

# The nature of immune responses to urinary tract infections

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## Abstract

The urinary tract is constantly exposed to microorganisms that inhabit the gastrointestinal tract, but generally the urinary tract resists infection by gut microorganisms. This resistance to infection is mainly ascribed to the versatility of the innate immune defences in the urinary tract, as the adaptive immune responses are limited particularly when only the lower urinary tract is infected. In recent years, as the strengths and weaknesses of the immune system of the urinary tract have emerged and as the virulence attributes of uropathogens are recognized, several potentially effective and unconventional strategies to contain or prevent urinary tract infections have emerged.

**Subject terms:** Adaptive immunity Bacterial infection Innate immunity Urinary tract infection

## Key points

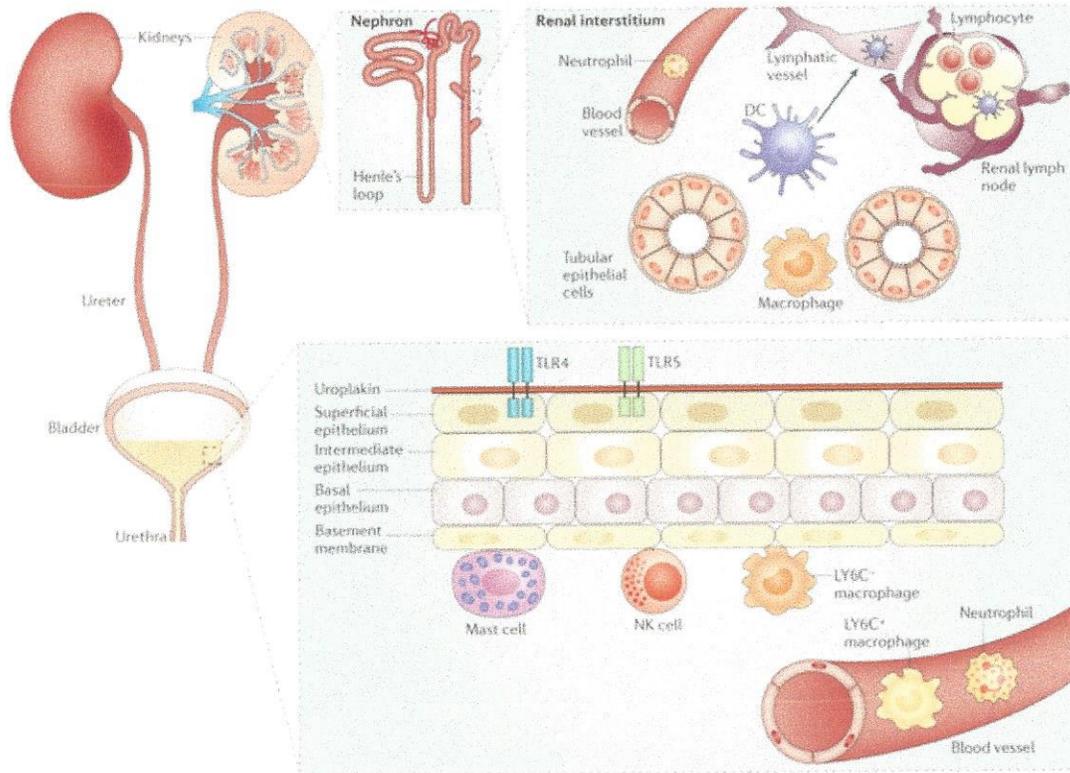
- An overarching theme of the immune system in the bladder seems to be balancing the need to respond promptly to microbial challenge with the need to rapidly curtail inflammatory responses, as the structural integrity of the epithelial barrier is disrupted during prolonged immune responses.
- Bladder epithelial cells not only alert the immune system during infection but also directly mediate bacterial clearance by secreting antimicrobial compounds into the urine and by expelling invading bacteria back into the bladder lumen to reduce intracellular load.
- Crosstalk between different subsets of macrophages in the bladder coordinates the precise recruitment and onset of neutrophil responses, and thereby reduces harmful inflammatory reactions.
- Mast cells seem to have a dual role in immune regulation in the urinary tract. They promote early mobilization of immune cells into the bladder and are central to terminating these pro-inflammatory responses presumably when the bladder epithelial barrier is disrupted. However, this homeostatic action often results in blunted adaptive immune responses.
- Although neutrophils are the predominant immune cells mediating bacterial clearance in the bladder, excessive neutrophil responses can cause damage to the bladder tissue and predispose this organ to persistent infections.
- Several unconventional, but potentially effective, strategies have been described that can boost immune defences of the bladder to contain or prevent urinary tract infections.

## Introduction

*the quality of mucus a structural issue*

The urinary tract comprises the kidneys, ureters, bladder and urethra and, with the exception of the urethra, most of this tract is perceived to be sterile. Protection from microbial colonization is mediated by various soluble factors that are secreted into urine and by anatomical barriers such as the glycoprotein plaque uroplakins<sup>1</sup> and a layer of hydrated mucus<sup>2</sup>. In addition, the urinary tract is lined by epithelial cells and various resident immune cells that further protect against infection (Fig. 1). These barriers prevent pathogens from entering the urinary tract and from establishing persistent infection.

