TYPE 2–MEDIATED IMMUNITY

Adapt(ed) to repair — T_H2 immune responses in the bladder promote recurrent infections

A new study reports that T_H2-coordinated tissue repair takes precedence over long-term protective immunity in urinary tract infections. Although effective in the interim, this can lead to recurrent infections and bladder dysfunction.

John R. Lukens and Paras K. Anand

While urinary tract infections (UTIs) are some of the most common infections to afflict humans[1], surprisingly little is known about how the immune system is deployed to handle infections that reach the bladder. In particular, the involvement of T cells in UTIs remains poorly characterized, and we also do not completely understand why humans are especially prone to recurrent infections of the urinary tract. In this issue of *Nature Immunology*, Wu et al. report that $CD4^+$ T cells are preferentially skewed to a T helper (T_H) type 2 cell response to coordinate epithelial repair during UTIs[2]. The T_H2 response plays a vital role in helping to ensure that deleterious epithelial leakage does not occur in the bladder during UTIs. However, a T_H2-skewed response can also increase susceptibility to reinfection, promote aberrant epithelial thickening and cause progression to bladder dysfunction.

During infection, the immune system has to walk a fine line to ensure adequate control of the pathogen while also minimizing collateral damage to vital organ systems that can be caused by unrestrained immune responses. A salient and timely example of this is the COVID-19 pandemic, where immune-mediated damage to the lungs is suspected to be a major cause of morbidity and mortality[3]. Likewise, immune responses in the bladder must also be carefully kept in check to prevent overzealous epithelial destruction that would result in the internal release of urine, which contains high levels of noxious chemicals and can cause considerable damage to the surrounding tissue.

UTIs are a severe public health problem affecting around 150 million people, mostly women, worldwide each year. In the US alone, UTI-associated health care costs are more than US$3 billion annually[4]. While uropathogenic *Escherichia coli* (UPEC) remains the most common cause of UTIs, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus* and *Enterococcus faecalis*, among others, can also cause serious infections[5]. A typical UTI starts when UPEC residing in the gut flora reach the urethra. The UPEC start inhabiting the urethra and subsequently ascend toward the bladder lumen (referred to as cystitis), where further colonization is mediated by bacterial adhesins. In some people, UPEC invasion goes no farther and the pathogen is removed from the urinary tract through the action of antimicrobial agents and proinflammatory cytokines, including interleukin (IL)-1, IL-6 and IL-8, released by bladder epithelial cells. In other people, UPEC invades bladder epithelial cells by activating Rho-GTPases to induce actin rearrangements in the epithelial cells. However, an increase in cyclic AMP levels caused by innate sensing of bacterial LPS results in the expulsion of UPEC from epithelial cells[6]. The pathogen, in turn, eludes this by forming intracellular bacterial communities, to which the patient responds by shedding superficial layers of the bladder epithelium through a process known as exfoliation. While this process can acutely terminate UPEC infection, it inevitably exposes the deeper uroepithelium to invasion, wherein UPEC establishes membrane-bound quiescent intracellular reservoirs. UPEC within quiescent intracellular reservoirs survive as persisters, which are refractory to antibiotic therapy and have the ability to trigger a recurrent infection[4,5,7]. Indeed, following initial infection, ~25–30% of patients experience recurrent infections, suggesting they have ineffective adaptive immune responses to UTIs[4].

To examine this anomaly in the adaptive immune response during UTI, Wu et al. took an in vivo approach to determine the contributions of T_H1 and T_H2 immune cells by employing mice that lacked *Ifng* and *Il4*, respectively. In their studies, the authors found that *Ifng*$^{-/-}$ mice displayed susceptibility to bladder infection that was further exacerbated with reinfection. By contrast, lack of *Il4* offered protection when mice were reinfected with UPEC, suggesting that T_H2 cells limit bacterial clearance[2].

