver [18]. The driver is widely expressed during embryogenesis, including developing nephrons, ureteric bud, mesonephric tubules, Wolffian duct, and Mullerian duct. In adult mice, the expression is restricted to renal tubules [18]. The lack of the α-IC specificity may complicate the data interpretation. A more specific driver is the V-ATPase B1-Cre, which is selectively active in all IC (-IC, β-IC and non-A/B cells) within the collecting duct and most cells of the connecting segment. About half of the PC of the connecting segment also expressed Cre. In other words, the V-ATPase B1-Cre also lacks the α-IC specificity [19]. Generation and characterization of new Cre drivers are required to overcome these limitations. One attractive approach is to create AE1-Cre line since AE1 is a well-established α-IC marker [20]. However, erythrocytes express a different isoform of AE1. If AE1-Cre is active in the progenitors of erythrocytes, erythropoiesis and even the whole immune system may be affected upon deletion of genes of interest, which in turn, may complicate analysis and interpretation of data, particularly those related to the immune response to UTIs. In addition, using loss of histone. H3 K79 dimethylation as a tracing marker, we have shown that most of ICs including α-IC and β-IC as well as principal cells are derived from the $Aqp2^{+}$ progenitors in $Dot1l^{AC}$ mice, which have disrupted Dot1l in

Aqp2-expressing cells [21]. Therefore, disruption of any genes critical for PC and IC specification may result in abnormal development of collecting duct and defects in the defense of UTIs. Another direction for future studies is to determine if α-IC plays a similar role in different types of reflux or obstructive uropathies, which are characterized by repetitive and severe urinary infections, and progressive interstitial infiltrates in the kidney.

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References

1. Litwin MS, Saigal CS, Yano EM, Avila C, Geschwind SA, Hanley JM, et al. Urologic diseases in America Project: analytical methods and principal findings. The Journal of urology. 2005; 173:933–937. [PubMed: 15711342]
2. Carlsson S, Wiklund NP, Engstrand L, Weitzberg E, Lundberg JO. Effects of pH, nitrite, and ascorbic acid on nonenzymatic nitric oxide generation and bacterial growth in urine. Nitric oxide: biology and chemistry/official journal of the Nitric Oxide Society. 2001:580–586. [PubMed: 11730365]
3. Paragas N, Kulkarni R, Werth M, Schmidt-Ott KM, Forster C, Deng R, et al. alpha-Intercalated cells defend the urinary system from bacterial infection. The Journal of clinical investigation. 2014; 124:2963–2976. [PubMed: 24937428]
4. Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, et al. Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature. 2001; 414:454–457. [PubMed: 11719807]
5. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002; 415:389–395. [PubMed: 11807545]
6. Hiratsuka T, Nakazato M, Minematsu T, Chino N, Nakanishi T, Shimizu A, et al. Structural analysis of human beta-defensin-1 and its significance in urinary tract infection. Nephron. 2000; 85:34–40. [PubMed: 10773753]
7. Valore EV, Park CH, Quayle AJ, Wiles KR, McCray PB, Ganz T. Human beta-defensin-1: an antimicrobial peptide of urogenital tissues. J Clin Invest. 1998; 101:1633–1642. [PubMed: 9541493]
8. Serafini-Cessi F, Malagolini N, Cavallone D. Tamm-Horsfall glycoprotein: biology and clinical relevance. Am J Kidney Dis. 2003; 42:658–676. [PubMed: 14520616]
9. Parravicini E, Lorenz JM, Nemerofsky SL, O'Rourke M, Barasch J, Bateman D. Reference range of urinary neutrophil gelatinase-associated lipocalin in very low-birth-weight infants: preliminary data. American journal of perinatology. 2009; 26:437–440. [PubMed: 19263333]
10. Kumpers P, Hafer C, Lukasz A, Lichtinghagen R, Brand K, Fliser D, et al. Serum neutrophil gelatinase-associated lipocalin at inception of renal replacement therapy predicts survival in critically ill patients with acute kidney injury. Critical care. 2010:14.
11. Bagshaw SM, Bennett M, Haase M, Haase-Fielitz A, Egi M, Morimatsu H, et al. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. Intensive care medicine. 2010; 36:452–461. [PubMed: 19956924]
12. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell. 2002; 10:1033–1043. [PubMed: 12453412]
13. Barasch J, Mori K. Cell biology: iron thievery. Nature. 2004; 432:811–813. [PubMed: 15602535]
14. Mori K, Lee HT, Rapoport D, Drexler IR, Foster K, Yang J, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. The Journal of clinical investigation. 2005; 115:610–621. [PubMed: 15711640]
15. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature. 2004; 432:917–921. [PubMed: 15531878]

16. Berger T, Togawa A, Duncan GS, Elia AJ, You-Ten A, Wakeham A, et al. Lipocalin 2-deficient mice exhibit increased sensitivity to Escherichia coli infection but not to ischemia-reperfusion injury. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103:1834–1839. [PubMed: 16446425]

17. Paragas N, Qiu A, Zhang Q, Samstein B, Deng SX, Schmidt-Ott KM, et al. The Ngal reporter mouse detects the response of the kidney to injury in real time. Nat Med. 2011; 17:216–222. [PubMed: 21240264]

18. Shao X, Somlo S, Igarashi P. Epithelial-specific Cre/lox recombination in the developing kidney and genitourinary tract. J Am Soc Nephrol. 2002; 13:1837–1846. [PubMed: 12089379]

19. Miller RL, Lucero OM, Riemondy KA, Baumgartner BK, Brown D, Breton S, et al. The V-ATPase B1-subunit promoter drives expression of Cre recombinase in intercalated cells of the kidney. Kidney Int. 2009; 75:435–439. [PubMed: 19052537]

20. Sabolić I, Brown D, Gluck SL, Alper SL. Regulation of AE1 anion exchanger and H(+)-ATPase in rat cortex by acute metabolic acidosis and alkalosis. Kidney Int. 1997; 51:125–137. [PubMed: 8995726]

21. Wu H, Chen L, Zhou Q, Zhang X, Berger S, Bi J, et al. Aqp2-expressing cells give rise to renal intercalated cells. J Am Soc Nephrol. 2013; 24:243–252. [PubMed: 23308014]