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Tissue Immunity in the Bladder

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Abstract

The bladder is a major component of the urinary tract, an organ system that expels metabolic waste and excess water, which necessitates proximity to the external environment and its pathogens. It also houses a commensal microbiome. Therefore, its tissue immunity must resist pathogen invasion while maintaining tolerance to commensals. Bacterial infection of the bladder is common, with half of women globally experiencing one or more episodes of cystitis in their lifetime. Despite this, our knowledge of bladder immunity, particularly in humans, is incomplete. Here we consider the current view of tissue immunity in the bladder, with a focus on defense against infection. The urothelium has robust immune functionality, and its defensive capabilities are supported by resident immune cells, including macrophages, dendritic cells, natural killer cells, and γδ T cells. We discuss each in turn and consider why adaptive immune responses are often ineffective in preventing recurrent infection, as well as areas of priority for future research.

INTRODUCTION

The bladder is a hollow, spherical organ in the lower pelvis. It is part of the urinary tract, which comprises paired kidneys, each with a connecting ureter that transports urine to the bladder, where it is stored prior to voiding via the urethra (1). The urinary tract is responsible for expelling metabolic waste and surplus water from the body; this inevitably requires proximity to, and an interface with, the external environment, providing a portal for infection. Although the bladder has historically been viewed as sterile, there is a growing body of evidence that points to a urinary microbiome that is present in homeostasis (2–4). Therefore, the bladder's mucosal surface, like that of the gut, must resist invasion by pathogens while permitting colonization by commensal microbes. In light of this, and the increasing awareness of the importance of tissue immunity in nonlymphoid organs across the body, there is significant interest in delineating immune cell heterogeneity and function in the urinary tract.

Tissue immunity in the kidney has been reviewed in detail elsewhere (5–7). In this review we focus on immunity within the lower urinary tract, and specifically within the bladder, summarizing the current knowledge of resident and infiltrating immune populations within the bladder and describing their roles in homeostasis and disease. We first provide an overview of the unique structure and function of the bladder. We then discuss the key immunological challenges to which it is subject, describe the immunological capability of the urothelium, and finally detail the composition and function of innate and adaptive immune cells within the bladder.

THE BLADDER—STRUCTURE AND PATHOLOGY

Anatomy

The bladder wall includes several distinct layers, from the outermost serosa, which interfaces with the peritoneal cavity, to the muscularis propria, lamina propria, and an inner lining of epithelium (urothelium) (**Figure 1**)—all specialized for the unique physical environmental challenges of the lower renal tract (8). Embedded within these layers is a network of blood and lymphatic vessels, visceral and motor neurons, and several populations of tissue-resident immune cells. The urothelium sits at the interface between the urinary space and the underlying bladder wall, forming a barrier resistant to ion, solute, and water influx as well as pathogens. It is further divided into three distinct layers (**Figure 1**): a basal layer composed of cuboidal cells anchored to a basement membrane via desmosomes, an intermediate layer of pyriform cells, and a superficial layer of highly specialized hexagonal cells known as umbrella cells. These multinucleated cells fuse as they rise to the surface, forming sealed tight junctions that create a protective and impermeable barrier (1, 8). A glycoprotein layer of uroplakins adds to the barrier, forming a superficial plaque covering the apical surface of the umbrella cells (9).

Disease of the Bladder

The bladder faces several immunological challenges. Because of its relative proximity to the urethral orifice in the perineum, particularly in women, it is exposed to microbes (frequently gram-negative bacteria) that originate in the lower gastrointestinal tract and migrate across the perineum to the urethra and upward into the bladder. Other pathogens show tropism for the bladder, most notably the helminth *Schistosoma haematobium*, endemic in some areas of Africa and the Middle East. Additionally, the bladder may be subject to chronic inflammation, termed sterile interstitial cystitis, and is also a common site of malignancy.

Urinary tract infections (UTIs) are among the commonest infections worldwide, with over 150 million cases per year. The financial impact on health care systems is substantial, with

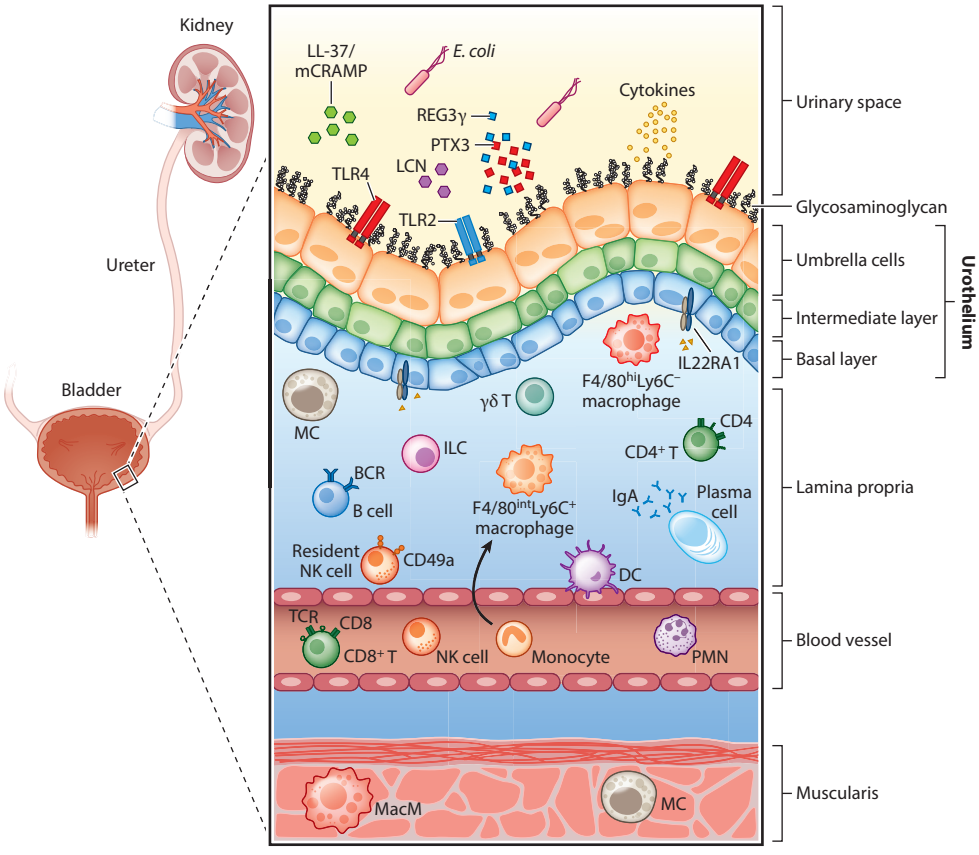


Figure 1

Functional anatomy and immunity of the bladder in homeostasis. The urinary tract consists of paired kidneys; a connecting ureter for each to conduct urine; and the bladder, which stores the urine prior to voiding. Together the kidneys maintain homeostasis by excreting waste products of metabolism and maintaining water and electrolyte balance. The bladder comprises distinct layers, from the outermost serosa to the muscularis propria, the lamina propria, and an inner lining of epithelium (urothelium). The urothelium can be further divided into a basal layer composed of cuboidal cells, an intermediate layer of pyriform cells, and a superficial layer of specialized umbrella cells, all of which form a surface impermeable to the external environment. In homeostasis the bladder is protected by a series of passive defenses including a mucinous layer of glycoproteins coating the superficial surface of the umbrella cells, a succession of TLRs, and antimicrobial peptides. Recent studies have demonstrated that the bladder contains a dense network of tissue-resident immune cells within the lamina propria and muscularis. The principal immune cells within the bladder are antigen-presenting cells, including F4/80^{hi}Ly6C^{-} resident macrophages and CD11b^{+} and CD103^{+} DCs. The lamina propria also contains NK cells expressing markers of residency such as CD49a, MCs, CD4^{+} αβ T cells, γδ T cells, ILCs, B cells, and IgA-secreting plasma cells. Neutrophils and CD8^{+} T cells are likely to be predominantly intravascular in the homeostatic bladder, while monocytes can be recruited from the circulation and differentiate into F4/80^{int}Ly6C^{+} inflammatory macrophages. CX$_3$CR1^{lo} (MacM) macrophages are positioned within the muscularis along with a population of MCs. Abbreviations: BCR, B cell receptor; DC, dendritic cell; ILC, innate lymphoid cell; LCN, lipocalin; MC, mast cell; mCRAMP, murine cathelin-related antimicrobial peptide; NK, natural killer; PMN, neutrophil; PTX3, pentraxin 3; TCR, T cell receptor; TLR4, Toll-like receptor 4.