**Figure 1: Organization of immune-competent cells along the urinary tract.**



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A variety of cell types are responsible for initiating immune responses along the urinary tract. The upper urinary tract is comprised primarily of the kidneys, and the filtration function of the kidneys is performed by hundreds of thousands of nephrons, each of which is composed of a glomerulus and a double hairpin-shaped tubule. Many of the immune-competent cells, including dendritic cells (DCs) and macrophages, are aggregated in the interstitium in close proximity to both the tubular epithelium and blood vessels. In addition, there is a large network of lymphatic vessels in these organs, which connects to the renal lymph nodes. The ureters, bladder and urethra constitute the lower urinary tract, and several layers of stratified epithelial cells that line the bladder function as the first line of defence. The major resident immune cells in the bladder include mast cells and LY6C<sup>-</sup> macrophages. These cells are located underneath the basal epithelium and function as sentinels to sense infection and recruit neutrophils and LY6C<sup>+</sup> macrophages to the bladder. NK, natural killer; TLR, Toll-like receptor.

*the means feces !! fecal contamination*

The majority of urinary tract infections (UTIs) in healthy individuals are caused by uropathogenic *Escherichia coli* (UPEC) that originate from the gut<sup>3</sup>. Uropathogens express common virulence factors that enable them to successfully establish infection in the urinary tract (Box 1). However, most of the time the urinary tract resists infection, and if infection is established, it remains contained within this tract unless predisposing conditions exist<sup>4</sup>.

**Box 1: Virulence attributes of uropathogens**

More than 80% of urinary tract infections (UTIs) in healthy individuals are caused by uropathogenic *Escherichia coli* (UPEC), with the remainder being caused by other enteric Gram-negative bacteria and some Gram-positive bacteria<sup>3</sup>. Most of these bacteria originate from the gut and reach the urinary tract by progressive colonization via the perineal region<sup>3</sup>. Uropathogens express certain key virulence factors that enable them to successfully establish infection in the urinary tract; these include adhesive fimbriae, which enable bacteria to adhere avidly to specific receptors on the urothelium, and flagella that enable bacteria to swim along the urinary tract including 'upstream' from the bladder to the kidneys<sup>67</sup>. Uropathogens also secrete toxins, such as haemolysin and cytotoxic necrotizing factor, which disrupt the epithelial barrier and enable access to the underlying tissue<sup>68</sup>, and siderophores, which enable bacteria to chelate iron that is important for growth<sup>69</sup>. Bacterial survival in the urinary tract is also promoted by their expression of cell surface capsules, which enable them to resist the bactericidal actions of complement and phagocytic cells<sup>70</sup>. Arguably, the capacity to penetrate the highly impregnable bladder epithelial barrier and seek refuge in bladder epithelial cells (BECs) is a critical initiating step of infection in the urinary tract. Uropathogens seem to achieve this feat by hijacking the innate capacity of BECs to regulate bladder volume. Once UPEC become intracellular, they are protected from elimination by urine or the immune system, enabling them to initiate an acute infection. If untreated, UTIs tend to be self-limiting and naturally resolve within several days<sup>71</sup>. However, studies in mice reveal that even after the infection is resolved, an appreciable population of UPEC persists within BECs in a quiescent phase for extended periods of time<sup>70</sup>. It is assumed that recurrent infections occur when some of these intracellular bacteria escape into the bladder where they rapidly grow in the urine to initiate another bout of infection.

QIG Deal!!

steal IRON

**Reduce**

An important function of the urinary tract is to store urine for substantial periods of time, and as urine contains toxic and noxious compounds, it has to be contained within a tight epithelial barrier. Thus, the immune responses of the urinary tract need to balance the response to microbial challenge with the need to rapidly curtail inflammatory responses to maintain the structural integrity of the epithelial barrier. This 'balancing act' can result in premature termination of inflammatory responses, leading to the persistence of residual bacteria, and can potentially give rise to chronic or recurrent infections, which are remarkably common in the urinary tract<sup>5</sup>.

UTIs are more prevalent in women than men, and the incidences have increased substantially over the past 30 years<sup>6</sup>, mainly owing to an increase in the population of elderly and immune-compromised individuals, as well as the rise in the use of indwelling urinary catheters<sup>7</sup>. The annual cost of managing and treating UTIs in the United States is now estimated at approximately US\$3 billion<sup>4</sup>, and the treatment of UTIs has become a serious clinical challenge due to the growing incidences of multi-drug resistant uropathogens and the high frequency of recurrent UTIs<sup>6</sup>. Thus, there is keen interest in improving our understanding of the nature of the immune responses in the urinary tract and determining whether some of these activities could be targeted to combat UTIs. Here, we review the components of the immune system in the urinary tract and discuss potential novel approaches for targeting the

immune system to prevent and limit UTIs. As the immune responses of the kidneys are fairly well characterized and reviewed elsewhere<sup>8, 9</sup>, we focus our discussion on the bladder. The immune responses of the bladder have a central role in determining the outcome of UTIs, as the bladder stores urine — which is a potentially permissive medium for bacterial growth — for extended periods of time.