To better understand the dynamics of T_H1 and T_H2 immune responses, Wu et al. studied mice engineered to express fluorescent versions of either interferon (IFN)-γ or IL-4[2]. The method used to infect these mice with UPEC induced spontaneous retrograde flow to the kidneys, causing pyelonephritis. Measurement of the fluorescence signal from the two reporter cytokines revealed that, while the kidneys displayed a more balanced immune response, the immune response in the bladder was atypical, with only IL-4^+ and not IFN-γ^+ $CD4^+$ T cells spiking during reinfection with UPEC, confirming their initial hypothesis.

The authors then followed exfoliation and regeneration of bladder epithelial cells (BECs) in mice by taking distinct labeling approaches. They discovered that the process of exfoliation is rapid, happening within one day of UPEC infection in wild-type and *Il4*$^{-/-}$ mice. This was followed by a steady regeneration of the superficial epithelium by day 3 in wild-type mice. By contrast, mice that lacked IL-4 failed to regenerate the epithelium and maintain barrier integrity, as indicated by unrestricted access of trypan blue and labeled dextran to the bladder epithelium.

Exfoliation of the superficial epithelial cells is initiated by NLRP3-inflammasome-dependent IL-1β secretion and the subsequent induction of the proinflammatory form of cell death known as pyroptosis[4,8]. IL-1β is a potent chemoattractant that is converted to its biologically active form by the action of the cysteine protease caspase-1 in the NLRP3 inflammasome. Secreted IL-1β recruits additional leukocytes to the affected site to initiate healing responses or infection control. In a previous study,

