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A Dynamic Interplay of Innate Immune Responses During Urinary Tract Infection

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
GAG, glycosaminoglycan; IBC, intracellular bacterial community; ILC, Innate lymphoid cell; MPO, myeloperoxidase; NET, neutrophil extracellular trap; NKT, natural killer T; PRR, pattern recognition receptor; PTX3, pentraxin 3; rUTI, recurrent urinary tract infection; UPEC, uropathogenic Escherichia coli; UTI, urinary tract infection; α-GalCer, α-galactosylceramide.

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ABSTRACT

Urinary tract infections (UTIs) represent one of the most prevalent bacterial infections globally, manifesting in diverse clinical phenotypes with varying degrees of severity and complications. The mechanisms underlying UTIs are gradually being elucidated, leading to an enhanced understanding of the immune responses involved. Innate immune cells play a crucial defensive role against uropathogenic bacteria through various mechanisms. Despite their significant contributions to host defense, these cells often fail to achieve complete clearance of uropathogens, necessitating the frequent prescription of antibiotics for UTI patients. However, the persistence of infections and related pathological symptoms in the absence of innate immune cells in animal models underscore the importance of innate immunity in UTIs. Therefore, the host protective functions of innate immune cells, including neutrophils, macrophages, mast cells, NK cells, innate lymphoid cells, and γδ T cells, are delicately coordinated and timely regulated by a variety of cytokines to ensure successful pathogen clearance.

Keywords: Urinary tract infections; Innate immunity; Cytokines; Chemokines; Host-pathogen interactions

INTRODUCTION

The urinary system comprises the upper urinary tract, including the kidneys and ureters, and the lower urinary tract, which encompasses the bladder and urethra. In clinical practice, lower urinary tract infections (UTIs) are more common than upper UTIs (1,2). This higher incidence is attributed to the proximity of the lower urinary tract to the gastrointestinal tract, exposing it to various bacteria. Patients who experience 2 or more infections within 6 months, or 3 or more within a year, are defined as having recurrent UTIs (rUTIs) (3). Since UTIs are typically treated with antibiotics, patients with rUTIs have a higher risk of developing antibiotic resistance to uropathogens (3).

A prominent pathogen causing UTI is uropathogenic *Escherichia coli* (UPEC), which accounts for 80% of infections (4). Among the fimbriae expressed by UPEC, FimH unit forms particularly strong bonds with uroplakin, which is expressed on urothelial cells, facilitating attachment and proliferation of UPEC on the epithelial surface (5). Although

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UPEC is classified as an extracellular pathogen, it has the ability to invade cells and establish intracellular bacterial niches within urothelial cells, thereby contributing to persistent and recurrent infections.

During the initial stage of UTIs, UPEC attaches to and colonizes epithelial cells, necessitating hosts to establish a solid physical barrier that hinders or inhibits the attachment of pathogens during the early infection stages. Umbrella cells, also known as superficial cells, secrete hyaluronic acid and sulfated glycosaminoglycans (GAGs) to form an extracellular matrix, which generates a GAG layer (6). Thus, the GAG layer in the bladder hinders the attachment and invasion of pathogens, thereby serving as a passive defense mechanism for the host.

Although passive defense mechanisms such as impeding bacterial colonization, are crucial in combating UPEC infections, effective host defense against pathogens necessitates active defense mechanisms. In the human body, immune cells undertake these defensive functions. Unlike other pathogens, bacteria can rapidly proliferate under suitable environmental conditions. Therefore, host is often vulnerable to infections until adaptive immunity takes effect. Innate immune cells make up for this short lag in responses and associated weakness. Clinically, rUTIs are significant as often immune responses directed at pathogens are associated with chronic infections. This involves primarily adaptive immune cells, with the role of immune effector cells being regulated by adaptive immunity. While this adaptive immunity during rUTI is beyond the scope of this review, detailed explanations can be found in the following excellently written review papers (7,8).

This review aims to emphasize the critical nature of the innate immune cells against UTIs, seeking to improve the current understanding of their contribution during infection. The innate immune response against invading pathogens is a synchronized process that involves a delicate balance of innate immune cells, various cytokines, and chemokines, all coming together to safeguard the bladder against the uropathogens. This review intends to introduce how innate immune cells and urothelial cells interact with immune effector cells to regulate defense mechanisms against these infections.

MAIN

The urinary bladder harbors a diverse array of innate immune cells, including tissue-resident innate immune cells such as macrophages, innate lymphoid cells (ILCs), dendritic cells, mast cells, and γδ T cells, which play defensive roles (**Fig. 1**). In addition, bladder epithelial cells also assist in innate immune functions. However, when these immune cells are insufficient to deal with rapidly proliferating bacterial infections, the body mobilizes non-resident immune effector cells from the bloodstream, such as neutrophils, monocytes, and NK cells, to actively participate in host defense (**Fig. 1**). Therefore, the presence of diverse immune cells in bladder tissues, complemented by the mobilization of bloodstream-immune cells when tissue-resident cells are insufficient, underscores the intricate and dynamic nature of the body's defense mechanisms against bacterial infections in the urinary tract.

