

Type 2-mediated immunity

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

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New studies report that T_H2-coordinated tissue repair takes precedence over long-term protective immunity in urinary tract infection. Although effective in the interim, this can lead to recurrent infections and bladder dysfunction.

While urinary tract infections (UTIs) are some of the most common infections to afflict humans¹, 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_H2 response in UTIs to coordinate epithelial repair². 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 skewed T_H2 response also can 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 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 mortality³. 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 surrounding tissue.

UTIs are a severe public health problem affecting around 150 million people, mostly women, each year worldwide. In the US alone, UTI associated health care costs are more than US\$3 billion annually⁴. While uropathogenic *Escherichia coli* (UPEC) remains the most common cause for UTIs, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, and *Enterococcus faecalis* among others can also cause serious infections⁵. A typical UTI starts when UPEC residing in the gut flora reaches the urethra. The UPEC starts inhabiting the urethra and subsequently ascends towards the bladder lumen (referred to as cystitis) where further colonization is supported by bacterial adhesins. In some people, UPEC invasion goes no farther and the pathogen is removed from the urinary tract through the action of anti-microbial agents and pro-inflammatory cytokines including 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 by innate sensing of bacterial LPS results in the expulsion of UPEC from epithelial cells⁶. The pathogen, in turn, eludes this by forming intracellular bacterial communities (IBCs) to which the host responds by shedding superficial layers of the bladder epithelium by a process known as exfoliation. While this process can acutely terminate UPEC infection, it inevitably exposes deeper uroepithelium to invasion wherein UPEC establishes membrane-bound quiescent intracellular reservoirs (QIRs). UPEC within QIRs 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 the patients experience recurrent infections suggesting ineffective adaptive immune responses to UTIs⁴.

To examine the anomaly in the adaptive immune responses during UTI, Wu et al. took an *in vivo* approach to finding out the contributions of T_H1 and T_H2 immune cells by employing mice that lacked either *Ifng* or *Il4*. In their studies, the authors found that *Ifng*^{-/-} mice displayed susceptibility to bladder infection that was further exacerbated during reinfection. In contrast,

lack of *Il4* offered protection when mice were reinfected for a second time with UPEC, suggesting that T_H2 cells limit bacterial clearance².

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 IFN- γ or IL-4². These animals were infected with UPEC in a way to induce spontaneous retrograde flow to kidneys causing pyelonephritis. Collection of 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⁺, but not IFN- γ ⁺, CD4 T cells spiking during reinfection with UPEC thus confirming their initial hypothesis.

The authors next followed exfoliation and regeneration of bladder epithelial cells (BECs) in mice by taking distinct labelling approaches. They discovered that the process of exfoliation is rapid, happening within 1 day of UPEC infection in WT and *Il4*^{-/-} mice. This was followed by a steady regeneration of superficial epithelium by day 3 in WT mice. By contrast, mice that lacked IL-4 failed to regenerate epithelium and maintain barrier integrity as noticed by unrestricted access to bladder epithelium by trypan blue and labelled polysaccharide, dextran.

Exfoliation of the superficial epithelial cells is initiated by NLRP3 inflammasome-dependent IL-1 β secretion and the subsequent induction of pro-inflammatory form of cell death known as pyroptosis^{4,8}. IL-1 β is a potent chemoattractant which by the action of a cysteine protease caspase-1 in the NLRP3 inflammasome is converted to its' biologically active form. Secreted IL-1 β recruits additional leukocytes to the affected site to initiate healing responses or infection control. In a previous study, Abraham and colleagues demonstrated that during UPEC infection caspase-1-dependent IL-1 β 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 pyroptosis⁹. Wu et al. next employed this chymase-deficient mouse model to test the correlation between exfoliation and T_H2 immune responses in the bladder. Intriguingly, mice that lacked this chymase (and thus exfoliation) aborted T_H2-mediated bias suggesting a skewed T_H2 adaptive response only when it was first preceded by exfoliation². This shows that while the initial T_H2 response is apt and is intended to repair epithelium, the immune system fails to identify and balance responses to recurrent infections of the bladder.

Wu et al. further then showed a specialized subset of CD301b-expressing dendritic cells (DCs) orchestrated the activation of T_H2 immune responses in bladder draining lymph nodes (BLNs) (Figure 1). To better understand how CD301b⁺ DCs are capable of preferentially inducing a T_H2 response at the mechanistic level, they turned their attention to a potential role for co-stimulatory ligand expression by DCs. In particular, OX40L, PD-L2, and ICOSL expression by DCs have been shown to potentiate T_H2 cell differentiation¹⁰. 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 remodelling and subsequent impairments 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 pre-existing memory CD4⁺ T cells are recruited to become T_H2 effector cells following the host'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 3 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 home 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 bladder¹¹; however, whether this microbiota community contributes to bladder physiology or immunity requires further investigation. It is feasible that the modulation of the bladder microbiome may offer a novel avenue to treat recurrent UTIs. Notably, improved strategies are greatly needed to treat UTIs as the current standard of care – repeated antibiotics therapy – can cause kidney damage and lead to the emergence of antibiotic-resistant bacteria.

It is well-recognized that recurrent UTIs are more common in females than males. It has been presumed that differences in reproductive tract anatomy largely account for the marked sex bias seen in recurrent UTIs. However, this may warrant revisiting given the findings presented by Wu et al.². 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 females¹². 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.

Their findings also possess important translational implications. A notable discovery made during this work was the identification that CD301b⁺ DCs and their expression of OX40L orchestrates 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. Based on their work, targeting CD301b⁺ DCs and OX40L may offer novel approaches to limit immune-mediated collateral damage and promote tissue healing in a wide range of disease conditions.

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Figure Legend

Figure 1. T_H2 immunity during urinary tract infection promotes bladder epithelial repair while rendering the host more susceptible to reinfection. Impairment in bladder epithelial repair function following exfoliation in urinary tract infection (UTI) can cause deleterious leakage of noxious urine into the surrounding tissue. To put this to action, 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 (M ϕ) produce growth factors that coordinate the epithelial repair. While T_H2 -skewed immune responses to UTI help to limit detrimental bladder leakage, the flipside of this is that it 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.

