

targeting podocytes in disease models or other kidney cell types in mice.^{7,8} Gene therapy to treat common or genetic kidney disease, which afflict ~15% of the worldwide population, will require cross-species-based AAV selection strategies, including validation across multiple scales (from cellular to organ level) before clinical translation. Expanding the AAV toolbox for targeting the kidney could provide new therapeutic options for these patients, thereby reducing the morbidity and mortality of kidney diseases.

DISCLOSURE

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translational science

Uromodulin, neutrophils, and control of urinary tract infection

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KEYWORDS: dipstick; neutrophil extracellular traps; pyelonephritis; uromodulin; uropathogenic *Escherichia coli*

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Uromodulin is a kidney-specific protein abundantly excreted in normal urine that plays multiple roles in salt absorption and blood pressure control, regulation of innate immunity, and protection against kidney stones and urinary tract infections (UTIs).¹ In the urine, uromodulin forms fishbone-like filaments with a backbone and laterally protruding arms harboring high mannose glycans that associate with uropathogenic *Escherichia coli* (UPEC) and mediate bacterial aggregation, preventing adhesion and allowing clearance of bacteria by micturition.² The protective effect of uromodulin levels on the incidence of UTIs has been verified in prospective cohorts,³ and may be relevant for pathogen-driven adaptation of the uromodulin (*UMOD*) gene locus through human evolution.⁴ The mechanisms of the antibacterial effects of uromodulin and its potential interactions with other protective

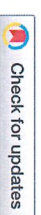
systems in the urinary tract have not been elucidated.

A hallmark of UTIs is the presence of neutrophils in the urine, which are crucial for host defense against uropathogens. The host defense role of activated neutrophils involves complex cellular events leading to the formation of neutrophil extracellular traps (NETs) that are made of decondensed DNA strands coated with cytotoxic effector proteins. In turn, these structures can trap and neutralize pathogens in a process termed NETosis.^{5,6} A critical step in NET formation involves the action of the peptidyl arginine deiminase type 4 (PAD4) enzyme, which mediates histone citrullination, enabling DNA decondensation and ejection from the cell (Figure 1).⁶

Although UPEC are most often confined to the bladder, causing cystitis, they can occasionally ascend the ureters and cause pyelonephritis,

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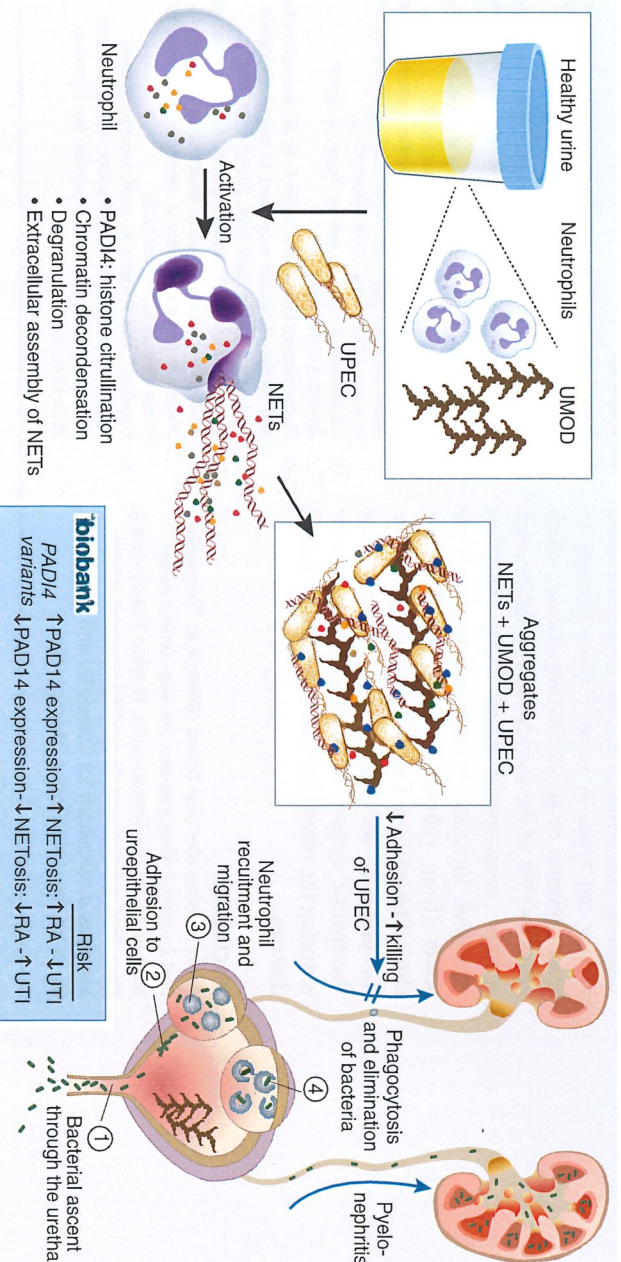