estimated costs exceeding 6 billion USD annually for treatment and management (10). UTIs can affect the upper (pyelonephritis) or lower (cystitis) urinary tract—a distinction that is pertinent to both management and prognosis. Cystitis is exceedingly common and affects over half of women and 5% of men in the United States at least once in their lifetime (10). Typical symptoms include urinary frequency, pain on passing urine (dysuria), and lower abdominal pain. The proportion of patients with cystitis that progresses to pyelonephritis is low (<2%) (10), with a variety of host and pathogen factors influencing susceptibility to ascending infection. In 75–95% of uncomplicated UTIs, uropathogenic *Escherichia coli* (UPEC) is the causative organism. Other prevalent gram-negative and -positive organisms include *Klebsiella pneumoniae* (6.2%), *Staphylococcus saprophyticus* (6%), *Enterococcus faecalis* (5.3%), *Proteus mirabilis* (2%), and *Pseudomonas aeruginosa* (0.9%) (11). Despite prompt diagnosis and initiation of appropriate antibiotic therapy for UTI, a subset of patients will go on to develop renal scarring as a result of recurrent pyelonephritis (12–14) and ultimately progress to end-stage renal failure (15). Indeed, the underlying diagnosis for approximately 10% of patients requiring dialysis for end-stage kidney failure in the United Kingdom is chronic pyelonephritis (16), with substantial socioeconomic costs.

The bladder is a common site of cancer, accounting for up to 3% of cancer diagnoses globally in 2020 (17), with a historical incidence three to four times higher in men than in women (18). However, despite a lower incidence, women show earlier tumor progression and invasion (19), with sex hormones likely playing a role in variable outcomes (20, 21). The majority of patients have non-muscle-invasive bladder cancer (NMIBC) (22), where intravesical delivery of bacillus Calmette-Guérin (BCG) after transurethral resection is the gold-standard treatment (23). BCG immunotherapy induces robust immune cell infiltration into the bladder (24) and provides useful information on the nature of tissue immune responses to local challenge with pathogen-associated molecules.

BLADDER TISSUE IMMUNITY

Nonimmune Structural Cells

Immune function within organs is not limited to immune cells; nonimmune tissue cells can also play a part. For example, we showed that pelvic epithelial cells in the kidney express antimicrobial peptides (AMPs) and neutrophil-recruiting chemokines that defend the kidney from ascending infection (6). Krausgruber et al. (25) profiled a wide range of mouse organs and demonstrated varying expression of immune mediators, as well as cytokines and chemokines, in epithelia, fibroblasts, and endothelia, that provided so-called structural immunity. Although the bladder was not included in this study, urothelial cells display a number of unique adaptations to prevent the invasion of bacteria translocating from the gastrointestinal tract (**Figure 1**). Firstly, the uroplakin plaques produced by urothelium confer a physical barrier to microorganisms. Furthermore, urothelial cells are coated with a proteoglycan- and glycosaminoglycan-rich mucous layer that contributes to antibacterial defense (26, 27). Urothelial cells can also produce a number of AMPs, including uromodulin (also known as Tamm-Horsfall protein), which binds type 1 pili expressed by UPEC, preventing adhesion to the urothelium (28). Other AMPs include REG3γ, lipocalin 2 (LCN2), lactoferrin, S100A9, pentraxin 3 (PTX3), and cathelicidin. REG3γ is the most upregulated AMP in the murine bladder after UPEC challenge, and increased concentration of its human ortholog, hepatocarcinoma-intestine-pancreas (HIP)/pancreatitis-associated protein (PAP) has also been found in human urine during UTIs (29). However, *Reg3γ*-deficient mice showed no impairment in bladder UPEC defense in terms of increased bacterial colony-forming units (CFUs), suggesting functional redundancy (29). LCN2 sequesters iron, an element critical for UPEC survival, consistent with the observation that *Lcn2*-deficient mice show increased bacterial counts in the bladder

after UPEC challenge (30). Similarly, lactoferrin, another iron chelator, is increased in the bladders of infected mice, and administration of lactoferrin reduces bacterial burden and neutrophil infiltration (31). Cathelicidin is cleaved to produce the AMP LL-37 in humans or murine cathelin-related AMP (mCRAMP) in mice. Cathelicidin is expressed by bladder urothelial cells, and mCRAMP-deficient mice have increased bacteria in the bladder after UPEC challenge (32). PTX3 is capable of opsonizing bacteria to enhance macrophage phagocytosis, and *Ptx3*-deficient mice were found to be more susceptible to bacterial cystitis (33). This study suggested that PTX3 secretion by urothelial cells was mediated via cell-intrinsic Toll-like receptor 4 (TLR4)-MyD88 signaling (33).

Urothelial cells are not only able to respond to pathogen-associated molecules via TLR4 engagement but may also be instructed by resident or infiltrating immune cells (**Figure 1**). For example, human primary urothelial cells both express the membrane-associated IL-22 receptor subunit IL22RA1 and respond to ex vivo IL-22 stimulation by producing S100A9 and LCN2 (34).

The urothelial barrier is also able to exfoliate in response to bacteria binding to the uroplakins on the surface of umbrella cells (35), leading to shedding of bacteria into the urinary space (**Figure 2**). This process is dependent on bacterial type 1 pili and epithelial cell caspase activation (36). Other physical methods of expulsion include exocytosis of bacteria enclosed within Rab27b-expressing fusiform vesicles (37). Autophagy may also play some role in UPEC elimination, as *Atg3*-deficient mice show increased bacterial loads in the bladder early after infection (38). In summary, the urothelium has substantial immune capability and plays a central role in bacterial defense.

Fundamental Principles and Experimental Models of Immune Cell Tissue Residency

Despite the immune function of urothelium, bladder homeostasis and defense also require tissue-resident immune cells. There is increasing appreciation, supported by murine experimental models, that many innate and adaptive immune cells reside within nonlymphoid organs and do not recirculate (39). Initially, it was shown that antigen-specific $CD8^+$ T cells, and later that $CD4^+$ T cells, entering tissues during challenge persist long after the resolution of infection (40–42). These long-lived tissue-resident memory (Trm) cells enable rapid clearance of a secondary infection with the same pathogen. In addition, some innate and innate-like immune cell subsets are seeded to organs during development and renew through homeostatic proliferation or via replenishment from local hematopoietic progenitors (43). The exemplar innate tissue-resident cell type is the macrophage. Studies of tissue macrophages have defined principles that can be applied to define residency. Broadly, these are long-lived, self-renewing populations with tissue-specific specialization and specific roles in organ homeostasis.