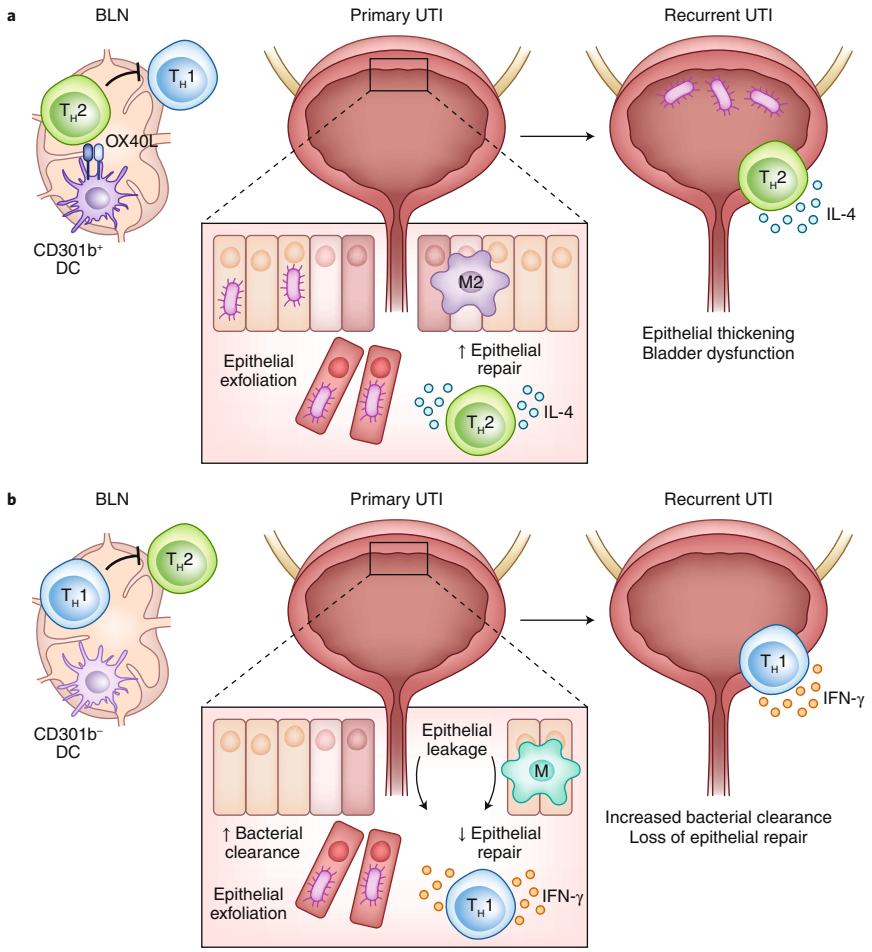

Fig. 1 | T_H2 immunity during urinary tract infection promotes bladder epithelial repair while rendering the host more susceptible to reinfection. Impairment of bladder epithelial repair function following exfoliation in urinary tract infection (UTI) can cause deleterious leakage of noxious urine into the surrounding tissue. To prevent such epithelial leakage in the bladder, the immune system deploys T_H2 cells that are potent inducers of epithelial repair. a, CD301b-expressing dendritic cells (DCs) in the bladder draining lymph nodes (BLNs) prime UTI-pathogen-specific T_H2 cells in an OX40L-dependent fashion, while restricting T_H1 responses. T_H2 cells traffic to the bladder and respond to epithelial exfoliation. Local activation of T_H2 cells and M2 macrophages produces growth factors that coordinate the epithelial repair. While T_H2-skewed immune responses to UTI help to limit detrimental bladder leakage, the flipside is that this renders the host more susceptible to recurrent UTIs, maladaptive epithelial thickening and impaired bladder control. b, Blockade of CD301b⁺ DCs or OX40L promotes the generation of IFN-γ-producing UTI-pathogen-specific T_H1 cells, while restricting T_H2 cell responses. T_H1 cells are more effective in orchestrating UTI-pathogen clearance and limiting reinfection of the bladder. However, T_H1-dominated responses are ineffective at epithelial repair, and this can lead to hazardous bladder leakage. M, macrophage.

Abraham and colleagues demonstrated that, during UPEC infection, caspase-1-dependent IL-1β secretion by BECs results in recruitment of mast cells to the superficial epithelium. BEC uptake of a mast-cell-released granule-associated chymase caused exfoliation by caspase-1-dependent pyroptosis[9]. Wu et al. next employed this chymase-deficient mouse model[9] to test the correlation between exfoliation and T_H2 immune responses in the bladder. Intriguingly, mice that lacked the granule-associated chymase (and thus exfoliation) had a significantly weakened T_H2-mediated bias, suggesting a T_H2-skewed adaptive response only occurred when it was first preceded by exfoliation[2]. This shows that, while the initial T_H2 response is apt and is intended to repair the epithelium, the immune system fails to identify and balance responses to recurrent infections of the bladder.

Wu et al. then showed that a specialized subset of CD301b-expressing dendritic cells (DCs) orchestrated the activation of T_H2 immune responses in bladder draining lymph nodes (BLNs) (Fig. 1). To better understand how CD301b⁺ DCs are capable of preferentially inducing a T_H2 response at the mechanistic level, the authors turned their attention to costimulatory ligand expression by DCs. In particular, OX40L, PD-L2 and ICOSL expression by DCs has been shown to potentiate T_H2 cell differentiation[10]. Using antibody-based blockade approaches, OX40L, but not PD-L2 or ICOSL, was found to play a critical role in the mounting of T_H2 cell responses during UPEC infection. Lastly, they found that recurrent UPEC infection leads to excessive epithelial remodeling and subsequent impairment in bladder capacity.

While these studies have begun to crack the code of what underlies the high rates of reoccurrence in UTIs, a number of important areas of future exploration remain. Intriguingly, Wu et al. report that preexisting memory CD4⁺ T cells are recruited to become T_H2 effector cells following the patient's first exposure to UPEC. They demonstrate that these memory T cells reside in the BLNs and that they quickly spring into action to aid in bladder epithelial repair within the first three days of infection. From their studies leveraging germ-free mice, they speculate that these memory CD4⁺ T cells were presumably first primed by the endogenous microbiota and then homed to the BLNs. In addition to the usual commensal microflora suspects in the gut, the possibility also exists that microbes found in the bladder are responsible for educating memory T cells. Indeed, recent studies have identified the presence of commensal microbes in the healthy human bladder[11]; however, whether this microbiota community contributes to bladder physiology or immunity requires further investigation. It is feasible that modulation of the bladder microbiome may offer a new avenue for treating recurrent UTIs. Improved strategies are greatly needed to treat UTIs, as the current standard of care — repeated antibiotics therapy — can cause kidney damage and can lead to the emergence of antibiotic-resistant bacteria.