Neutrophils

Although neutrophils are innate immune cells, they exhibit a behavior distinct from other tissue-resident innate immune cells by primarily circulating in the bloodstream and swiftly migrating to sites of infection upon sensing inflammatory signals, thereby exerting

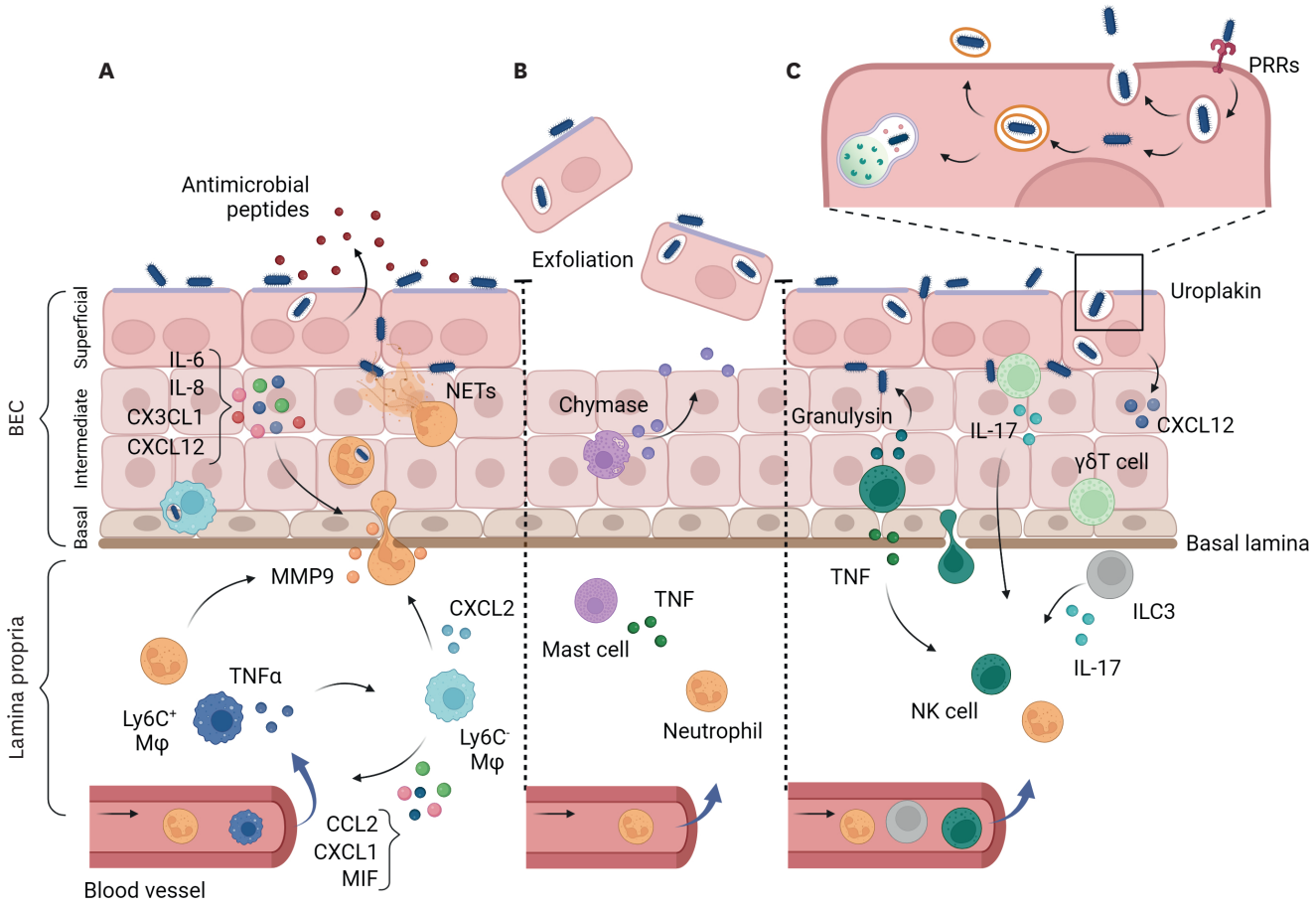

Figure 1. Dynamic response of innate immune cells in the infected bladder. A schematic representation elucidating various interactions among innate immune cells within the bladder upon infection by uropathogenic bacteria. The infection prompts an immediate mobilization of various innate immune cells, such as Mφ, neutrophils, mast cells, NK cells, innate lymphoid cells, and γδ T cells. This dynamic response is orchestrated through the action of cytokines and chemokines. The response can be categorized in 3 phases: (A) UPEC invasion is detected by PRRs on the BEC surface, such as TLR4 and TLR5, which trigger an expedited response involving the production of cytokines (IL-6, IL-8, SDF-1/CXCL12, and CX3CL1) and the secretion of AMPs. Neutrophil recruitment is prominently driven by SDF-1 and is further assisted by IL-17, TNF-α, CXCL1, CXCL2 and CXCL5. IL-8 helps in recruiting neutrophils, and IL-6 promotes the expression of CX3CL1, which then recruits Mφ. IL-6 also enhances AMP production. The resident Ly6C⁻ Mφ secrete CXCL1 and MIF to recruit neutrophils and CCL2 to recruit Ly6C⁺ Mφ. Upon TNF-α stimulation, they also secrete CXCL2, which triggers neutrophils to secrete MMP9, aiding in the degradation of the basement membrane and initiating their transepithelial movement towards the site of infection. (B) Infected BECs secrete IL-1β to induce mast cell recruitment. Mast cells secrete TNF-α to recruit neutrophils and release chymases through degranulation which play a key role in the exfoliation of the infected BECs. However, as the infection progresses, they switch to a more immunomodulatory phenotype by secreting IL-10. (C) SDF-1 is a primary recruiter of NK cells during UPEC infection. NK cells secrete granulysin to induce bacterial death during UPEC infection. Both γδ T cells and ILC3 prominently recruit neutrophils via IL-17 secretion.
BEC, bladder epithelial cell; SDF-1, stromal cell-derived factor 1; Mφ, macrophage; MMP9, matrix metalloproteinase-9; MIF, migration inhibitory factor.