Two approaches have been applied in mice to establish the tissue residency status of an immune cell. The most robust of these is the parabiosis model (44), in which the circulatory systems of two mice expressing a congenic surface marker (most often CD45.1 versus CD45.2) are surgically joined. After four weeks, equal numbers of donor (e.g., CD45.1) and recipient (e.g., CD45.2) cells would be expected for any recirculating leukocyte population, whereas tissue-resident populations remain donor derived (42, 45–47). The second, more pragmatic, approach is the use of an in vivo intravascular anti-CD45 antibody to label circulating immune cells prior to organ harvest. Theoretically, tissue-resident cells outside of the vasculature remain unlabeled (48). These approaches have been used to identify tissue-specific markers expressed on organ-resident populations that are largely absent from peripheral circulating cells. For Trm cells these include CD69; integrin α_E (CD103); and the α_1 subunit of the $\alpha_1\beta_1$ integrin, CD49a (49–51). In humans, assessing tissue residency is more challenging, but T cells isolated from nonlymphoid organs express some of

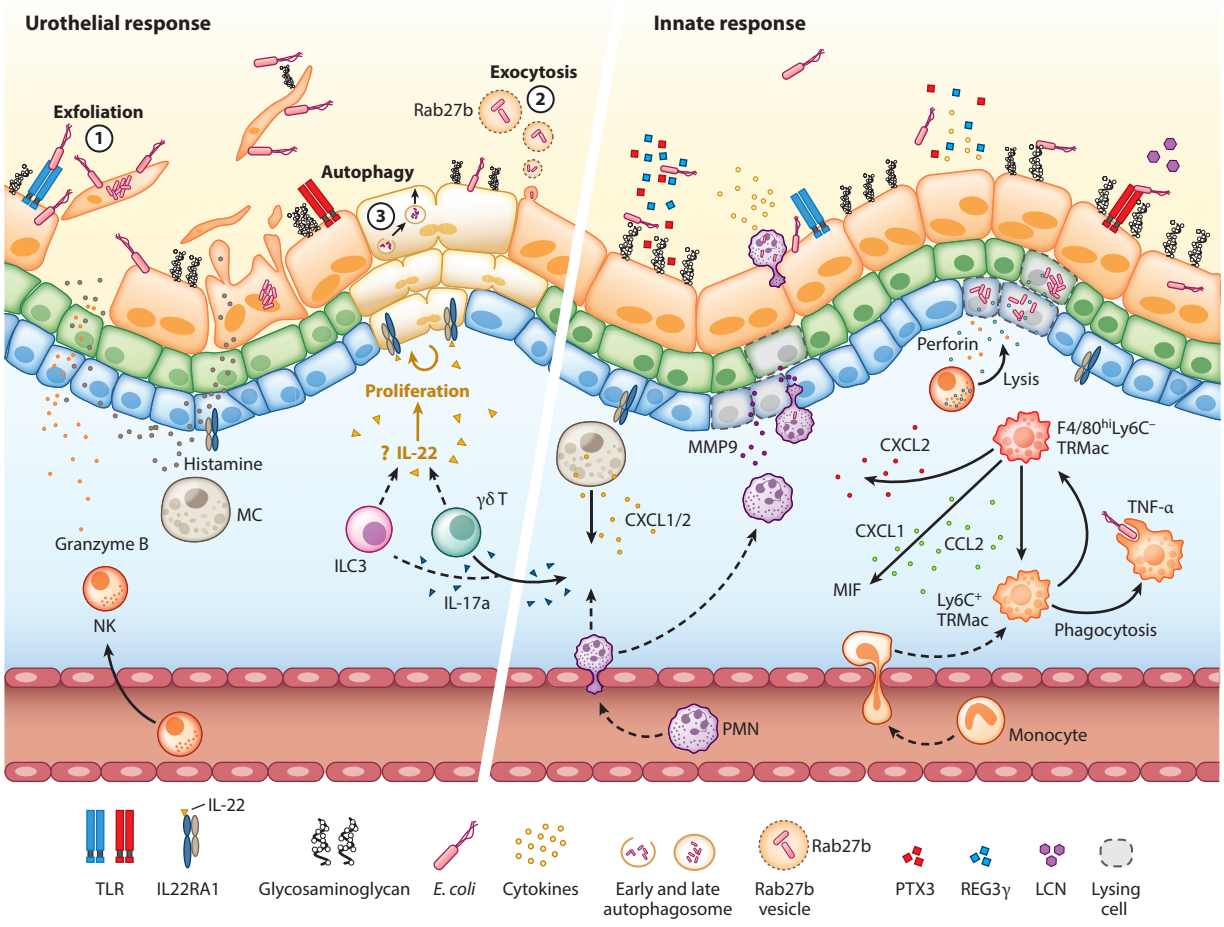


Figure 2

Structural and innate immune responses in the bladder. The immune response to UPEC in the bladder is delivered by both structural and innate immune cells. The urothelium is the first line of defense and has a number of unique adaptations to combat bacterial invasion. A tight-knit and impermeable layer of umbrella cells lines the bladder and is covered in a mucinous, rich glycosaminoglycan layer. TLRs on bladder epithelial cells sense bacterial components such as lipopolysaccharide and via a cascade of signaling pathways secrete antimicrobial peptides (LCN, PTX3, REG3γ, and cathelicidin), and chemokines such as CXCL1 and CXCL2. Similarly, the urothelium expresses the membrane-associated interleukin IL-22 receptor subunit IL22RA1 and as such can respond to IL-22, stimulating production of antimicrobials as well as proliferation and survival cues. Several other processes can be exploited for bacterial defense, including (①) bladder epithelial cell exfoliation following bacterial interaction with uroplakin and subsequent shedding of infected cells, (②) exocytosis of bacteria enclosed within Rab27b-expressing fusiform vesicles, and (③) induction of cellular autophagy to eliminate intracellular bacteria. A coordinated cellular response soon follows, with MCs and NK cells degranulating in response to distress signals from bladder epithelial cells and augmenting exfoliation and the recruitment of neutrophils and Ly6C$^+$ macrophages to the bladder. Neutrophils and inflammatory Ly6C$^+$ macrophages are the principal phagocytes in the response to urinary tract infection; indeed, resident F4/80^{hi}Ly6C$^-$ macrophages orchestrate their recruitment by the release of the chemokines CCL2, MIF, and CXCL1. Ly6C$^+$ macrophages produce TNF, which in turn stimulates the production of CXCL2 by resident Ly6C$^-$ macrophages and facilitates transepithelial migration of neutrophils via secretion of MMP9, enhancing access to UPEC. Abbreviations: ILC3, group 3 innate lymphoid cell; LCN, lipocalin; MC, mast cell; MMP9, matrix metalloproteinase 9; NK, natural killer; PMN, neutrophil; PTX3, pentraxin 3; REG3γ, regenerating islet-derived protein 3 γ; TLR, Toll-like receptor; TRMac, tissue-resident macrophage; UPEC, uropathogenic *Escherichia coli*.

these markers (52, 53). For example, CD69 is detectable on skin-resident T cells in humans (54, 55). However, there are discrepancies in the phenotypes of Trm cells in murine versus human organs (55), and different organs imprint distinct, tissue-specific transcriptional programs, phenotypes, and functions on resident T cells (52, 56). A similar residency program, with parallel markers, is likely in other tissue-resident cells, including natural killer (NK) cells (57). In contrast, tissue-resident macrophages are extremely heterogeneous (58); many are specialized for performing specific organ homeostatic functions within their microanatomical niche. To summarize, identification and study of bona fide tissue-resident subsets rely on the two experimental systems discussed above in model organisms and are challenging in humans and have rarely been applied to the bladder.

Bladder-Resident Immune Cells

Despite the high prevalence of bladder infection, there is a relative paucity of data profiling bladder-resident immune cells, in either homeostasis or disease, compared with other mucosal organs, such as the gut. This may relate to the historical perception that the urinary tract is sterile in homeostasis. However, several studies have provided some insights into the immune compartment in mouse bladder (59–61). Healthy murine bladders contain 30,000–50,000 $CD45^+$ cells, as assessed by flow cytometry (59, 60), but in the absence of a parabiosis study or premortem intravascular CD45 labeling, the extent to which these cells are circulating versus residing in tissue is unclear. In addition, flow cytometry assessment of single-cell suspensions may underrepresent the number of tissue immune cells up to a hundredfold, depending on the ease with which the organ can be dissociated (39). With these caveats, published flow cytometry studies found that the majority (70%) of bladder immune cells were antigen-presenting cells. The most abundant of these antigen-presenting cells were $F4/80^+CD64^+$ macrophages, constituting 40% of immune cells, while $CD11b^+$ and $CD103^+$ dendritic cell (DC) subsets were 15% and 5% of immune cells, respectively. The remaining 30% of bladder immune cells included NK cells, mast cells (MCs), $CD4^+$ αβ T cells, and γδ T cells (59) (**Figure 1**).