Figure 1 | Neutrophil extracellular traps, uromodulin, and control of urinary tract infections. Uromodulin (UMOD) and neutrophils are present in normal, uninfected urine. Activation of neutrophils, for instance by uropathogenic *Escherichia coli* (UPEC), induces the formation of neutrophil extracellular traps (NETs). This process, called NETosis, is triggered by the citrullination of histone arginines by the peptidyl arginine deiminase 4 (PAD4) enzyme, leading to chromatin decondensation. Extracellular strands of decondensed DNA complexed with histones and neutrophil granule proteins form NETs, which aggregate with uromodulin and UPEC. In turn, these aggregates decrease bacterial adhesion to the urothelium and increase antibacterial activity. This action helps to prevent ascending urinary tract infections (pyelonephritis). Variants in *PAD4* are associated with autoimmune inflammatory diseases, like rheumatoid arthritis (RA). In the UK Biobank, *PAD4* variants associated with high NETosis and increased risk of RA show a decreased susceptibility of UTI, and vice versa.

with potentially severe complications, including bacteremia. In a recent study, Stewart *et al.* investigated a potential role of NETosis interacting with uromodulin in the defense against UTIs and, more specifically, in protecting against UPEC ascent and development of pyelonephritis.⁷

What did the study show?

First, by a proteomic analysis of urinary extracellular vesicles (EVs), the authors showed that EVs isolated from the urine of healthy individuals contain neutrophil and histone proteins, compatible with NETs present in the uninfected urinary tract. They next showed the presence of neutrophils in both male and female urine samples. Urinary neutrophils are morphologically different from blood neutrophils, with some presenting features characteristic of early-phase NETosis, including evidence of histone citrullination. Most urine neutrophils appeared intact but rapidly underwent NETosis when stimulated with known activators. These analyses demonstrated the presence of neutrophils and NETosis in the uninfected urine, with a potential role in antibacterial response at baseline.⁷

Knowing the protective role of uromodulin against UPEC, the authors demonstrated that urine neutrophils induced to form NETs bind uromodulin, forming large aggregates when UPEC are added. The interaction between uromodulin and NET was significantly decreased by the deglycosylation of uromodulin. Infiltrates of neutrophils undergoing NETosis and containing uromodulin and UPEC were documented in mouse models of cystitis and pyelonephritis. The functional importance of NETosis in the defense against UPEC and ascending UTI was demonstrated using the *PAD4* inhibitor GSK484, which inhibits NETosis. GSK484-treated mice showed more severe pyelonephritis, including lower NETosis in pelvic neutrophil aggregates and a reduction in uromodulin aggregates, compared with PBS-treated mice. *In vitro*, uromodulin alone was effective in reducing UPEC growth, an effect that was amplified when NETs were combined with uromodulin. In the presence of uromodulin alone, large aggregates of bacteria and polymeric uromodulin were observed; with the addition of NETs, bacterial death, initially low, increased in these aggregates.

Using population genetics, the authors identified common *PADI4* GWAS variants associated with rheumatoid arthritis (RA), which carry opposing effects on the risk of autoimmune disorder (RA) and infection (UTI) in the UK Biobank. *PADI4* genotypes increasing the risk of RA, associated with increased *PADI4* expression, reduce the risk of UTI. Conversely, *PADI4* variants decreasing the risk of RA, associated with decreased *PADI4* expression and potentially decreased NET formation, increase the risk of UTI. Although these observations could not discriminate between the risk of cystitis or pyelonephritis, they suggest that genetically driven altered *PADI4* expression may impact the propensity to UTIs.

Last, the authors showed that the number of NETosing neutrophils in urine, rather than the absolute number of neutrophils, affects the result of the leukocyte esterase (LE) dipstick test that is widely used to detect UTIs.⁷

Why is it important?

Collectively, the studies of Stewart *et al.* support an important role of NETosis in a defense mechanism that can rapidly be activated to prevent ascending infection with uropathogenic *E coli* (i.e., reducing the risk of pyelonephritis). Mechanistically, they suggest that NETs interact with glycosylated uromodulin to form a scaffold that aggregates and kills UPEC in the urine, in a synergistic way (Figure 1).⁷ Another recent study has confirmed the role of uromodulin as a regulator of NETosis in the urinary tract in the context of UTI.⁸ Open questions include the impact of environment or disease state on the biochemistry of

uromodulin and its capacity to interact with NETs and bacteria, the potential role of NETs in uromodulin-associated kidney diseases, and the factors driving neutrophils in uninfected urine.

DISCLOSURE

All the authors declared no competing interests.

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