antimicrobial actions (9,10). As neutrophils exit the bloodstream and migrate through tissue, they encounter barriers such as the basement membrane, which is composed of laminins expressed by basal epithelial cells, which could impede their recruitment. However, neutrophils can secrete metalloproteinase-9, which facilitates their penetration of the basement membrane (11). Having traversed this barrier, neutrophils navigate through multiple layers, including basal and intermediate cells, to reach superficial epithelial cells. Considering that colonization and invasion by UPEC predominantly occur in superficial epithelial cells, the migration of neutrophils can represent the most proactive and dynamic immune defense mechanism against bacterial invasion. The dynamics of neutrophils in response to bacterial infection in the bladder have been systematically investigated using murine models. Neutrophil infiltration typically peaks at 6 h post-infection, followed by a gradual decline in influx attributed to a reduction in bacterial load or response to anti-inflammatory cytokines (12).

Infiltrating neutrophils primarily exert antimicrobial effects through phagocytosis, using reactive oxygen species such as superoxide anion, hydrogen peroxide, hydroxyl radical, and hypochlorous acid, as well as neutral proteases such as elastase and cathepsin G, to intracellularly eradicate bacteria (13,14). CD300a, expressed on neutrophils, appears to trigger neutrophil activation and mediate UPEC killing via phagocytosis (15). Although the neutrophils infiltrating the site of infection possess intrinsic capabilities to kill pathogens, the signaling mechanism mediated by TLR4 and MyD88 triggers the secretion of pentraxin 3 (PTX3), thereby enhancing the phagocytic ability of neutrophils against UPEC and directly influencing phagosome maturation (16). The bladders of PTX3-deficient mice exhibited a significantly higher number of bacteria than normal mice, highlighting the importance of the *PTX3* gene in controlling infection (16). Additionally, clinical studies have further revealed an association between *PTX3* gene polymorphisms and acute cystitis and pyelonephritis (16).

In response to TLR stimulation, neutrophils undergo degranulation, releasing azurophilic granules along with other granule types, that help eliminate microbes within tissues. During this process, neutrophils release nuclear and plasma membrane components, leading to the formation of chromatin scaffolds of neutrophil extracellular traps (NETs) (17). This scaffold efficiently captures and exerts antimicrobial activity against invading pathogens while also minimizing damage to the surrounding host cells. The DNA scaffold of NETs primarily contains histones, myeloperoxidase (MPO), elastase, and calprotectin, which exhibit both direct antimicrobial effects and pro-inflammatory effects (18). During the process of NETosis, which is triggered by stimuli such as LPS, histone citrullination can occur, leading to the secretion of these proteins (19). In a pyelonephritis mouse model, UPEC has been reported to evade innate immunity by secreting a virulence factor known as TcpC, thereby inhibiting NETosis (20). Such neutrophil NET formation holds clinical significance in patients with UTI, as evident by its presence in urine sediment, which readily decomposes structurally via deoxyribonuclease I, forming structures containing neutrophil effectors such as histones and MPO (21,22). In this context, pyuria, a common symptom in patients with UTIs, is observed as a result of the innate immune response aiming to eliminate the infecting pathogens and protecting the bladder tissues. Although neutrophils are effective immune effector cells against bacterial infections, they can also cause collateral damage to host cells. Therefore, innate immune cells have evolved precise mechanisms to regulate the recruitment and activity of neutrophils, enabling an immediate response to bacterial infections while limiting excessive neutrophil activity to minimize host tissue damage.

Macrophages

As the most abundant innate immune cells in the bladder, macrophages play a multifaceted role in protecting tissue from bacterial infection (23,24). The robust phagocytic capabilities of macrophages and dendritic cells are activated upon the infiltration of uropathogens subsequent to the breach of superficial epithelial cells within the bladder mucosa (**Fig. 2**). These bladder tissue resident macrophages phagocytose bacterial pathogens and regulate neutrophil influx during UTIs (25). Their sentinel role in pathogen recognition and direct killing mediated by pattern recognition receptors (PRRs) is key to reducing the initial bacterial burden during the early stages of infection. Although neutrophils are highly effective in killing pathogens, their antimicrobial substances are not pathogen-specific owing to the nature of innate immune cells. The nonspecific toxic substances released by neutrophils can induce bladder tissue damage and chronic inflammation (26). Therefore, finely tuned signaling cells are necessary to regulate neutrophil influx. Following recognition of UPEC through TLRs on bladder epithelial cells, IL-8 secretion induces neutrophil