One of the drawbacks of flow cytometry assessment of immune populations is that a limited number of surface markers can be simultaneously assessed, and the selection of these markers relies on prior knowledge of canonical cell type–specific markers. To enable a more unbiased approach to profile the tissue immune landscape, single-cell RNA sequencing (scRNA-seq) is increasingly applied (62). Studies have revealed large numbers of diverse immune cells within healthy renal tissue, including macrophages, DCs, B cells, T cells, NK cells, and MCs (6, 7, 63). Many of these kidney immune cells expressed markers of tissue residency, consistent with murine studies confirming kidney residency using parabiosis or intravascular CD45 labeling (39, 48, 64, 65). The *Tabula Muris* (66) provided a compendium of single-cell transcriptomes across multiple organs, including the murine bladder, although representation of $CD45^+$ cells was limited. scRNA-seq has also been utilized to provide an overview of the tumor microenvironment in human and murine bladder cancer (61, 67). These studies included a small number of healthy control bladder tissues, providing a limited snapshot of immune cell heterogeneity in homeostasis. A more recent scRNA-seq study of sorted CD45 cells from young and old mouse bladders revealed a much richer immune landscape than previously appreciated, with monocytes, three subsets of macrophages, four DC subsets, NK cells, group 2 innate lymphoid cells (ILC2s), γδ T cells, $CD4^+$ and $CD8^+$ T cells, B cells, and plasma cells identified (68) (**Figure 1**).

Given that bladder infection is more common in females than in males, an obvious question is whether there are sex-based differences in tissue immunity. Zychlinsky Scharff et al. (60) reported that overall immune cell count and composition in the bladder were comparable in male

and female mice aged 5–12 weeks. However, they found that female mice had more robust innate responses following UPEC challenge. For example, 23 of 26 cytokines detected in bladders were significantly increased by infection in female mice, compared with 7 of 26 in male mice, with IL-6, IL-17, and the neutrophil-recruiting chemokine CXCL1 notably different between the sexes at 24 h after infection. A comparison of sex-related differences in human bladder immune cells has not been undertaken. Age-related changes in tissue immunity are well documented in multiple organs, including the kidney (6, 69), and the recent single-cell atlas of aging organs, the *Tabula Muris Senis*, captured some immune cells in both the kidney and bladder (70). Ligon et al. (68) provided a more rigorous comparison of young and old bladders, using bulk transcriptomics to identify extensive differences in genes of immunological relevance. There was notable enrichment of antigen presentation, B and T cell activation, cytokine-receptor interactions, and intestinal IgA production pathway genes. Furthermore, there may well be an interaction of age and sex, with sex differences in bladder-resident immune populations developing with increasing age and infection history.

In summary, there is currently evidence that the bladder houses an array of innate and adaptive immune cells. These may play varying roles in health and in disease, particularly in UTI and bladder cancer. We now consider these different cell types and their functions in detail.

INNATE IMMUNE CELLS OF THE BLADDER

Macrophages

Until relatively recently, the dogma in macrophage ontogeny was that all tissue-resident populations were derived from blood monocytes via a committed bone marrow precursor (71, 72). This view was definitively challenged by two fate-mapping studies demonstrating that microglia (73) and Langerhans cells (74) are exclusively of embryonic origin, with a variable representation of yolk sac–derived macrophages in other organs, including kidney, liver, pancreas, and spleen (74). In these fate-mapping studies, yolk sac–derived macrophages in mice were found to be exclusively F4/80^{hi} and transcriptionally distinct from those of hemopoietic origin (F4/80^{lo}). Several more recent studies have shown that the relative proportion of each of these subsets in adult murine tissues is organ, age, and perturbation dependent (74–76).

Macrophages demonstrate substantial functional diversity and serve roles in development, homeostasis, tissue repair, and immunity (77, 78). They are required for normal skeletal development (79) and angiogenesis (80). Yolk sac macrophages are also critical for some physiological functions of organs. For example, in the heart, a subset of macrophages act to buffer calcium ions within the conducting system (81), and in the colon, muscularis macrophages regulate steady-state peristaltic activity (82). Studies of macrophages in the bladder have focused on their immunological functions.

A network of F4/80^{+} macrophages within the lamina propria of the murine bladder was first described in 1984 by Hume and colleagues (83). These macrophages were often situated next to blood vessels with processes extended into the epithelium, drawing comparisons with Langerhans cells (84). Schiwon et al. (85) further characterized resident F4/80^{+}Ly6C^{-} macrophages in the naive murine bladder as the predominant antigen-presenting cell in homeostasis. Mariano et al. (86) identified bladder macrophages as F4/80^{+}CD64^{+} cells and noted heterogeneity of CX_3CR1 expression. The CX_3CR1^{hi} population was present in greater numbers in healthy bladder with higher F4/80 and MHC-II expression, whereas the CX_3CR1^{lo} cells showed higher expression of CD64 and CD11b and were positive for TIM4 and LYVE1, with similarities to macrophage subsets described in the lung, heart, fat, and dermis (87). These two populations were shown to hold specific spatial niches. The CX_3CR1^{hi} subset was positioned in the lamina propria (giving rise to the term MacL) and the CX_3CR1^{lo} subset in the muscularis (labeled MacM) (86). The ontogeny

of these subsets was defined using classical fate-mapping methods (73, 87). Overall, adult bladder macrophage subsets were derived from embryonic progenitors, including fetal hematopoietic stem cells (HSCs) and/or later yolk sac progenitors, as well as adult HSCs. Once established postnatally, these tissue macrophages were not rapidly replaced over the lifetime in the context of homeostasis.

Yu et al. (61) profiled both mouse and human bladders in homeostasis using scRNA-seq but did not identify macrophages. Rather, they described a large monocyte cluster as well as DCs, T cells, and B cells (61, 85). However, the differentially expressed genes presented for the monocyte clusters in this manuscript included *Lyz2* but also several canonical macrophage markers, including *C1qc*, *Csf1r*, *Ctss*, *Folr2*, *Cd68*, *Cd14*, and *Adgre1*. In the human bladder data presented, the monocyte annotated cluster included cells expressing monocyte-associated (*LYZ*, *AIF1*, and *CD14*), macrophage-associated (*C1QC*, *CD14*), and cDC2-associated (*SRGN*, *CLEC10A*, *CLEC7A*, and *CD83*) transcripts (6). Ligon et al. (68) used CD45$^+$ magnetic bead enrichment prior to scRNA-seq and identified a large population of bladder macrophages defined by *Adgre1* (F4/80) expression. Interestingly, two distinct subsets were identified based on expression of *Retnla*, which encodes a resistin-like molecule found on macrophages in the small intestine muscularis (88) and is also a marker for alternatively activated macrophages important in tissue repair (89, 90). A small additional macrophage cluster was present only in aged bladders and was highly enriched for *Cxcl13*, enabling the recruitment of CXCR5-expressing immune cells. It is unclear how these subsets relate to the MacM and MacL subsets previously defined by flow cytometry.

Schiwon et al. (85) reported that during UTI, resident macrophage number remained constant, but there was a marked increase in inflammatory monocyte–derived macrophages, denoted by the expression of marker Ly6C. Fluorescence labeled UPEC was used to show that these inflammatory macrophages have superior phagocytic capabilities compared to the resident population, and their depletion with clodronate led to more severe cystitis and a higher incidence of pyelonephritis (85). In contrast, Ly6C$^-$ tissue-resident bladder macrophages play an important role in coordinating the immune response to infection (91), via the recruitment of effector cells such as neutrophils and inflammatory monocytes, mediated by chemokines such as CXCL1, MIF, and CCL2 (92) (**Figure 2**). In the context of UPEC challenge, resident Ly6C$^-$ macrophages were the major source of these chemokines, and neutralization of MIF or CXCL1 in vivo resulted in a reduction in neutrophil and inflammatory monocyte recruitment and a corresponding increase in bacterial burden within the bladder (85). Conversely, inflammatory macrophages produced little cytokine, except TNF, with well described roles in defense against bacterial infection (93). Consistent with this, *Tnfr*$^{-/-}$ mice challenged with UPEC demonstrated impaired translocation of neutrophils into the bladder urothelium (the initial portal of entry of bacteria) and fewer neutrophils detectable in urine. Administration of recombinant TNF or TNF-sufficient Ly6C$^+$ monocytes to a *Tnfr*$^{-/-}$ mouse restored neutrophil migration into the uroepithelium, confirming the importance of Ly6C$^+$ monocyte–derived macrophages in this process.