### Innate immune responses

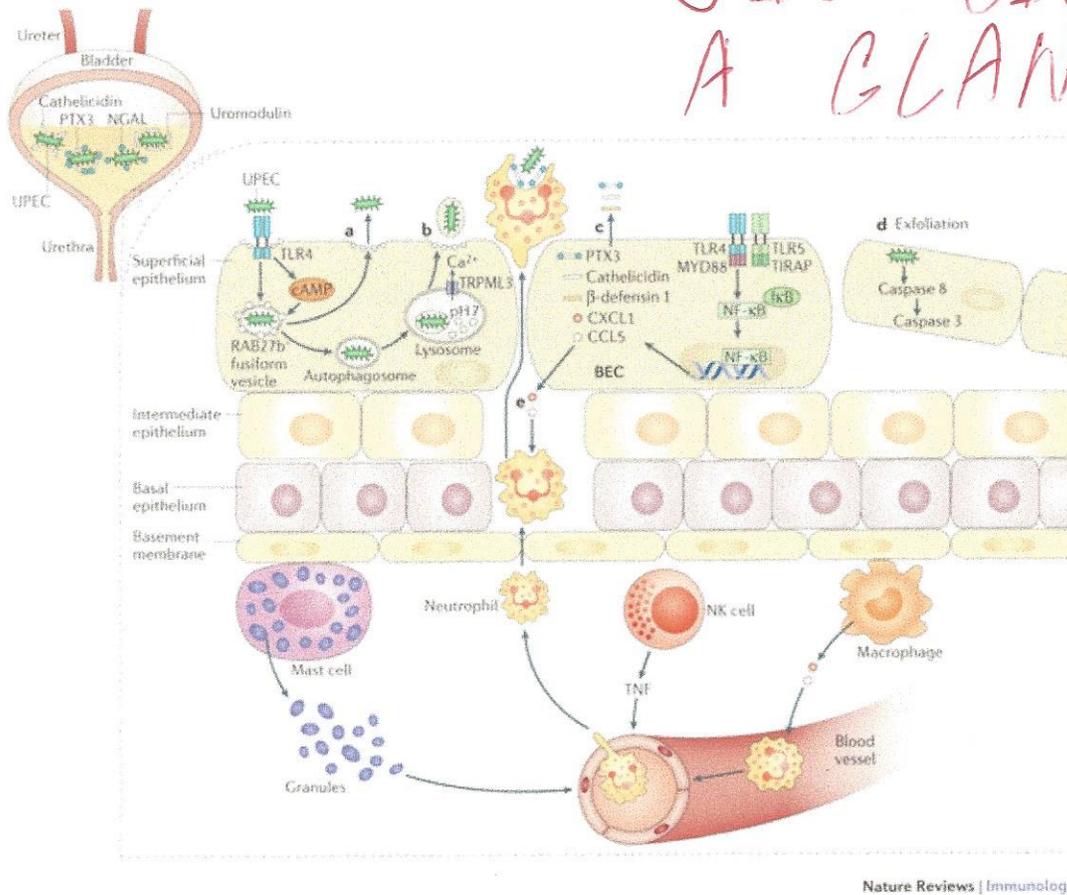
The innate immune system in the urinary tract comprises various resident and recruited cells that express a wide range of pattern recognition receptors (PRRs) — such as Toll-like receptor 2 (TLR2), TLR4, TLR5 and TLR11 — which enable early recognition of pathogens and transduce this signal to induce a rapid and robust pro-inflammatory immune response. These signalling events have been reviewed in detail<sup>10, 11</sup> and are therefore not described here. The importance of these signalling events is illustrated by the fact that individuals with genetic defects in components of these pathways have increased susceptibility to UTIs<sup>12</sup>. Although these immune responses are important, they have to be tightly controlled to ensure the retention or rapid recovery of the epithelial barrier. In this section, we review the main innate immune cells in the urinary tract. We focus on their unique antimicrobial activities and discuss the regulatory mechanisms that control over-reactive innate immune responses.

**Epithelial cells.** The epithelial cells lining the urinary tract are the first line of defence against pathogens. These cells secrete a plethora of soluble compounds ranging from pro-inflammatory cytokines to antibacterial agents. Interleukin-1 (IL-1), IL-6 (Ref. 13) and IL-8 (Ref. 14) are often the first cytokines to be detected in urine following infection, and they are important for the recruitment of phagocytes into the infected bladder or kidney tissue<sup>15</sup>. Uromodulin (also known as Tamm–Horsfall urinary glycoprotein) is specifically produced in the urinary tract by epithelial cells lining the ascending limb of Henle's loop in kidney nephrons<sup>16</sup>. Upon binding to UPEC, uromodulin prevents bacteria from interacting with the epithelial cell surface while simultaneously inducing them to aggregate, which facilitates early removal of UPEC in the urine<sup>17</sup>. Uromodulin has also been suggested to directly trigger TLR4 and induce the maturation of certain cells such as myeloid dendritic cells (DCs)<sup>18</sup>. Thus, uromodulin may also have an immune-modulatory function.

Some secreted factors from epithelial cells inhibit bacterial growth by eliminating important bacterial growth factors in the urine. The neutrophil gelatinase-associated lipocalin (NGAL) protein binds bacterial siderophores such as enterochelin<sup>19, 20</sup>. NGAL, which is produced by  $\alpha$ -intercalated cells in the kidney, was recently shown to restrict the growth of UPEC in the urinary tracts of both humans and mice<sup>21</sup>. During UPEC infection, mice deficient in NGAL or  $\alpha$ -intercalated cells showed increased susceptibility to infection compared with wild-type mice<sup>21</sup>. A variety of antimicrobial peptides (AMPs) — such as cathelicidin-related AMPs (for example, LL-37 (also known as CAMP))<sup>22</sup>,  $\beta$ -defensin 1 (Ref. 23) and ribonuclease 7 (Ref. 24) — are also secreted into the urine to restrict bacterial growth. In response to bacterial infection, bladder epithelial cells (BECs) and  $\alpha$ -intercalated cells function as the initial source of AMPs in the urinary tract; at a later stage, AMPs are secreted by recruited neutrophils (Fig. 2). The contribution of AMPs to protection against UTIs is indicated by the observation that cathelicidin-deficient mice are highly susceptible to both cystitis and pyelonephritis<sup>22</sup>. AMPs can also have immune-modulatory effects, such as increasing cytokine production and promoting neutrophil infiltration<sup>25</sup>. Pentraxins are an evolutionarily conserved protein family that can function as soluble PRRs. The level of pentraxin-related protein 3 (PTX3) in urine during UTIs is strongly associated with the severity of symptoms<sup>26</sup>, and genetic polymorphisms in the human *PTX3* locus correlate with increased susceptibility to acute pyelonephritis<sup>26</sup>. PTX3 is thought to bind bacterial surfaces, which leads to complement-mediated killing and increased uptake by phagocytes<sup>26</sup>.