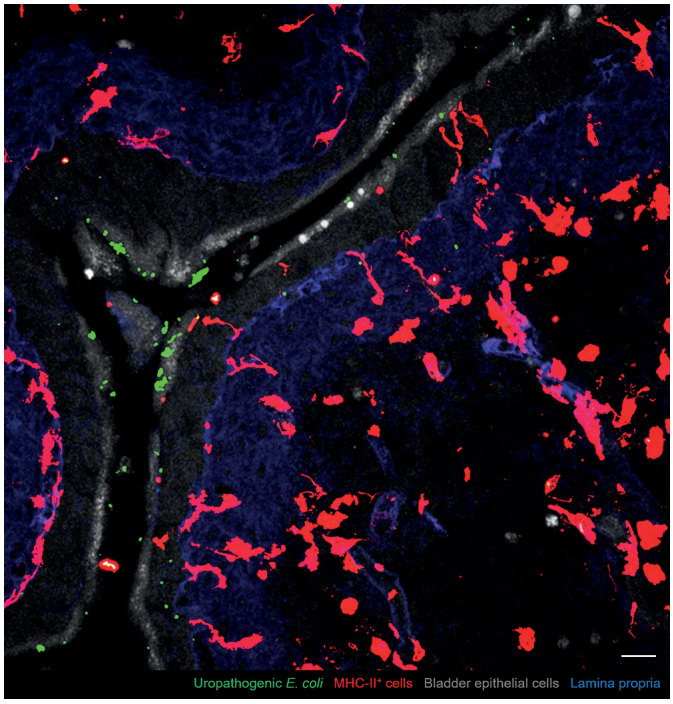

Figure 2. Phagocytes in the bladder mucosa exhibit a host-protective response by phagocytosing or contacting UPEC. C57BL/6 female mice were infected by intravesical instillation of the UPEC CI5 strain (1×10^8 CFU/mouse). After 6 h post-infection, the harvested mouse bladder was cryosectioned and stained with specific antibodies for confocal microscopic imaging. MHC class II-positive cells (red) migrate to the bladder mucosa (bright grey) where colonization by UPEC (green) has occurred. Scale bar: 20 μm.

recruitment to the infected epithelial cells (27). In addition, neutrophil influx is regulated in a more sophisticated manner by tissue-resident macrophages, enabling flexible responses to bacterial infections (28-30).

During bacterial infections, the host defense roles of resident macrophages located in the lamina propria and detrusor muscle of the bladder include effective pathogen clearance through phagocytic activity. The defensive function of macrophages against UPEC has been demonstrated in experiments depleting monocytes/macrophages using clodronate liposomes (30,31). Macrophages that predominantly reside in the lamina propria and detrusor muscle directly engage in phagocytic activity against UPEC present in bladder epithelial cells, with those in the muscle being more active in phagocytosing UPEC (25). Specifically, upon IL-6 stimulation, epithelial cells secrete CX3CL1, signaling macrophages to migrate to the infected urothelial cell layer, actively contributing to host defense (32). Macrophages employ CD14 as a coreceptor for PRRs to recognize gram-negative bacteria, thereby regulating the expression of cytokines associated with immune cell influx. Additionally, UPEC infection is exacerbated in CD14 knockout mice (31). Furthermore, in instances where UPEC infection occurs and IL-6 expression is sufficient, macrophages play a crucial role in creating an environment within the bladder that is unfavorable for UPEC growth by sequestering free irons (33). Therefore, macrophages facilitate a rapid response and clearance of pathogens near infected epithelial cells of the bladder mucosa.

However, in infected tissues with appropriate temperature and nutrient conditions, the rapid proliferation of pathogenic bacteria frequently exceeds the phagocytic capacity of macrophages. Therefore, to compensate for their limited function, macrophages swiftly

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induce a neutrophil influx into the infected tissue. Macrophages present in the lamina propria and detrusor muscle of the bladder comprise various subsets (25). Upon recognition of pathogens, Ly6C$^-$ macrophages secrete chemokines, recruiting neutrophils and Ly6C$^+$ monocytes (or macrophages) into the bladder's lamina propria (30). However, for neutrophils gathered in the lamina propria to reach the superficial epithelial cell layer, where the main infection occurs, they must traverse the basement membrane, which is densely structured with laminin and collagen IV, presenting a barrier to movement (30). Activated by TNF, Ly6C$^-$ macrophages secrete CXCL2, which promotes the release of metalloproteinase-9 by neutrophils, thereby facilitating the degradation of the basement membrane and easing neutrophil passage (30). When combined, these regulatory mechanisms suggest that the sentinel function of macrophages primarily contributes by directing neutrophils to the epithelial layer in addition to directly killing pathogens, thereby contributing to host defense.

Macrophages distributed in the bladder exhibit distinct subsets based on their location, with RNA sequencing analysis revealing unique characteristics between those present in the lamina propria and those in detrusor layer (25). Particularly, lamina propria macrophages, being closer to the site of infection, exhibit higher expression of infection- and inflammation-related genes. In contrast, detrusor layer macrophages display higher endocytic activity and anti-inflammatory properties, maintaining elevated expression of genes involved in lysosome formation (25). Consequently, following the resolution of bacterial infection, bladder macrophages undertake the removal of apoptotic neutrophils and pathogens, as well as mitigate unnecessary inflammatory responses, thereby assuming a flexible role in bladder infection control.