Dendritic Cells

After macrophages, DCs are the most prevalent immune cell in the bladder. They account for around 25% of all CD45$^+$ cells (59, 86). Despite this, their role in bladder defense and homeostasis is unclear. Conventional DCs (cDCs) can be identified as CD11c$^+$MHC-II$^+$CD123$^-$ cells specialized for antigen uptake and presentation to T cells, providing the link between innate and adaptive immunity. cDCs can be broadly divided into cDC1s and cDC2s in humans. These are equivalent to CD8α$^+$/CD103$^+$ and CD11b$^+$ DCs, respectively, in the mouse (94). scRNA-seq analyses of murine and human bladders have largely found cDC2s (61, 66). CD45 enrichment enabled the identification of two distinct subsets of cDC2s (CD209^{hi} and Retnla^{hi}), as well as smaller numbers of cDC1s, plasmacytoid DCs, and migratory DCs (68).

In the kidney, CD11c$^+$ DCs were described as playing a key role in neutrophil recruitment during ascending pyelonephritis by secreting the chemokine CXCL2. Depletion of these cells using the CD11c-diptheria toxin receptor system led to delayed neutrophil influx and bacterial clearance in vivo (95, 96). However, these effects may not be exclusively related to DC depletion, as CD11c may also be variably expressed by tissue macrophages. This caveat is pertinent to comparisons between the role of DCs in bladder and their role in kidney defense against bacterial infection. As discussed previously, in the bladder, resident F4/80$^+$Ly6C$^-$ macrophages are the major source of CXCL2 (85) and the predominant phagocytic APCs at early time points. However, from 24 h after infection, DC bacterial phagocytosis increased, and there was an interplay between the relative phagocytic activities of bladder DCs and macrophages that influenced the magnitude of the adaptive immune response generated (59) (**Figure 3**). Specifically, depletion of bladder macrophages prior to primary infection resulted in improved adaptive immune responses to a rechallenge, the latter in a macrophage-replete environment, leading to a 2-log reduction in bladder bacterial counts. DC phagocytosis of UPEC was increased by a factor of two in macrophage-depleted mice, indicating that bladder macrophages may reduce adaptive immune priming by DCs by preferentially sequestering antigen, as described in other mucosal organs (97).

Mast Cells

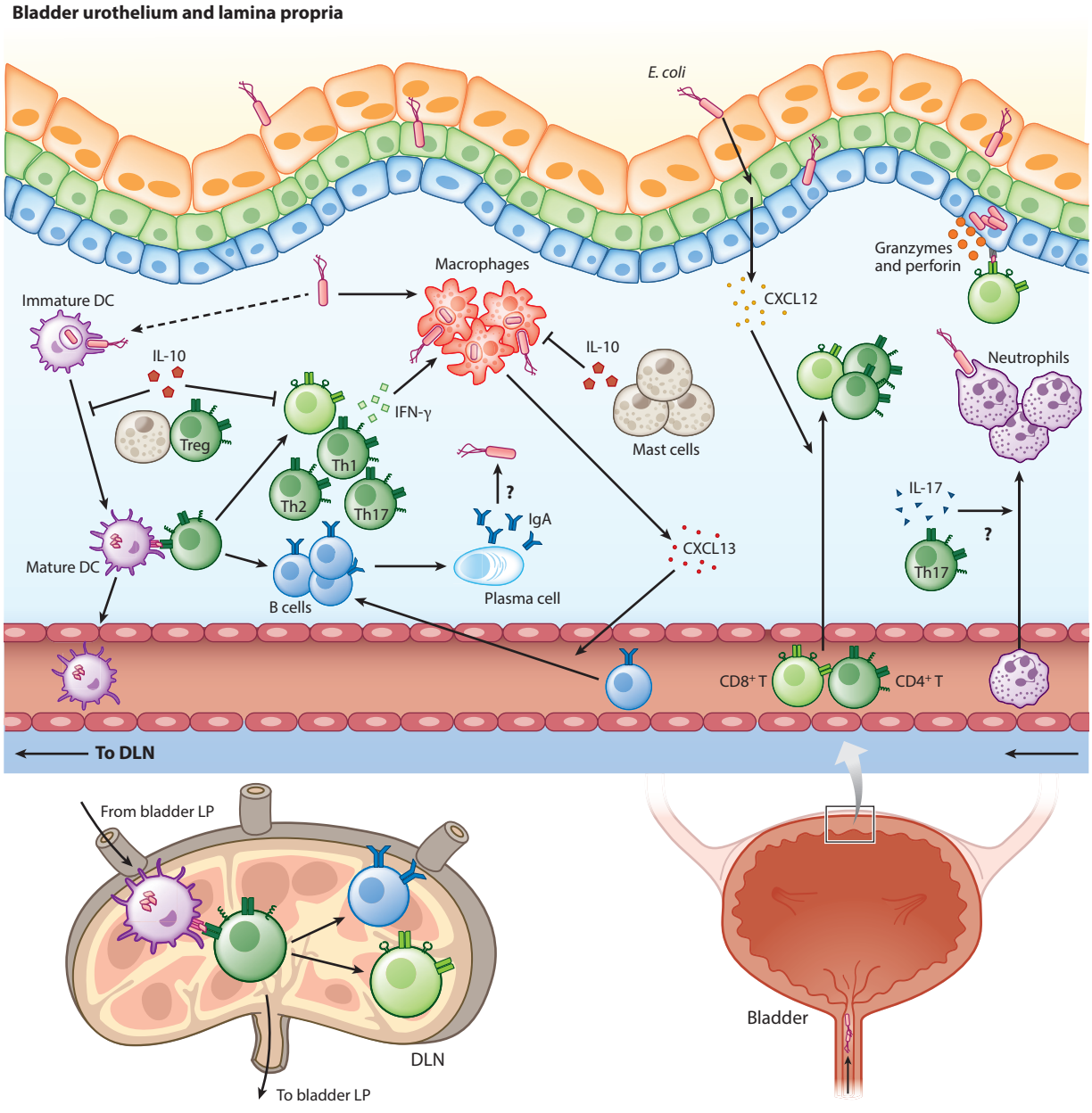
MCs are tissue-resident granulocytes that inhabit most organs in the body and have key defensive and immunoregulatory functions in border tissues (98, 99). Within the bladder, MCs are located beneath the urothelium, in close proximity to blood and lymphatic vessels, as well as within the muscularis layer (100). Until recently the prevailing dogma was that MCs were derived from bone marrow HSCs through circulating intermediates that terminally mature in their tissue of residence (101–103). In 2018 Gentek et al. (104) successfully defined the ontogeny of MCs using the CDh5-Cre^{ERT2} fate-mapping system: Yolk sac–derived precursors are progressively replaced by HSC-derived MCs later in development. Interestingly, as seen in macrophage biology (73), the replenishment dynamics of MCs are organ specific; for example, in the skin, MCs are predominantly yolk sac derived whereas in the tongue and peritoneal cavity, they are of both yolk sac and hematopoietic origin. In the skin, yolk sac–derived and adult HSC–derived MCs displayed both phenotypic and transcriptional differences (104). While the specific ontogeny of bladder MCs in homeostasis and disease has not yet been studied, it is likely that populations of both yolk sac– and HSC-derived MCs are present, as observed in the intestine (105).

Whatever their origin, bladder MCs modulate both innate and adaptive immune responses to bacterial challenge. MCs may be activated via TLR and Fc receptor ligation, releasing proinflammatory mediators and chemokines from cytoplasmic granules, including tryptase, TNF, CXCL1, and CXCL2 (106, 107). Mice deficient in MCs demonstrated impaired neutrophil recruitment and activation and bacterial clearance from the bladder (108). MCs also interact with the bladder epithelium and are critical for urothelial cell exfoliation (**Figure 2**). Specifically, urothelium-derived IL-1β recruits MCs to infected urothelium, where they release granule-associated chymases that promote caspase-1-dependent cytolysis and exfoliation upon uptake by urothelial cells (109).