Figure 2: Scope of innate immune responses in the bladder.

JUST GIVE A GLANCE !!



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A robust and complicated network of innate immune responses can be initiated in the bladder in response to infection. **a** | Following invasion by uropathogenic *Escherichia coli* (UPEC) and encapsulation within RAB27b<sup>+</sup> vesicles in bladder epithelial cells (BECs), Toll-like receptor 4 (TLR4) recognizes intracellular UPEC and increases intracellular cyclic AMP (cAMP) levels. This leads to exocytosis of RAB27b<sup>+</sup> vesicles harbouring UPEC and expulsion of the intracellular UPEC back into the lumen of the bladder. **b** | When intracellular UPEC escape the first wave of expulsion by breaking the RAB27b<sup>+</sup> vacuole, these bacteria are targeted by autophagy and delivered into the lysosomes, which in turn are manipulated by UPEC to lose their degradative capacity. These malfunctioning lysosomes are sensed by a lysosomal transient receptor potential mucolipin 3 channel (TRPML3) and trigger lysosome exocytosis, resulting in bacterial expulsion. **c** | Upon sensing the presence of pathogens by TLR4 and subsequent signalling, a wide range of soluble factors are also secreted by BECs, including antimicrobial peptides (such as cathelicidin and  $\beta$ -defensin 1), antimicrobial proteins (such as pentraxin 3 (PTX3)) and chemokines (such as CXC-chemokine ligand 1 (CXCL1) and CC-chemokine ligand 5 (CCR5)). **d** | Bacterial infection initiates caspase 3- and caspase 8-dependent apoptosis of infected BECs, which shed into the bladder lumen; this represents another effective mechanism to reduce bacterial load. **e** | Coordinated cellular immune responses in the bladder are shown. The resident sentinel immune cells — such as mast cells, natural killer (NK) cells and macrophages — can sense the presence of the infections and secrete various cytokines to recruit other innate immune cells from the bloodstream, especially neutrophils, to clear the infections. I $\kappa$ B, NF- $\kappa$ B inhibitor; MYD88, myeloid differentiation primary response protein 88; NGAL, neutrophil gelatinase-associated lipocalin; NF- $\kappa$ B, nuclear factor- $\kappa$ B; TIRAP, Toll/IL-1R domain-containing adaptor protein; TNF, tumour necrosis factor.

Following UPEC invasion of BECs, the bacteria are encapsulated in RAB27b<sup>+</sup> fusiform vesicles, which intrinsically have exocytic properties, and as a consequence, BECs can then expel intracellular bacteria back into the extracellular medium without loss of viability<sup>27</sup>. *In vitro* studies of UPEC-infected BECs reveal that bacterial

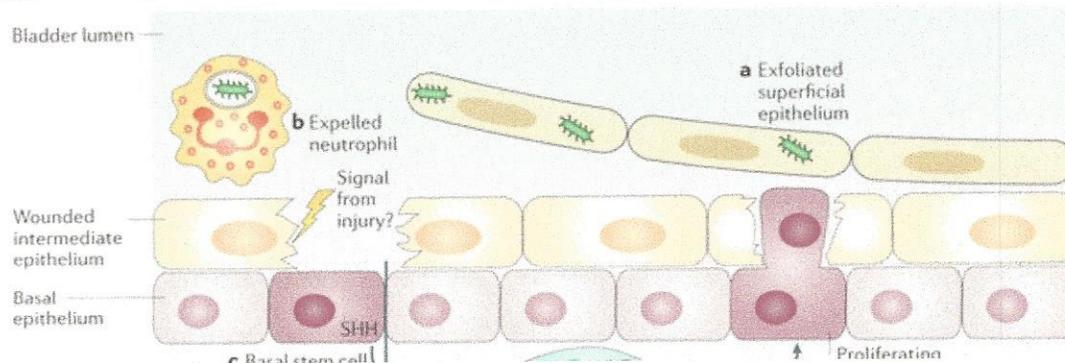
expulsion can be detected within minutes of infection; the rate seems to peak at 4 hours post-infection and by 24 hours, up to 70% of the infecting bacteria are expelled from BECs<sup>27</sup>. The underlying mechanism involves mobilization of multiple exocytic activities within BECs. One pathway involves TLR4 signalling that leads to increased intracellular levels of the second messenger molecule cyclic AMP (cAMP), which in turn triggers spontaneous expulsion of RAB27b<sup>+</sup> vesicles (some of which contain bacteria)<sup>28</sup>. A second exocytic pathway is activated when intracellular bacteria that escaped the first wave of expulsion, by breaking out of RAB27b<sup>+</sup> vacuoles, are recognized and captured by autophagy<sup>29</sup>. Autophagy normally leads to the degradation of bacteria, but UPEC can block acidification and survive within lysosomes<sup>29</sup>. Studies in cultured human BECs have shown that the presence of malfunctioning lysosomes containing UPEC is promptly sensed by transient receptor potential (TRP) mucolipin 3 (TRPML3), which is a cation channel that is expressed on the lysosome, and this sensing triggers the spontaneous exocytosis of these lysosomes<sup>29</sup>. This capacity of BECs to exocytose lysosomal contents seems to be an innate homeostatic mechanism to remove lysosomal contents that are not degraded. In contrast to bacteria exocytosed in the first wave of expulsion, bacteria expelled from lysosomes are encapsulated within host membranes, which prevents reattachment of UPEC to the bladder wall and ensures bacterial removal in urine<sup>29</sup>. Thus, infected BECs seem to use various components of their export machinery to reduce bacterial load, and *in vivo* studies in mice show that inhibition of autophagy or blockade of calcium flux can substantially increase the bacterial load in the bladder and, conversely, boosting the activity of other components, such as intracellular cAMP, can markedly reduce bacterial load<sup>27</sup>.