It is well recognized that recurrent UTIs are more common in females than in males. It has been presumed that differences in reproductive tract anatomy largely account for the marked sex bias seen with recurrent UTIs. However, this may warrant revisiting, given the findings presented by Wu et al. Sex differences also exist in T_H1–T_H2 skewing of CD4⁺ T cells, with greater T_H1 responses observed in males and T_H2-biased responses commonly seen in females[12]. In light of these new

findings, it will be interesting to ascertain whether differences in T_H1–T_H2 skewing between the sexes contribute to the sexually dimorphic nature of UTIs.

The authors' findings also possess important translational implications. A notable discovery made during this work was that CD301b$^+$ DCs and their expression of OX40L orchestrate the induction of T_H2 responses. The overwhelming majority of bacterial infections are known to incite T_H1-dominated responses. As a result, very little is currently known about the regulation of T_H2 immunity during bacterial infection. On the basis of Wu and colleagues' work, targeting CD301b$^+$ DCs and OX40L may offer new approaches to limit immune-mediated collateral damage and promote tissue healing in a wide range of disease conditions. ❐

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Competing interests

The authors declare no competing interests.

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GERMINAL CENTERS

Directing traffic in the germinal center roundabout

B cells undergo iterative rounds of somatic hypermutation and selection for high-affinity antigen binding in the germinal center microenvironment. Two new studies provide insights into the temporal and spatial control mechanisms that act within B cells and follicular dendritic cells to jointly govern B cell differentiation and cell traffic within the GC.

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In T cell–dependent immune responses, antigen-activated B cells undergo repeated cycles of immunoglobulin-gene somatic hypermutation and high-affinity antibody selection before differentiating into memory B cells and plasma cells. The making of highly specific B cells is a rapid, dynamic process that occurs in a confined histological environment in the lymph nodes called the germinal center (GC), which consists of two zones — a dark zone (DZ) and a light zone (LZ) — that contain B cells with different biological functions, called centroblasts, also known as DZ B cells, and centrocytes, also known as LZ B cells. Recent studies have provided a good understanding of the dynamic movements of B cells between the DZ and LZ and of the molecular control of the major stages of GC B cell differentiation[1]. However, GC B cells show a marked phenotypic heterogeneity, and the transitions between different cell developmental stages remain incompletely defined. In addition, it is largely unclear how B cell–interacting reticular cells (BRCs) within the GC microenvironment function to direct these movements. Two studies in this issue of *Nature Immunology* aim to solve this puzzle. Pikor et al.[2] used a chemokine reporter system combined with single-cell transcriptomics to molecularly characterize DZ follicular dendritic cells (FDCs) and define their function in the GC reaction. Kennedy et al[3]. identified two functionally distinct, spatially separated B cell populations in the DZ and uncovered their relationship to LZ B cells, thus providing new insights into how GC B cells segregate and transition between diverse biological programs.

Stromal cells within lymphoid tissues provide an architectural framework that facilitates the spatial organization required for effective immune responses[4]. Recent work using single-cell transcriptomics has highlighted the heterogeneity within lymphoid stromal cell populations, most notably in T cell areas of the lymph node[5]. However, little is known about the heterogeneity and molecular identity of BRCs, due to difficulties in the isolation and enrichment of these cell types required for molecular analysis. BRCs are thought to primarily consist of FDCs, occupying the center of the B cell follicle, and marginal reticular cells (MRCs), which reside in the outer part of the follicle beneath the subcapsular sinus.

Within the GC microenvironment, DZ–LZ polarization is thought to be accomplished by differential distribution of BRCs expressing the chemokines CXCL13, which signals through CXCR5, in the LZ and CXCL12, which signals through CXCR4, in the DZ[6,7]. FDCs are one type of BRC present in the primary follicle, where they mediate critical organizational functions, partly due to their high expression of CXCL13. Following immunization, FDCs increase in number and occupy the GC LZ. FDCs can harbor antigen for long periods of time, a function that is critical for the