Later after bacterial bladder challenge, MCs appear to shift to more tolerogenic functional programs producing the immunoregulatory cytokine IL-10 (106), which in turn tempers adaptive immune responses (**Figure 3**). Indeed, MC-deficient mice had increased numbers of mature DCs in draining lymph nodes following bladder challenge with UPEC, phenocopying IL-10-deficient mice (108). It is unknown whether these opposing MC functions, initially promoting innate defense but subsequently suppressing adaptive responses, are mediated by MCs of differing ontogeny.

Innate Lymphoid Cells

ILCs are a heterogeneous immune cell population that expresses IL-2Rα and IL-7Rα but, unlike T and B cells, lacks antigen-specific receptors (110). ILCs are categorized into three major groups based on cytokine profile and transcription factor dependence. Group 1 ILCs (ILC1s) require T-bet for development and function; produce IFN-γ and TNF in response to IL-12, IL-15, and IL-18; and contribute to immunity against intracellular bacteria and viruses. ILC2s are dependent



(Caption appears on following page)

Figure 3 (*Figure appears on preceding page*)

Adaptive immune responses to UTI caused by UPEC in the bladder. UPEC stimulates the urothelium to produce CXCL12 in a FimH-dependent manner, which attracts CXCR4-expressing T cells from the circulation. UPEC is phagocytosed by both macrophages and DCs in the lamina propria. Macrophages may preferentially sequester bacterial antigen, reducing uptake by DCs and subsequent activation of the adaptive immune response. On uptake of bacteria or bacterial antigens, DCs mature and are able to present antigen to cognate $CD4^+$ T cells. This T cell priming may occur directly within the lamina propria or after DC migration into the draining lymph node. Primed and activated $CD4^+$ T cells help activate $CD8^+$ T cells and B cells via IL-2 and costimulatory molecules. $CD8^+$ T cells participate in direct killing of infected cells containing intracellular bacterial communities through production of granzymes and perforin. Activated B cells either in the lamina propria or recruited from the circulation via macrophage-produced CXCL13 (specifically observed in aged mice) proliferate and differentiate into IgA-secreting plasma cells. IgA may be able to neutralize UPEC, although the precise role of IgA in UTI is unclear. Activated cytotoxic T cells; Th1, Th2, and Th17 cells; and Tregs may be present in the lamina propria during UTI. Th1 cells produce IFN-γ, which may activate and polarize M1 macrophages. Th17 cells produce IL-17, which may contribute (alongside γδ T cells) to the recruitment of neutrophils. Mast cells direct an immunoregulatory program, producing IL-10, which dampens adaptive responses. Th2 cells and Tregs (via IL-10) likely help to dampen the adaptive response and restore and maintain tissue homeostasis. Abbreviations: DC, dendritic cell; DLN, draining lymph node; LP, lamina propria; Th1, type 1 T helper; Treg, regulatory T cell; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.

on GATA3, RORα, and TCF-1 for development and produce the Th2 cytokines IL-5, IL-9, IL-13, and amphiregulin in response to epithelium-derived alarmins IL-33, IL-25, and thymic stromal lymphopoietin (TSLP) (111). Their major functions are anti-helminth immunity and maintenance of tissue homeostasis, as well as priming of adaptative immunity via MHC-II. ILC3s are dependent on the transcription factor RORγt and are the major producers of type 17 cytokines, including IL-17A, IL-22, GM-CSF (granulocyte-macrophage colony-stimulating factor), and TNF, in response to IL-23 and IL-1β (112). As such, they play pivotal roles in barrier integrity at mucosal surfaces and promote killing of extracellular pathogens.

ILCs were first described in the naive bladder in 2019 (defined as $CD90^+CD3^-CD4^-NK1.1^-MHC\text{-}II^-CD11b^-$) (60). Interestingly, there were greater numbers of ILCs in male naive bladders compared to female ones. Following UPEC challenge, there was a modest increase in ILCs in both sexes, but it was of greater magnitude in females. However, one caveat to this observation is that ILC numbers in this study were not normalized for bladder weight. The authors did not perform a detailed interrogation of specific ILC subsets, but they did show that $CD4^+$ ILC3s (defined as $CD90^+CD25^+CD4^+CD3^-NK1.1^-MHC\text{-}II^-CD11b^-$) were present during homeostasis and increased after UTI (60). ILC3s are a potential source of IL-17 in the bladder. This cytokine is important in defending against UTI since IL-17-deficient animals are susceptible to increased infection. γδ T cells are suggested to be a major source of IL-17 (113). However, $Rag2^{-/-}\gamma c^{-/-}$ female mice (lacking αβ T cells, γδ T cells, and ILC3s) were unable to clear bacteria from the bladder after challenge (60), in contrast to $Rag2^{-/-}$ mice (deficient in αβ T cells and γδ T cells) (59), implicating ILC3s as important players in type 17 immune responses in bladder defense (**Figure 2**). ILC1s have also been described in murine bladder, identified as $CD45^+CD3^-CD11b^-TCR\gamma\delta^-Tbet^+ROR\gamma t^-$ and present at 24 h after UPEC challenge, although data from naive bladders were not reported (86). Interestingly, bladder ILC1s were increased in number in macrophage-depleted mice that were rechallenged with UPEC four weeks after the initial infection compared with controls. However, their IFN-γ expression remained unchanged (86). ILC2s have recently been identified in naive adult (3 months) and aged (18 months) murine bladders by scRNA-seq, with a skew toward greater numbers in older mice (68). In humans, a single study (114) has described the existence of urinary ILC1s ($Lin^-CD127^+CRTH2^-c\text{-}Kit^-$), ILC2s ($Lin^-CD127^+CRTH2^+$), and ILC3s ($Lin^-CD127^+CRTH2^-c\text{-}Kit^+$) in patients undergoing intravesical BCG therapy for non-muscle-invasive bladder cancer, but other urine single-cell studies emphasize that both bladder and kidney cells are detectable in urine (115). Therefore, a bladder origin for these ILCs cannot be unequivocally confirmed, but their marked increase

in the context of intravesical BCG makes this highly likely. Much work remains to unravel the phenotype and function of ILCs in the bladder, particularly in humans.

Natural Killer Cells

NK cells make up approximately 2% of immune cells within healthy, naive murine bladders (59), with 100–1,000 NK cells isolated per bladder and no difference between female and male mice that are healthy, naive, and 5–12 weeks old (60). These ILCs are best known for their roles in immunosurveillance, identifying virally infected or malignantly transformed cells that have downregulated MHC-I, which are recognized through the differential signaling of a broad array of inhibitory and activating receptors expressed on the NK cell surface (116, 117). Activation of NK cells directs target cell killing, characterized by the release of cytotoxic mediators such as perforin and granzyme, and proinflammatory cytokines, such as TNF-α and IFN-γ.