*MANY duplication*

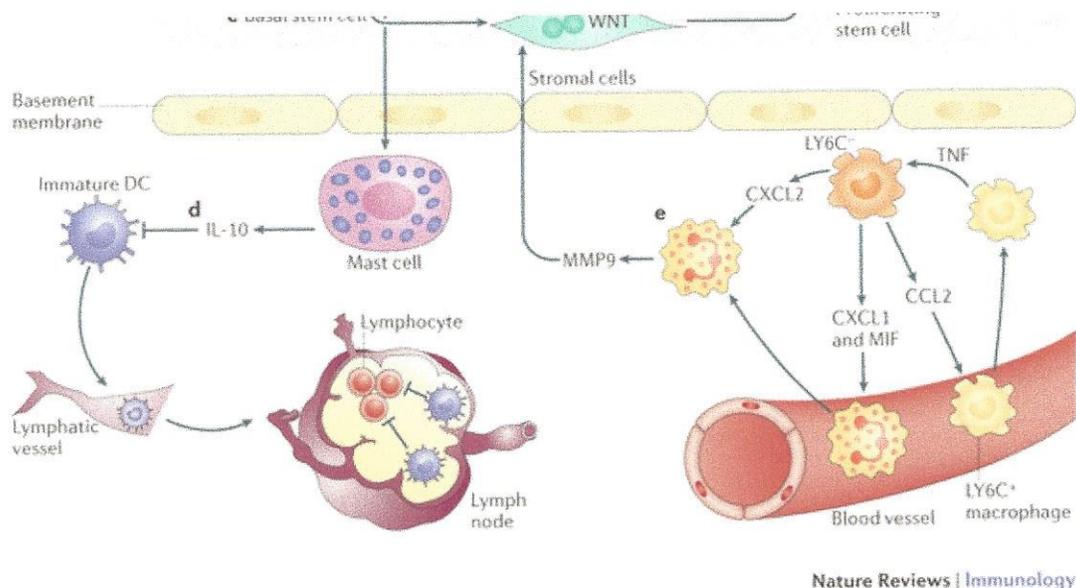
A more drastic mechanism used by the bladder to reduce bacterial load is shedding of the superficial epithelial cell layer. Observations in experimental mouse models and in patients with UTIs reveal that during the acute phases of UTIs, when the superficial BECs become heavily infected, BECs spontaneously shed in large numbers into the urine, which leads to significantly reduced bacterial numbers in the bladder<sup>30</sup>. Conceivably, massive shedding of BECs may also be a homeostatic action to temper their inflammatory responses, as excessive release of pro-inflammatory mediators can be harmful. Shedding of BECs leads to exposure of the underlying tissue to toxic components within urine, which could have severe consequences. To limit these harmful effects, the loss of the superficial epithelial cell layer is promptly followed by a shift of the urothelium from a quiescent phase to a highly proliferative state to restore the epithelial barrier<sup>31</sup>. When the stem cell-like progenitors at the basal layer of the urothelium sense injury in the overlying epithelium caused by exfoliation, they secrete sonic hedgehog (SHH), which activates the WNT signalling pathway. In turn, activation of WNT stimulates the proliferation of urothelial cells and stromal cells and the restoration of a tight epithelial barrier<sup>32</sup> (Fig. 3). This rapid turnover of cells protects the deeper tissue from toxic substances in the urine, and it enables efficient bacterial clearance while limiting pro-inflammatory responses.

*Fascinating*

**Figure 3: Mechanisms to curtail inflammatory responses in the bladder following infection.**



*see this!*



Infections of the superficial epithelium can eventually lead to extensive exfoliation of bladder epithelial cells (part a), resulting in loss of the physical barrier. Each type of immune cell possesses specialized strategies to prevent excessive inflammation and to maintain the tissue integrity. Neutrophils are rapidly expelled into the urine, possibly to reduce the tissue damage from their toxic granules (part b). Local stem cells and stromal cells underneath the intermediate epithelium sense damage to the tissue and initiate a proliferation programme to regenerate the tissue barrier (part c). Local mast cells, which were previously active in mediating a pro-inflammatory immune response, sense the damaged epithelium and switch to mediating anti-inflammatory responses to facilitate regeneration of the epithelium (part d). This switch in activity is achieved by secreting large amount of interleukin-10 (IL-10). Local IL-10 suppresses the activation of dendritic cells (DCs) but not their migration to the iliac lymph nodes. Immature DCs are unable to induce substantial antibody responses in the lymph nodes. Intimate crosstalk between LY6C<sup>-</sup> and LY6C<sup>+</sup> macrophages provides a 'double-safe' checkpoint to ensure precise initiation of neutrophil responses (part e): local LY6C<sup>-</sup> macrophages release CC-chemokine ligand 2 (CCL2), chemokine CXC-chemokine ligand 1 (CXCL1) and macrophage migration inhibitory factor (MIF) to recruit LY6C<sup>+</sup> macrophages and neutrophils from the bloodstream. Upon sensing the infection, LY6C<sup>+</sup> macrophages secrete tumour necrosis factor (TNF), which acts on local LY6C<sup>-</sup> macrophages to trigger their production of CXCL2, which induces neutrophils to cross the basal membrane. MMP9, matrix metalloproteinase 9; SHH, sonic hedgehog.