Mast cells

Mast cells are predominantly found in the lamina propria and detrusor of the bladder, with a significant portion distributed around blood vessels (34). During degranulation, mast cells release substances such as proteases (chymase, tryptase, and carboxypeptidase), histamine, and serotonin, which can induce various physiological responses. In particular, during UPEC infection, mast cell degranulation plays a crucial role in active immune defense. Upon UPEC infection, mast cells secrete chymase and tryptase into the epithelial cells of the bladder, directly contributing to the detachment of bladder epithelial cells and the disruption of the urothelial barrier (35,36). Bladder epithelial cells are the initial site of UPEC infection, and a significant portion of UPEC forms quiescent intracellular reservoirs within these cells, thereby contributing to recurrent infections. During this process, infected epithelial cells undergo exfoliation, previously attributed solely to cell death induced by UPEC infection (37). Recent findings suggest that epithelial exfoliation is facilitated by mast cell-derived chymase, which activates caspase-1 and induces cytolytic cell death, thereby promoting detachment. Notably, this phenomenon of infected epithelial cell exfoliation and urinary voiding represents an effective host defense mechanism that reduces the number of UPEC within the bladder.

An intriguing aspect of mast cells is their role in host defense during the early stages of infection by inducing exfoliation. However, as the infection progresses, these cells undergo a functional switch towards immunoregulation. During the late stages of infection, activated mast cells secrete various cytokines, including IL-10, among other cytokines, through *de novo* synthesis. Comparative studies of mast cell conditional knock-out mice that lack production of IL-10 and control mice revealed that IL-10 plays a key role in dampening adaptive immunity (38). Notably, in the absence of IL-10 secretion, mast cell conditional knock-out mice exhibited lower levels of persistent infection compared with control mice,

indicting their role in suppressing adaptive immunity while contributing to the maintenance of persistent infection (38). This shift in mast cell function from promoting exfoliation to regulating immune responses underscores the dynamic and context-dependent nature of mast cell-mediated host defense mechanisms during infection. In fact, the function of IL-10 in suppressing adaptive immunity may not typically align with the host's defense against bacterial infections. However, considering that mast cell chymase-mediated epithelial cell exfoliation disrupts the barrier function of the bladder through invasive defense mechanisms, the effect of IL-10 secretion in reducing inflammation can be considered to play a beneficial role in tissue regeneration. Bladder exfoliation, particularly resulting in the loss of barrier function, can lead to severe pain upon urine contact. Therefore, a series of processes aimed at inhibiting the inflammatory response can be considered a trade-off that does not significantly compromise bladder homeostasis.

Lastly, whether the time-delayed responses of mast cells represent sequential activation of different functions within a single mast cell population or if multiple distinct subsets of mast cells with opposing functions are involved remains unclear. Mast cell fate mapping studies have revealed morphological and transcriptomic differences between mast cells derived from the yolk sac and those derived from the bone marrow (39). Moreover, an analysis of mast cell populations using single-cell RNA sequencing has identified subsets of mast cells with distinct signature genes (40). While these findings suggest the presence of functionally diverse mast cell subsets, further research is required to determine whether these mast cell subsets elicit different responses during UPEC infection.

NK cells

NK cells are known for their crucial role in combating viral infections and suppressing tumors, but they have also been reported to play a role in host defense against bacterial infections. This defensive function extends to both intracellular and extracellular bacterial infections, with well-documented roles in defending against pathogens such as *Bacillus* and *Mycobacterium* (41-43). The host defense function of NK cells during UTIs caused by UPEC is also known to be significant (44). During UTI infection, bladder epithelial cells secrete stromal cell-derived factor 1, initiating immune cell influx, including recruitment of NK cells (45,46). NK cells secrete cytotoxic factors such as perforin, granzyme, and granulysin, which induce damage and distortion of bacterial cell walls, suppress energy generation, and exhibit antibacterial effects against pathogenic bacteria (42). Specifically, FimH component of UPEC's type I fimbriae acts as a ligand for TLR4, leading to activation of NK cells through the downstream signaling mechanism involving TLR4-MyD88. This activation results in the secretion of TNF-α by NK cells (44,47), which in turn plays a crucial role in controlling bacterial bladder infections in the body (44). Interestingly, researchers observed that depletion of NK cells by using an NK cell-neutralizing antibody did not impact the bacterial clearance in the infected bladder (48). However, NK cells secrete cytokines such as IFN-γ and GM-CSF, creating an inflammatory environment that helps maintain effective immune regulation (49,50).

Secretion of hemolysin A by UPEC significantly impairs the host defense function of NK cells, primarily attributed to the cytotoxicity of hemolysin A (44). Hemolysin A secretion by UPEC is a strategy to evade host defense being mounted by the innate immune cells. However, whether NK cells exhibit their intrinsic function, such as controlling the number of intracellular bacteria, during bladder infections has not been reported. Although NK cells are well-known for their role in viral infections, which involves secretion of IFN-γ and

inducing the death of infected host cells containing intracellular pathogens, there is currently no evidence from animal studies demonstrating whether NK cells regulate quiescent intracellular bacterial niches or control persistent infection by uropathogens. However, it is plausible that the normal function of NK cells in overcoming intracellular survival by uropathogens contributes to the recovery of several patients who do not progress to persistent or rUTIs. However, experimental data to prove this hypothesis is lacking.