Human NK cells consist of two major subsets: $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$. The $CD56^{dim}$ subset is the stereotypical cytotoxic NK cell subset and constitutes over 90% of circulating NK cells (118). A further increase in the proportion of circulating $CD56^{dim}$ NK cells is observed with increasing age (119). NK cells with a tissue-resident signature have been identified in a number of organs, including the liver, lung, spleen, lymph nodes, and uterine decidua. These largely consist of the $CD56^{bright}$ subset, which is characterized by lower cytotoxicity and higher cytokine production (57, 120). This is paralleled to some extent in mice, in which tissue-resident NK cells (confirmed by parabiosis) are $CD49a^{+}DX5^{-}$ and express genes associated with tissue retention and immune tolerance, while circulating NK cells are largely $CD49a^{-}DX5^{+}$ and display higher levels of cytotoxic activity (121). Parabiosis experiments have shown that $CD49a^{+}DX5^{-}$ NK cells are also resident within murine kidneys, where they account for 15–20% of NK cells (64). Kidney-resident $CD49a^{+}$ NK cells had increased expression of adhesion and activation proteins CD44 and CD160, in addition to reduced expression of inhibitory receptors, including KLRG1 (64). In this study, tissue-resident NK cell frequency (assessed by expression of CD49a) was comparable across common inbred mouse strains (B6, NOD, BALB/c, and 129) within kidneys and a range of other organs (lung, liver, spleen, uterus), but bladder was not assessed. Tissue-resident NK cells in the kidney and other organs have reduced expression of AsGM1, allowing circulating NK cells to be selectively depleted by an anti-AsGM1 antibody (64). In contrast, all NK cells can be depleted using anti-NK1.1 (122) or anti-NKp46 (123) antibodies. Together, these are useful tools for studying the distinct roles of circulating and tissue-resident NK cells in different disease models and have been used to show that kidney-resident NK cells are key mediators of ischemic reperfusion injury (64). Two populations of human kidney NK cells have been identified, and these broadly align with the $CD56^{bright}$ and $CD56^{dim}$ subsets, with the $CD56^{bright}$ subset expressing higher levels of tissue-resident markers, including CD69 (6). Although NK cells have been identified in healthy bladders, the relative contribution of these NK cell subsets to bladder-resident populations is unknown.

Although NK cell heterogeneity in the bladder has not been well defined, NK cells are expanded in murine models of UTI and are key in defense (60, 124). In particular, NK cells have been shown to increase in female, but not male, mouse bladders after UTI (60). This increase appears to be due to infiltration of circulating NK cells, which occurs as early as 2 h after infection, directed by the CXCR4/CXCL12 axis (125). UPEC infection (strain CFT073, which expresses hemolysin) stimulates FimH-dependent secretion of CXCL12 by the urothelium, which attracts CXCR4-expressing immune cells, including NK cells, T cells, and neutrophils, from the circulation. Interruption of the CXCL12/CXCR4 axis decreased immune cell recruitment to the site of infection and hindered bacterial clearance (125). Depletion of NK cells using anti-NK1.1

antibody in this model also resulted in a moderate, but significant, increase in bacterial load at 24 h after infection, suggesting NK cells play a role in early clearance (125) (**Figure 2**).

The mechanism by which NK cells are able to reduce bacterial load in early infection is unclear, but it may involve inflammatory cytokine production. NK cells have been shown to directly recognize UPEC and secrete TNF-α in response to FimH and LPS ligation of TLR4 (124, 126). TNF-α has a central role in acute bladder inflammation during UTI, reducing bacterial load and, in particular, the formation of intracellular bacteria communities (IBCs) by promoting exfoliation of infected bladder epithelial cells (127). Thus, NK cells may protect against acute infection through direct recognition of UPEC in a FimH- and TLR4-dependent manner, resulting in the production of TNF, which stimulates shedding of infected urothelial cells. Increased TNF has also been observed in patients with UTIs (128); however, the contribution of NK cells to this observation is unclear in human UTI. Compared to controls, a higher proportion of mice with depleted NK cells after treatment with anti-NK1.1 antibody were infected upon initial challenge, supporting a role for NK cells in the early elimination of bacteria. However, there was no difference in infection rates after day 8, and the resolution of infection was unaffected, suggesting a limited role for NK cells later in infection (60). Furthermore, UPEC is able to adhere to, and kill, NK cells through hemolysin A (129), which is expressed by around half of UPEC strains causing pyelonephritis and a third of those causing cystitis (130). Therefore, NK cells may be unable to effectively contribute to defense against these strains. Even in infections with an NK cell–sensitive UPEC strain, IBCs were still identified, supporting the conclusion that NK cells are insufficient for complete clearance of infection (124).

NK cells are enriched in human bladder cancer, constituting 5–40% of intratumoral lymphocytes (131). And the efficacy of intravesical BCG in NMIBC relies partly on NK cells, which, once activated by BCG, kill tumor cells via perforin (132). Consistent with this, intravesical BCG has reduced efficacy against bladder tumors in NK cell–deficient beige mice (133).

Innate-Like T Cell Populations

Innate-like T cells are a collection of unconventional T cells, including γδ T cells, natural killer T (NKT) cells, and mucosal-associated invariant (MAIT) cells, that express T cell receptors (TCRs) with limited diversity and feature prominently at barrier sites, including the gut, skin, and lung (134, 135). NKT cells recognize glycolipid antigens presented by the MHC-I-like molecule CD1d, playing roles in endogenous lipid sensing and defense against bacterial infection. MAIT cells are activated by bacterial riboflavin metabolites presented by the MHC-I-like molecule MR1. γδ T cells recognize antigens presented on a range of MHC-I-like molecules, in addition to some soluble ligands.

Most barrier tissues contain γδ T cells, which are seeded early during embryonic development and maintained in residence throughout life (135). Distinct epithelial and mucosal tissues harbor distinct γδ T cell populations, which are also heavily influenced by the microbiota (135). γδ T cells constitute 1–4% of immune cells in healthy murine bladders and are significantly enriched in male bladders compared with female bladders at baseline (59) (**Figure 1**). Although T cells have been identified in human bladders based on CD3D and CD3E expression, the precise composition of human bladder T cells, particularly the representation of unconventional T cells, is unclear (61).

Mouse bladder γδ T cell counts are higher in males at baseline, and they significantly increase 24 h after UPEC UTI in females but not males (60). When female mice were treated with testosterone and then challenged with UPEC, their bladder γδ T cell counts were lower than those of untreated female control mice, supporting a role for sex hormones in determining the differential representation of bladder immune populations and subsequent responses to UTI (60).

Female mice were able to clear infection better than male or testosterone-treated female mice, and higher levels of IL-17 in females were suggested to play a role (60). In support of this, IL-17 neutralization induced chronic infection in female mice, and IL-17$^{-/-}$ mice had more bacterial CFUs than wild-type counterparts at three and four days after infection. However, IL-17 supplementation in male mice was not sufficient to clear infection (60). γδ T cells can express high levels of IL-17 in some contexts, including in UTI, potentially playing a key role in recruiting neutrophils to the infected bladder (113) (**Figure 2**). Indeed, γδ T cell–deficient mice were more susceptible to acute bacterial cystitis (136). In mouse models of BCG therapy for NMIBC, infiltrating neutrophils appear to be essential for antitumor activity (137), and IL-17-producing γδ T cells may be required for their recruitment. In γδ T cell–deficient mice, BCG had no impact on survival in a bladder cancer model (138).

Although 5–15% of immune cells in naive murine bladders are T cells, CD4^{+} T cells account for only 2–4% of immune cells. CD8^{+} T cells were undetectable by flow cytometry, with the majority of T cells being CD4^{-}CD8^{-}, suggestive of innate-like T cell populations, including MAIT and NKT cell populations (59). Although MAIT cells and NKT cells are important in protection from infection at other mucosal and barrier sites, their presence and roles within the bladder are relatively unclear. MAIT cells have been identified in urine from UTI patients, and their migration into the bladder in mouse models of UTI was associated with decreased bacterial load (139). Although MAIT cells are abundant in human blood (5–10% of circulating T cells), they also possess a CD69^{+}/CD103$^{+/-}$ tissue-resident phenotype in the liver, lungs, and kidney (140). However, it is currently unclear whether infiltrating or tissue-resident MAIT cells are involved in bladder infection. Similarly, the phenotype and roles of NKT cells within bladder homeostasis and disease have not been studied.

Adaptive Immunity in the Bladder

Although innate immune responses in the bladder have recently received some attention, particularly in the context of infection (141), much less is known about the role of adaptive immunity in the bladder. However, recurrent bacterial cystitis is not uncommon in women, suggesting that memory responses (a hallmark of adaptive immunity) may be suboptimal.