**Neutrophils.** Neutrophils are the first immune cells to be recruited to the bladder following UTIs, and they have a predominant role in bacterial clearance<sup>33</sup>. They respond to CXC-chemokine ligand 1 (CXCL1) and other chemoattractants produced by superficial bladder epithelial cells, macrophages and mast cells after activation of PRRs by various bacterial products. In mouse models of UTI, neutrophils can be detected in urine as early as 2 hours post-infection, and their numbers reach a peak by 6 hours<sup>34</sup>. The number of neutrophils closely parallels the bacterial burden in the urinary tract, and as bacterial numbers decrease, so do the number of neutrophils<sup>35</sup>. Upon entry into urinary tract tissue, neutrophils control the infection via multiple mechanisms, and this is facilitated by various soluble factors in the urine, such as pentraxins<sup>26</sup>. However, owing to the release of reactive oxygen species and other cytotoxic products, activated neutrophils are also responsible for substantial toxicity to surrounding bladder tissue. In fact, excessive neutrophil responses — including the increased expression of cyclooxygenase 2 (COX2; also known as PTGS2) — cause inflammatory damage to the bladder tissue and predispose the bladder to persistent infections<sup>36</sup>. To reach bacteria in the bladder lumen, activated neutrophils cross multiple layers of epithelial cells, including the usually impermeable superficial epithelial cell layer<sup>33</sup>. Presumably, this activity could limit the cytotoxicity of neutrophils by removing them from the bladder tissue into the urine where they can be

removed from the body during voiding.

**Macrophages.** A substantial population of macrophages resides in the submucosa of the urinary tract, and more cells are recruited to these sites following infection<sup>37</sup>. Upon activation, these macrophages produce crucial cytokines and chemokines that modulate the activity of these and other immune cells in the vicinity, which markedly influences the timing and intensity of inflammatory responses during UTIs<sup>38, 39</sup>.

Crosstalk between different subsets of macrophages in the bladder was recently shown to coordinate the precise recruitment and onset of neutrophil responses<sup>40</sup>. Resident macrophages in the bladder are mainly LY6C<sup>-</sup> and function as sentinels at this site. Following infection, these macrophages secrete the chemokines CXCL1 and macrophage migration inhibitory factor (MIF) to recruit neutrophils, and CC-chemokine ligand 2 (CCL2) to recruit LY6C<sup>+</sup> macrophages<sup>40</sup>. Upon extravasation and reaching the epithelial region, recruited neutrophils need local tumour necrosis factor (TNF) signalling to be able to cross the basal membrane of the epithelium; this requirement was evident from a study showing that transepithelial migration of neutrophils was absent in TNF-deficient mice<sup>40</sup>. Interestingly, the recently recruited LY6C<sup>+</sup> macrophages are the source of TNF, the effects of which are not directed at the neutrophils. Instead, TNF induces resident LY6C<sup>-</sup> macrophages to secrete a second wave of cytokines, mainly CXCL2, which triggers neutrophils to spontaneously produce matrix metalloproteinase 9 (MMP9) and initiate their transepithelial movement<sup>41</sup>. Thus, whereas the resident LY6C<sup>-</sup> macrophages function as the main pro-inflammatory cells, the recruited LY6C<sup>+</sup> macrophages have a key role in keeping neutrophils in close proximity before targeting the pathogen. Furthermore, the recruited LY6C<sup>+</sup> macrophages could also cause simultaneous activation of the surrounding microenvironment, as the locally generated TNF probably has a global activating effect. Paradoxically, studies have indicated that the infiltrating GR1<sup>hi</sup>LY6C<sup>+</sup> monocytes are dispensable for bacterial clearance in the bladder, suggesting that our current understanding may be incomplete<sup>37</sup>. In any case, crosstalk between bladder macrophages leads to mobilization of neutrophils into the epithelium and their subsequent activation alongside neighbouring immune cells. These immune responses ensure efficient bacterial clearance and also minimize unnecessary and damaging inflammation.

**Mast cells.** Mast cells are another resident immune cell type located underneath the uroepithelium in close proximity to blood and lymphatic vessels that traverse the mucosal region (Fig. 2). In the bladder, mast cells are also found in high numbers in the detrusor muscle region<sup>42</sup>. Mast cells have a pivotal sentinel and key immunomodulatory role during UTIs, which is partly due to their ability to release many pre-stored pro-inflammatory mediators — such as TNF, histamine and several chemokines — upon activation<sup>43</sup>. These mediators are stored within cytoplasmic granules that gradually release their cargo after being released extracellularly. Substantial amounts of histamine are detectable in the urine as early as 30 minutes after bladder infection in mice<sup>44</sup>. It is unclear how bladder mast cells become activated when the epithelium is still intact, but it is possible that products of stressed epithelial cells — which are known mast cell activators and include ATP, LL-37 and IL-33 — could contribute to this activation. In response to UPEC infection, mast cell-deficient mice show impaired neutrophil responses and decreased bacterial clearance compared with wild-type mice, which indicates an important role for mast cells in the early recruitment of neutrophils<sup>44</sup>. The number of mast cells in the mucosal region of the bladder markedly increases during bladder infection, suggesting a very dynamic role for these cells at this site<sup>44</sup>.