ILCs

ILCs lack antigen-specific receptors of T cells and secrete specific cytokines to activate or modulate mucosal immunity. Residing mainly on the mucosal surfaces of intestine or lungs, ILCs rapidly respond to pathogenic infections and regulate immune cell functions to play a defensive role against infections (51). Although their role in the bladder has been recently understood, bladder-resident ILC subsets mainly consist of ILC2 and ILC3, with ILC1 present in smaller numbers (52). In particular, bladder ILC3 exhibits a cellular phenotype similar to $CCR6^{+}$NKp46-LTi cells (48,52). During UPEC infection, ILC3 is believed to play a primary defensive role, as evidenced by increased UPEC burden in ILC3-deficient mice, highlighting the importance of bladder-resident ILC3 in UPEC infection (52). Single-cell RNA sequencing analysis of UPEC-infected mouse bladders revealed ILC3 as one of the IL-17 producers, indicating the crucial role of ILC3-mediated type 17 immunity in host defense, alongside γδ T cells and Th17 cells (53).

During UPEC infection of the bladder, concurrent functions of neutrophil influx mediated by ILC3-IL17A and macrophage-mediated neutrophil influx in the lamina propria can be considered to have overlapping roles. Additionally, it also serves as evidence of the significance of neutrophil influx in response to bacterial infection. Nevertheless, it is necessary to examine the differences in the role of IL-17A in these 2 distinct influx pathways. While Th17 cells can effectively respond to UPEC infections through IL-17 production, the time constraints involved in induction of adaptive immunity by Th17 cells against bacterial infections may necessitate entrusting this function to innate immune cells, reflecting a natural design in the body.

IL-17 stimulates epithelial, endothelial, and fibroblast cells to produce chemokines (CXCL1, CXCL2, and CXCL8), directly promoting neutrophil recruitment. Additionally, IL-17-stimulating endothelial cells increase the expression of ICAM-1, facilitating an easy migration of neutrophils across the endothelial barrier to the site of infection. Neutrophils recruited to the site of infection not only serve as survival signals but also enhance the bactericidal activity against the pathogens they encounter. Through these host defense mechanisms, IL-17 plays a crucial role in defending against UPEC, a pathogen commonly responsible for bladder infections. However, if IL-17 is not properly regulated after the resolution of bladder infection, it may lead to the development of various bladder diseases.

IL-17 dysregulation has been associated with excessive neutrophil accumulation in various organs, contributing to inflammatory autoimmune diseases such as psoriasis, rheumatoid arthritis, and inflammatory bowel disease (50,54). In the bladder, an increase in IL-17 levels has been reported in some disease conditions, most notably interstitial cystitis (55). Interstitial cystitis is a chronic condition characterized by bladder pain, urinary frequency, and urgency, with no clear known cause. However, elevated IL-17 levels in some interstitial cystitis patients suggest a potential association between IL-17-mediated inflammation and the pathophysiology of the disease. While interstitial cystitis is not typically associated with

infections, numerous patients diagnosed with interstitial cystitis have a history of recurrent bladder infections (56). Dysregulation of IL-17 signaling following previous infections could potentially lead to symptoms of interstitial cystitis after the infection has resolved. However, systematic studies are required to further investigate this relationship. In summary, IL-17 plays a crucial role in mediating immune responses at inflammatory and infection sites by recruiting neutrophils, but its dysregulation can also contribute to the pathology of inflammatory diseases.

γδ T cell

γδ T cells occur in peripheral tissues such as the skin, intestines, and lungs, and they are also present in significant numbers in the bladder. For instance, in uninfected murine bladders, they constitute 1%–4% of the immune cell population (23). Strategically positioned to swiftly respond to invading bacterial pathogens that breach these barriers, these cells are characterized by their ability to rapidly produce large amounts of cytokines, exerting an influence on the surrounding cells and tissues. Notably, γδ T cells have been reported as a major source of IL-17 production in the bladder (57). As previously stated, IL-17 plays a crucial role in neutrophil-mediated bacterial defense, making γδ T cell deficiency in mice phenotypically associated with decreased host defense against bacteria (57,58). Following UPEC infection, IL-17A is secreted in the bladder of infected mice as part of the innate immune response, contributing to host defense efficacy by reducing bacterial burden, as evidenced by studies using IL-17A$^{-/-}$ mice or anti-IL-17 neutralizing antibody mediated IL-17 depletion (48,57). Furthermore, IL-17A$^{-/-}$ mice exhibit a sharp decrease in the number of neutrophils infiltrating the infected bladder, resulting in a diminished host protective role against bacterial infections (57).

Given the predominant presence of γδ T cells in the bladder mucosa, we can reasonably anticipate their direct response upon the initiation of UPEC invasion into the epithelial layer. γδ T cells recognize non-peptide antigens via the γδ TCR or are activated by stress-induced signals. However, the mechanisms through which they recognize UPEC or how UPEC, while forming quiescent intracellular reservoirs in host epithelial cells, induces stress signals that may be associated with γδ T cell activation are not well understood. Further research is required to determine whether γδ T cells are activated directly by stress-induced signals during UPEC infection.