T Cells and Cellular Adaptive Immunity

Conventional αβ T cells express a highly variant, somatically rearranged αβ TCR in conjunction with either the CD8 (cytotoxic T cells) or CD4 (helper T cells) coreceptors and recognize a vast array of peptide antigens expressed on MHC-I or MHC-II molecules, respectively. In flow cytometry studies, CD4^{+} T cells were a minority population and CD8^{+} T cells were undetectable in healthy murine bladder (59); however, a more recent scRNA-seq experiment confirms the presence of both (68). Following infection, there is a substantial increase in CD4^{+} T cell number in the bladder in female mice in particular (60), with Th1, Th2, and Th17 cells and Tregs (regulatory T cells) detectable (86). The appearance of Tregs is notable, and may reflect an effort to terminate excessive immune responses to maintain urothelial integrity, but it may have negative effects in terms of limiting antibacterial and antitumor responses. Yu et al. (61) identified T cells in human bladders using scRNA-seq, but their study annotated both CD3D- and CD3E-expressing cells as CD4^{+} T cells and did not distinguish subclusters.

In a mouse model of UTI caused by UPEC isolate CFT073, mice developed strain-specific immunity and were protected from reinfection with the same strain, but not from infection with UPEC cystitis isolate UTI89. Depletion of T cells in this model impaired clearance of infection and increased susceptibility to reinfection, although this was not observed when CD4^{+} or

CD8$^+$ T cells were depleted in isolation (142). Furthermore, although T cells mediated protection against chronic and same-strain recurrent cystitis caused by CFT073 UPEC, depletion of T cells did not affect the incidence of chronic or recurrent cystitis caused by UTI89 (59, 142). In the UTI89 UTI model, macrophages subverted adaptive immune responses by sequestering UPEC from DCs, preventing antigen presentation to T cells and the generation of adaptive immunity (59). Therefore, the relative susceptibility of different bacterial strains to macrophage versus DC phagocytosis may have a significant effect on the ability to generate T cell memory responses (**Figure 3**). Additionally, the mechanisms of T cell–mediated protection are also currently unclear, but given that Th1 and Th17 cells were detectable in murine bladder after infection (86), their effector cytokines may augment neutrophil recruitment (IL-17) or activate macrophages and CD8$^+$ T cells through IFN-γ.

In contrast to the case of UTI, T cells are known to play an important antitumor role in bladder cancer, with CD4$^+$ T cells potentially contributing to tumor cell killing (143). BCG therapy produces a local inflammatory reaction in the bladder mucosa that is characterized by infiltration of a large number of CD4$^+$ and CD8$^+$ T cells, both major contributors to therapeutic efficacy of BCG (144).

B Cells and Humoral Immunity

Despite the growing body of evidence indicating the presence of B cells and plasma cells in a variety of both mouse and human nonlymphoid organs (for example, skin and lung) (145, 146), until recently no study had specifically examined whether B cells reside in urinary bladder during homeostasis. Ligon et al. (68) revealed B cells and IgA$^+$ plasma cells in murine urinary bladders at steady state in a scRNA-seq study of CD45$^+$ cells. Subsequent flow-cytometry validation confirmed that B cells constituted up to 10% and 40% of live CD45$^+$ bladder cells from young and aged mice, respectively. Although the majority of bladder B cells were naive, two smaller clusters expressed markers consistent with innate-like B cells and atypical memory or age-associated B cells, some sharing the same immunoglobulin-variable gene, suggesting local clonal expansion. Interestingly, B cells were highly enriched in aged bladders and were colocalized with T cells in TNF-α-dependent, but microbiome-independent, tertiary lymphoid structures, promoting differentiation of IgA$^+$ plasma cells. This finding was further supported by identification of a small macrophage cluster in aged mice only with high expression of *Cxcl13*, a potent B cell–attracting chemokine. Although this landmark study provided the first direct evidence that B cells are part of the bladder immune landscape in homeostasis and aging, their role in local organ immunity is unclear. Furthermore, this study did not utilize parabiosis or intravascular labeling, so it may not have faithfully assayed tissue-resident cells.

Similar to what occurs in the gastrointestinal tract, plasma cells situated in the lamina propria of the healthy urinary tract produce secretory IgA (sIgA) (147, 148). IgA secretion increases with age even in germ-free animals (68), but studies exploring its homeostatic or protective role in the urinary tract are limited (149–151). In humans, both symptomatic cystitis and asymptomatic bacteriuria induce local specific sIgA production (151–153). Surprisingly, one study reported no increase in annual UTI incidence in a small cohort of patients with selective IgA deficiency ($N = 5$) and no disturbance in urinary sIgA in patients with recurrent UTIs (151).

The adaptive immune response to bladder infections tends to be limited, generating few or no serum UPEC-specific antibodies (108, 149, 153–155). This failure to mount a humoral response may relate to the abundance of phagocytosing, tissue-resident macrophages, as previously discussed (59), but it may also be influenced by local IL-10 production by MCs in later stages of cystitis, tempering the inflammatory response and promoting tissue integrity and regeneration at

the expense of adaptive memory (108) (**Figure 3**). Chan et al. (108) did not evoke any significant serum anti-UPEC IgG recall response or protection in a naive host by adoptive splenocyte transfer. On the other hand, in a cystitis-only model with ovalbumin-expressing UPEC, Thumbikat et al. (156) achieved similar levels of protection by adoptive transfer of splenocytes, splenic T cells, or serum from previously infected mice. The differences between these studies in the magnitude of adaptive immune responses to cystitis only might reflect differences in experimental setup (e.g., bacterial strain, inoculum size, or technique) or support the conclusion that bacteria also adopt varying strategies to avoid promoting adaptive immune responses in the bladder (157, 158).

Evidence for the role of B cells and humoral immunity in cystitis prevention or resolution is far from clear, in particular because of the incomplete distinction between cystitis with and without pyelonephritis in available studies. Hopkins et al. (159) reported that unlike SCID (severe combined immunodeficiency) mice, T cell–deficient mice had no increased UTI susceptibility, suggesting that T-independent antibody responses may be important. On the other hand, a significantly lower proportion of infected bladders were observed in B cell–deficient mice (muMT^{-} or JHD$^{-/-}$) 12–24 h after UPEC inoculation, indicating a potential regulatory role of B cells in early cystitis (136). However, the dynamics of bladder B cells and plasma cells during cystitis and following its resolution remain largely unknown.

There have been considerable efforts to induce both systemic and local protective humoral immunity by vaccination against either single virulence factors (e.g., FimH, flagellin, siderophores, or α-hemolysin) or whole uropathogenic bacteria or their lysates (91, 160). Some of these vaccination efforts in humans showed modest short-term efficacy, but their long-term effect requires clarification (161). Most vaccination strategies aim to induce systemic immunity against uropathogens by parenteral administration, but this strategy may have limited effect on the generation of bladder plasma cells and local antibody. Billips et al. (162) achieved a hundredfold reduction in bacterial colonization after challenge in mice vaccinated with live-attenuated UPEC instilled intravesically, but bladder B cell and antibody responses were not profiled and the protective effect was lost eight weeks after vaccination. Recently, Wu et al. reported that the addition of a Th1-skewing adjuvant to intravesical vaccines helped to overcome the Th2-biased bladder immune responses to infection, and this combination was more efficacious than the same vaccine administered subcutaneously (163, 164). Whether these local vaccination approaches induce long-lived, protective, bladder-resident memory B cells and plasma cells is yet to be determined.

CONCLUSIONS AND FUTURE DIRECTIONS

Despite the progress made in understanding bladder immunity in the last decade, several questions remain. One key limitation of the majority of studies of murine bladder immune cells is the failure to use an intravascular label. Thus, additional work is needed to more rigorously assess bona fide extravascular tissue-resident cells in health and murine models of infection and cancer. scRNA-seq studies have begun to shed light on cellular heterogeneity in a more unbiased way. However, immune cells are often underrepresented in these data sets, and future studies will benefit from strategies to enrich for immune cells, for example, by sorting CD45-expressing cells. Overall, many of the data available on bladder tissue immunity are from murine models, and our understanding of the immune landscape of human bladder remains limited. In particular, an assessment of sex differences in bladder immune cells, and how these change over the lifetime of a human, is required to better understand, prevent, and treat bacterial infection and cancer. Given the increasing emphasis on studying human immunity, and the availability of single-cell technologies and their applicability to human tissues, exemplified by the Human Cell Atlas project (165), there is a realistic prospect of rapid progress in the next decade.

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