Whereas mast cells function as pro-inflammatory immune cells during the early phase of infection, mouse models of UTIs have revealed that when the infection progresses to a later stage, typically 6–12 hours post-infection, mast cells start to produce anti-inflammatory cytokines such as IL-10 to suppress inflammatory responses<sup>45</sup>. This switch

seems to occur in parallel with the breakdown of the epithelial barrier and could facilitate regeneration of the epithelium. There are typically two subsets of mast cells in the bladder (mucosal and connective tissue types), but it is unknown if this anti-inflammatory role is ascribable to one or both of these subsets. Nevertheless, mast cells in the bladder seem to have a dual role in immune regulation, presumably to balance the disparate needs of host defence and tissue homeostasis.

**Other innate immune cells.** Whereas natural killer (NK) cells are known to have a key role in clearing viral infections, their importance during bacterial infection is less well characterized. Recently, NK cell-depleted mice were reported to be particularly susceptible to UPEC infection<sup>46</sup>. Although the protective actions of NK cells were attributed to their production of TNF, the underlying basis of how this controls the UPEC infections remains unclear<sup>46</sup>. DCs have been shown to be highly active during UTIs<sup>47</sup>, but their specific contribution to innate immune responses in the urinary tract remains to be determined.

$\gamma\delta$  T cells are an intriguing group of resident immune cells in the urinary tract. Mice deficient in  $\gamma\delta$  T cell receptors (TCRs) are markedly more susceptible to UTIs than wild-type mice<sup>48</sup>. A study on the susceptibility of IL-17-deficient mice to UTIs showed that  $\gamma\delta$  T cells were the source of IL-17 (Ref. 49). Whether IL-17 production is the primary contribution of  $\gamma\delta$  T cells to host defence against UPEC is unclear. Innate lymphoid cells are increasingly being recognized for their contribution to mucosal immunity at various sites, but their importance in the urinary tract has not been defined.

Polymodal chemosensory cells located in the mammalian urethra represent another cell type that has recently been implicated in immune surveillance in the urinary tract<sup>50</sup>. Upon exposure to UPEC, these brush cells activate a signalling cascade composed of taste-specific G protein  $\alpha$ -gustducin, phospholipase C $\beta$ 2 and the TRP cation channel melanostatin 5 (TRPM5)<sup>50</sup>. These cells are thought to sense putative bitter receptor-activating substances associated with UPEC and release acetylcholine to communicate with neighbouring cells<sup>50</sup>. The importance of this activity in relation to the overall immune responses of the urinary tract is currently unknown.

In summary, the innate immune cells in the urinary tract rapidly respond and evoke robust immune responses during UTIs. These responses are tightly controlled, but sometimes the innate responses of the bladder are prematurely terminated, which can have detrimental effects on the host, similar to what was recently shown for the development of inadequate adaptive immune responses to infection, as described below.

### **Adaptive immune responses**

Whereas the wide-ranging innate immune responses of the urinary tract are highly responsive to infections, the adaptive immune responses, particularly in the bladder, tend to be limited. UTIs that progress to the kidneys can lead to the productions of antibodies specific for the infecting agent, but patients with infections limited to the bladder inexplicably fail to induce an antibody response<sup>51</sup>. This apparent defect in the antibody response of the bladder could be a major reason for the remarkable recurrence of UTIs, especially following bladder infection.

This clinical observation was also recently reproduced in mouse models in which UTIs that were strictly limited to the bladder induced little or no antibody response to the infecting bacteria, whereas a substantial antibody response was induced during infections of both the bladder and kidneys<sup>45</sup>. The underlying basis of the inability of the bladder to mount an adaptive response was linked to increased local IL-10 production, as IL-10-deficient mice showed substantial antibody responses to bladder infection<sup>45</sup>. As previously discussed, mast cells are a major source of

IL-10 in the bladder following bacterial infection<sup>45</sup>. Although these secretory cells have an important role in initiating a vigorous immune response in the early phases of bladder infection, mast cells seem to reverse their activity approximately 6 hours post-infection by switching to IL-10 production to shut down this response<sup>45</sup>. Mast cell-generated IL-10 can prevent the expression of co-stimulatory molecules on DCs and thereby limit their capacity to function as effective antigen-presenting cells when they traffic to draining lymph nodes<sup>45</sup>. Hence, consistent with the role of mast cell-derived IL-10 in attenuating innate immune response, the inability of the bladder to mount an antibody response to bacterial infection could be a by-product of its attempt to prevent harmful adaptive immune responses to the contents of urine, as well as to facilitate the rapid regeneration of its epithelium following infection-induced damage.

### **Immunomodulatory therapies**

As the nature of the immune responses in the urinary tract has become clearer, this knowledge could be leveraged to develop novel and effective strategies for the prevention, treatment and/or management of UTIs. Several of these strategies are targeted at boosting innate immune responses, and some target adaptive immune responses. Adaptive immune responses targeted at various components of uropathogens have been found to be particularly protective in the urinary tract provided that they are induced at sites other than the bladder. However, the efficacy of these strategies is based almost exclusively on data from experimental mouse models.

