Published in final edited form as:

Kidney Int. 2019 September ; 96(3): 537–539. doi:10.1016/j.kint.2019.03.031.

Urinary Defence Begins in the Kidney

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Keywords

Intercalated Cells; UTI; Insulin; Diabetes; RNase7; RNase4; Lcn2 (NGAL)

Urinary defense begins in the kidney, quite literally! In the inaugural volume of the Journal of Urology published in 1917, Shohl and Janney demonstrated that urine pH 4.6 to 5.0 was lethal for several strains of *Escherichia coli*.¹ Even to this day, therapeutic acidification of the urine is often recommended for suppression of urinary tract infections (UTI).

These early insights prompted the notion that the kidney defends the urinary tract from infection, as summarized by the Urinology Think Tank held at the National Institutes of Health in 2015 (https://www.niddk-nih-gov.ezproxy.cul.columbia.edu/news/meetings-workshops/2015/urinology-think-tank_02-2015). Only the kidney's collecting ducts, and in fact only the intercalated cells (ICs), can reduce urine pH to achieve organismal H⁺ homeostasis and at the same time achieve bactericidal levels of urine acid.² We know this because defects in IC H⁺ATPases or in components operating in series (anion exchanger 1 or carbonic anhydrase II) prevent maximal urinary acidification.³ A distal renal tubular acidosis (dRTA) syndrome of kidney stones, nephrocalcinosis, and UTIs results from the acidification defect. The dRTA syndrome is a doppelganger for obstructive uropathy and for the vesicular-ureteral-reflux syndrome, which is also marked by alkaline urine coupled with repeated UTIs and kidney stones in children around the world. Like dRTA, obstructive uropathy and vesicular-ureteral-reflux result in a defect in the IC cells, as evidenced by the failure to maximally acidify urine during ammonium loading. Together, these observations suggest that acidification of the urine by ICs may not only have a primary function in waste removal, but may also be central to urinary defense.

Although the association of defective H⁺ physiology and repeated UTIs is a compelling correlation, it does not establish causation. However, the chance identification of the transcription factor Tfcp2l1 by Werth and Schmidt-Ott et al.⁴ provided a stronger link, because deletion of Tfcp2l1 both prevented the development of ICs and resulted in higher colony counts throughout the urinary system.² Hence, it appears that ICs are required for defense.

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Disclosure

Columbia University has licensed NGAL to detect kidney injury (AKI) to Abbott and Biopoint.

Now our story gets really interesting. Vandewalle et al.⁵ and then Paragas and colleagues^{2, 6} found that ICs can recruit urinary bacteria directly to their apical membrane, where the bacteria are exposed not only to H⁺ but also to an IC protein that specifically targets bacterial functions. This protein, called Lipocalin 2, NGAL or, better, Siderocalin, was shown by Goetz and colleagues to specifically bind 1 subgroup of siderophores, iron-chelating compounds that some bacteria use to siphon iron from mammalian hosts.⁷ Together, IC knockout models, IC-bacterial interactions, and IC secretions including both nonspecific sledge hammers (H⁺) and fine tools (Lipocalin 2), paint a clear picture that ICs are previously unrecognized members of the innate immune family.

However, to conclude the story at this point would suggest that ICs only make a halfhearted attempt to defeat bacteria. Because different bacteria have different pH sensitivities and Lipocalin 2 is surprisingly specific to a subset of siderophores, more evidence is required to connect the ICs to a broader spectrum of urologic infections.

This is where the exceptional work of J. D. Spencer and colleagues enters to further define the biology of ICs in immune defense. Their group has previously focused on an antimicrobial family, RNases 4,6,7, which is capable of killing a broad range of organisms (including *Escherichia coli* and *Pseudomonas*) by lysing cell membranes. A combination of positively charged residues and amphipathic sequences allows the RNases to interact with both the aqueous phase and with bacterial lipids to create a lethal pore, and RNase 7 has been shown to derive from human ICs.⁸

In their recent paper, Spencer et al. evaluated a critical clinical question: why do diabetics have more frequent UTIs?⁹ Any of the mechanisms described above could be the target of diabetes, and the presence of glucose in the urine could also stimulate the growth of bacteria, as has become evident with the use of SGLT2 inhibitors.

By sequentially examining a classical model of hyperglycemia (db/db mice), then a model of reduced insulin sensitivity (TallyHo mice), and finally an IC-specific deletion of the insulin receptor, Spencer and colleagues have eliminated the secondary contribution of glycosuria to focus directly on the role of insulin signaling. Rather than a developmental problem in IC number or a defect in urine acidification, they found that insulin is a critical signaling molecule that induces the expression of the IC antimicrobials RNase4 and Lipocalin 2 (Figure 1). As a result, diabetic mice have higher colony counts throughout the urinary system. This is most likely because of a cell autonomous signaling defect, as insulin stimulated RNase4 and Lipocalin 2 expression in isolated medullary cells, whereas direct inhibition of the insulin-signaling pathway by AKT inhibitors reduced their expression.

In sum, Spencer and colleagues have demonstrated that the IC-secreted antimicrobials are metabolically regulated, perhaps linking food intake (which may provide extra iron, sugar, and nitrogenous compounds to bacteria) with the production of prophylactic antimicrobials. In this light, insulin has joined the ranks of aldosterone and ADH as hormones that regulate the metabolic functions of the collecting ducts in response to dietary challenges. Their work has also reinforced the notion that urinary defense begins in the kidney, because the deletion of insulin receptors in ICs, like the deletion of ICs themselves, affected colony counts

throughout the urinary system. Hence, Spencer and colleagues have demonstrated that the kidney, and specifically the ICs, defend the urinary system from infection by secreting toxic chemicals into the urine flowing downstream from kidney to bladder.

Acknowledgments

This work was supported by the O'Brien Center for Benign Urology (1U54DK104309) and 2R01DK073462.

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Figure 1.

Stimulation of the intercalated cell results in the secretion of antimicrobials. Stimuli including food, Kp, glucose, and water drive the expression of aldosterone, insulin, and ADH, which, in turn, drive the secretion of chemical defenses including H_p, RNase, and NGAL. H_p and RNase have bactericidal activity, whereas NGAL is bacteriostatic. These data portray the intercalated cell as a member of the innate immune system.