Natural Killer T (NKT) cells

CD1d-restricted NKT cells play a crucial role in host defense against pathogens through innate immunity. When activated by α-galactosylceramide (α-GalCer), they possess the ability to eliminate bacterial infection, as demonstrated in UTIs caused by pathogen such as methicillin-resistant *Staphylococcus aureus* (59). In a UTI mouse model, administration of α-GalCer prior to bacterial infection resulted in robust preventive antimicrobial effects, which were attributed to the direct involvement of IL-12, IFN-γ, and TNF-α (59). While this study primarily observed the defensive function of NKT cells in an activated state induced by α-GalCer rather than direct activation of NKT cells in response to UTIs, the findings hinted at the correlation between cytokine secretion and type I response, potentially leading to antimicrobial effects. Although this study did not specifically investigate the specific mechanisms underlying the protective effects of cytokine production against bacterial infections, it is conceivable that similar mechanisms, such as enhanced phagocytosis by alveolar macrophages mediated by IFN-γ upon NKT cell activation in pulmonary infections (60), may also operate in UTIs. Further research is warranted to elucidate the precise

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mechanisms by which cytokines contribute to host defense against UTIs mediated by NKT cell activation.

Urothelial cells

The expression of uroplakin by bladder umbrella cells facilitates the attachment of UPEC fimbriae, thereby promoting infection, with the attached UPEC serving as a reservoir for persistent infection within the epithelial cells (61,62). While playing a role in providing a foothold for infection, bladder epithelial cells also actively engage in various defense strategies against infection by directly participating in innate immunity.

First, these defense mechanisms are facilitated by the presence of PRRs, which serve to alert bladder epithelial cells to the onset of infection (63). Particularly in UTIs, TLRs play a pivotal role as they recognize microbial components and initiate intracellular signaling, thereby triggering the secretion of various cytokines, chemokines, and AMPs. Although TLR4 and TLR5 are widely recognized as the most crucial subfamily members for host defense against UTIs, TLR2 and TLR11 also contribute to these functions to some extent (64). The significant decline in the ability of $TLR4^{-/-}$ mice to clear pathogenic bacteria from the bladder underscores the pivotal role of TLR4-mediated host responses in determining susceptibility to UTIs. While LPS is well-known as a pathogenic factor of UPEC that stimulates TLR4, other UPEC components such as FimH adhesin, type I, and P fimbriae can also be recognized by TLR4 (65,66). Furthermore, $TLR5^{-/-}$ mice are significantly more susceptible to UPEC due to a limited cytokine and chemokine response to flagellin during UPEC infection (67). Therefore, bladder epithelial cells seem to be designed to ensure host defense through a robust innate immune response by recognizing various types of pathogen-associated molecular patterns from UPEC.

Stimulation of TLRs in bladder epithelial cells triggers the production of cytokines that play a pivotal role in initiating innate immune responses. Among these cytokines, ILs have been extensively studied for their direct involvement. Specifically, IL-6 facilitates the activation of transcription factors, leading to the expression of AMPs such as Hamp, RegIIIβ, and RegIIIγ in the bladder epithelium (68). AMPs such as β defensin 1 (69,70), cathelicidin (71), and ribonuclease 7 (72) contribute to the formation of an antimicrobial barrier, thereby preventing microbial colonization and providing mucosal immunity. IL-8, also known as CXCL8, is a chemokine that is directly involved in the recruitment of neutrophils and is secreted, in response to TLR4 stimulation in bladder epithelial cells, serving as a key chemokine that forms a chemotactic gradient to facilitate the transepithelial migration of neutrophils into the infected bladder epithelial layer (73,74). Particularly, mice with deletion mutations in IL-8 receptor homologues experienced significant challenges in bacterial clearance in the bladder and kidneys, whereas patients with low expression of CXC chemokine receptor 1 exhibited a higher incidence of acute pyelonephritis, indicating a clinical association (74). Through these mechanisms, bladder epithelial cells play a role in inhibiting the formation of intracellular bacterial communities (IBCs) and ultimately contributing to direct UPEC clearance.

Bladder epithelial cells not only defend against bacteria through the influx of immune cells but also exert a physical defense mechanism by expelling pathogens outside the cell. Specifically, a considerable number of epithelial cells often expel intracellular UPEC that invades them. TLR4, stimulated by LPS, regulates this active defense mechanism (64,75). In other words, UPEC that invades bladder epithelial cells initially resides in $Rab27b^{+}$ vesicles

within the cell. Upon stimulation of TLR4, increased cAMP levels cause exocytosis of Rab27b^{+} vesicles containing UPEC, expelling the bacteria outside the cell (64). In addition, epithelial cells possess a second system for expelling intracellular UPEC outside the cell, which involves the mucolipin TRP channel 3. This mechanism facilitates the exocytosis of UPEC hidden in lysosomes, by expelling the bacteria outside the cell enclosed in exosomes (76). The existence of multiple pathways for expelling intracellular bacteria from bladder epithelial cells potentially serves as a series of defense mechanisms aimed at removing persistent UPEC, which demonstrates a strong ability to form IBCs.

Finally, lysosomal proteases present within epithelial cells also play a crucial role in bacterial defense. Despite the mechanisms of intracellular bacterial expulsion, these lysosomal proteases can be understood as a resistance mechanism against bacteria causing persistent infection within the cells. Specifically, cathepsin, one of the proteases expressed in epithelial cells, possesses bactericidal effects. Considering the higher bacterial burden in cathepsin$^{-/-}$ epithelial cells, it can be interpreted as a direct host defense mechanism (77). Furthermore, a recent study has shown that cathepsin D expression is not only regulated by commensal bacteria present in the bladder but also increases when *Lactobacillus crispatus* is introduced into the bladder, thereby enhancing host defense against UPEC (78). Taken together, we can conclude that bladder epithelial cells, employ various strategies to protect against infection, adapting their defense mechanisms based on the timing and location of the infection.