**Boosting bacterial expulsion from infected BECs.** Recent studies have revealed that the innate capacity of BECs to expel intracellular UPEC present in RAB27b<sup>+</sup> vesicles can be greatly accelerated by increasing intracellular cAMP levels<sup>27</sup>. For example, when UPEC-infected mice were treated systemically or intravesically with forskolin at various time points after infection, up to 90% of the bacterial burden was reduced compared with saline-treated mice<sup>27</sup>. Of note, forskolin treatment also reduced the secretion of the pro-inflammatory cytokine IL-6 (Ref. 27) and ameliorated the pathology associated with UTIs<sup>52</sup>. There are currently many drugs approved by the US Food and Drug Administration (FDA) that regulate intracellular cAMP levels by inhibiting phosphodiesterase 4 activity (for example, roflumilast and ibudilast), and these could potentially be used in combination with current antibiotic treatments for UTIs to increase therapeutic efficacy<sup>27</sup>. As this type of treatment is directed at signalling events in BECs, it is unlikely that uropathogens will develop resistance to the treatment, as is the case with antibiotics.

**Vaccination against bacterial virulence factors.** As the adaptive immune responses naturally elicited by bladder infections are limited, inducing memory responses by administering vaccines could also be an approach to protect against recurrent UTIs. The bacterial virulence factor fimbrial adhesin FimH has been used as an effective vaccine antigen in mouse models<sup>53</sup>. However, studies examining the efficacy of a FimH vaccine in humans were not continued because of limited immunogenicity and the lack of a safe and effective adjuvant<sup>54</sup>. With the emergence of several safe and efficacious adjuvants for human use<sup>55</sup>, it may now be appropriate to revisit the use of FimH vaccines in clinical trials. Recently, iron-chelating factors of UPEC were shown to be effective vaccine antigens in mice, and therefore these could provide another vaccine target<sup>56</sup>. Of note, the current immunization regimen involving the use of a single vaccine antigen has never evoked complete protection, as re-infection upon challenge still occurred in immunized mice, albeit at a significantly lower level<sup>57</sup>. Thus, there is a growing consensus that a multivalent vaccine directed at multiple uropathogenic virulence factors may be more effective.

**Exogenous regulation of hormones.** As elderly women are particularly prone to recurrent UTIs, ageing-

associated hormonal changes seem to be a predisposing factor. Furthermore, there is epidemiological evidence that oestrogen supplementation after menopause protects against UTIs<sup>58</sup>. Oestrogen supplementation can boost innate immunity by stimulating local production of AMPs and by strengthening the integrity of the urothelium<sup>58</sup>. Another recent study showed that administration of SR121463B — an antagonist of the vasopressin receptor V2, which is expressed in the kidney — stimulated local innate immune responses in the urinary tract in UPEC-infected mice. Thus, a hormone-based prophylactic strategy could be another effective strategy in elderly women who are prone to UTIs<sup>59</sup>.

*pH of Vaginal Canal acidic*

**Probiotics to exclude prospective uropathogens.** With the success of probiotic therapy and faecal transplantation in the gut, there is keen interest in using similar approaches to manage UTIs. As UTIs are often preceded by the presence of unhealthy microbiota in the vagina and urethra<sup>60, 61</sup>, a possible approach to prevent UTIs could be to normalize the vaginal and urethra microflora by direct administration of probiotics<sup>62</sup>. The use of strains that cause asymptomatic bacteriuria (ABU strains)<sup>63</sup> — such as *E. coli* strain 83972 or VR11 (Ref. 64) — to inoculate the urethra and even the bladder is a more provocative approach. ABU strains can potentially persist in the urinary tract for months or even years without evoking any destructive symptoms, while importantly excluding more virulent pathogens<sup>64, 65</sup>. Furthermore, ABU strains can selectively suppress host cells from inducing pro-inflammatory responses, and as a consequence, the host remains asymptomatic during infection. The effectiveness of this approach was recently revealed in patients prone to UTIs, and administration of a prototype ABU strain into the urinary tract of susceptible individuals protected them from superinfections by virulent UPEC strains<sup>65</sup>.

### Concluding remarks

The physical barrier and the antibacterial actions of urinary secretions and resident cells lining the urinary tract constitute an integrated multi-layered defence system that protects against most prospective pathogens. Paradoxically, the pathogens that typically cause UTIs are considered to be 'opportunistic pathogens' that are mostly of enteric origin, suggesting that these infections occur only because of a breakdown in one or more of the immune defences of the urinary tract. Although data for this notion are still limited, one major breakdown that we have highlighted here is the premature termination of innate immune responses to infection. Due to the overriding need to retain or restore the epithelial barrier, innate as well as adaptive immune responses in the bladder are often prematurely curtailed, predisposing individuals to either chronic or recurrent infections, often by the same bacterial strain. In addition, once infected, the architecture and cellular composition of the bladder is substantially affected, predisposing it to further infections<sup>66</sup>.

Several new but markedly different strategies to combat UTIs have emerged from studies carried out in experimental mouse models. With the sharp increase in multidrug resistance among UTIs, it is of paramount importance that these approaches are quickly advanced into the clinic for the treatment of UTIs in humans. However, determining which of these approaches is most appropriate for a particular patient may also depend on first identifying the specific defect in their immune system. Thus, in addition to isolating and characterizing the uropathogen, identifying the nature of the defect in the urinary immune system — for example, through screening for presence or absence of certain biomarkers and examining for single-nucleotide polymorphisms — may become a key requirement in the diagnosis of UTIs. This information should allow for the future tailoring of treatments for individual patients so that it is not only appropriate but also optimal.

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