CONCLUSION

Various pathogens can infect the urinary tract, but protection against bacterial infections requires a rapid host response compared to viral or fungal infections. This is because bacterial pathogens proliferate rapidly under suitable conditions, necessitating a vigorous innate immune response in the urinary tract during the initial stages of infection. The role of innate immune cells during UTIs is summarized in **Table 1**. Responses such as bacterial expulsion by bladder epithelial cells or the secretion of AMPs serve as host defense mechanisms to reduce the number of pathogens in the epithelial cell layer during the early stages of infection. Despite these initial responses, pathogens adapt to colonize and invade epithelial cells, prompting various components of the innate immune system to act, with neutrophils playing a pivotal role. Tissue-resident macrophages, γδ T cells, urothelial cells, mast cells, and other immune cells employ different strategies, ultimately aiming to recruit neutrophils to the site of infection, where they exhibit bactericidal effects against invading pathogens. Despite the stepwise responses of innate immune cells against UTIs, chronic infection may arise, possibly due to bacterial persistence within the urothelial cells during the initial stages of infection. Understanding why bacteria causing intracellular infections evade immune cells involved in adaptive immunity requires the establishment of appropriate animal models and the use of various immunological analysis methods. Despite the stepwise responses of innate immune cells to UTIs, chronic infection may occur, possibly attributed to pathogens hiding within urothelial cells during the initial stages of infection. Therefore, activating the innate immune response through various methods during the early stages of infection may serve as a crucial rate-limiting factor in preventing the exacerbation of chronic bladder infections.

Although antibiotic treatment is currently the best option for UTIs, several therapeutic options that boost the activation of host innate immunity are under investigation with the

Table 1. Functional roles of innate immune cells against uropathogens

Cells	Function	References
Macrophages	Two distinct populations of macrophages reside in the bladder	(25)
	Directly kill pathogens and recruit neutrophils via CXCL1 and MIF	(30)
Neutrophils	Release IL-1β, MMP9	(30,79,80)
	Release fibrous DNA-histone complexes, called NETs	(21,22)
	Exert antimicrobial effects via phagocytosis and ROS generation	(13)
	Enhanced expression of CD300	(15)
Mast cells	Activated by exosomes from bladder epithelial cells	(36)
	Secrete TNF, histamine; switch to anti-inflammatory later and maintain balance between pathogen clearance and tissue maintenance	(38,81)
	Secrete proteases such as chymase, tryptase etc. to induce exfoliation of BECs	(35,82)
NK cells	Produce TNF-α to aid in bacterial clearance	(44)
	Activated by FimH via IFN-γ and TNF-α	(47)
	Secrete perforin, granzyme, and granulysin to induce bacterial death	(83)
ILC3	ILC3 cells are of 3 types: $CCR6^+$ LTi-like cells (majority) secrete IL-17, NCR^+ ILC3, and NCR^- ILC3s	(48,52)
	Secrete IL-17 and recruit neutrophils	
γδ T cells	Play a regulatory rule during UTI; one of the main source of IL-17A in the bladder tissue during infection	(57)
NKT cells	Have the capacity to kill the bacteria upon activation by α-GalCer	(59)
Urothelial cells	Express TLRs for pathogen recognition	(67,80,84)
	Secrete proinflammatory cytokines such as IL-6, IL-8, etc.	(85,86)
	Secrete antimicrobial proteins such as cathelicidin-related AMPs, β-defensin 1, ribonuclease 7, etc.	(70-72)
	Secrete SDF-1/CXCL12 for recruiting NK cells, T cells, and neutrophils	(45)

MMP9, matrix metalloproteinase-9; ROS, reactive oxygen species; BEC, bladder epithelial cell; MIF, migration inhibitory factor; SDF-1, stromal cell-derived factor 1.

hope of reducing antibiotic resistance. One promising therapeutic approach involves utilizing commensal microbiota residing in the bladder. Studies have shown that commensal bacteria strains, such as *Lactobacillus crispatus*, have been effective in preventing the predominance of UTI-causing strains. This is achieved by augmenting the elimination of intracellular UPEC via the secretion of type I IFNs. These IFNs have downstream effects on infected bladder epithelial cells, enhancing the acidity and degradative proficiency of lysosomes that harbor UPEC (78). Another potential approach involves combining local vaccination with Th1-skewing adjuvants, which stimulate innate immune receptors, such as TLRs, on dendritic cells and B cells. Administration of UPEC lysates or a prominent UPEC antigen alongside the Th1-skewing adjuvant CpG oligodeoxynucleotides has shown significant bacterial clearance in infected bladders. This treatment also yielded promising results in mice with rUTI, indicating potential as a therapeutic tool for recurrent cases (87). More in-depth studies focusing on UTI pathogenesis and innate immune responses are needed to design therapeutics that can stimulate immune responses while complementing existing treatments. These refined strategies could provide more effective solutions against UPEC infections and prevent their progression to rUTIs.

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