Strapline: Bacterial pathogenesis

Title: Remodeling recurrent infection

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Standfirst:

Bacterial infection of the bladder can lead to mucosal remodeling and increased predisposition to recurrent infection, changing the way we view host susceptibility and providing new opportunities to develop novel therapeutics.

Main text:

Urinary tract infections (UTIs) are among the most common bacterial infections of humans and a significant burden on global healthcare. A major issue is recurrence, as approximately 25% of women who suffer UTI will experience recurrent UTI (rUTI) within six months of initial infection. The problem is magnified by emerging antibiotic resistance¹.

Most UTIs (~70%) are caused by uropathogenic *Escherichia coli* (UPEC), which possesses virulence factors that facilitate infection, including fimbriae that permit attachment to the uroepithelium, toxins that damage the host, systems to acquire iron in an iron-limited environment, and polysaccharide surface coatings that provide resistance against innate immune factors². Current knowledge of UTI pathogenesis is largely derived from mouse model studies³ that have elucidated processes of adherence, invasion, intracellular growth and exfoliation of UPEC into the bladder lumen during acute UTI², but few models have been applied to study rUTI⁴.

In this issue of *Nature Microbiology*, O'Brien et al⁵ show that an initial UTI can leave an enduring molecular imprint on the bladder through uroepithelial remodeling and transcriptional rewiring that comprises cyclooxygenase-2 (COX-2)-dependent inflammation. This discovery provides new insight into how initial infection can cause remodeling of the innate immune system and how this can influence the outcome of subsequent infections.

The authors showed that an initial bladder infection with UPEC resulted in two different outcomes: (i) spontaneous resolution that was associated with a reduced incidence of rUTI ('resolved' mice), or (ii) persistent (chronic) infection that led to

increased susceptibility to subsequent infection and a greater incidence of rUTI ('sensitized' mice). Sensitization was long-lasting (up to 6 months) and occurred in two different models of chronic UTI. Interestingly, sensitization increased susceptibility to different UPEC strains and *Klebsiella*, but did not increase susceptibility to the asymptomatic bacteriuria *E. coli* strain 83972 or the gut commensal *E. coli* strain Nissle 1917. Thus, some components of the UPEC virulence armory are required to cause rUTI in the sensitized bladder. It also remains to be determined if multiple repeat episodes of rUTI, as experienced by many women, can cause sensitization that predisposes to infection caused by less virulent strains.

By examining the mechanisms of bladder sensitization, the authors found that the uroepithelium in sensitized mice displayed altered architecture, distinct transcriptomic and proteomic signatures, and a predisposition for inflammation (Figure 1). The authors exploited these findings to explore rUTI prevention strategies in sensitized mice: they found that targeting COX-2 as a checkpoint for inflammation or vaccination with the FimH adhesin of type 1 fimbriae could prevent severe rUTI and acute and chronic cystitis, respectively. The authors conclude that COX-2 expression in the bladder during the first 24 hours following UTI, as noted in prior studies^{6,7,8}, is the likely determinative factor of acute outcomes in sensitized mice⁵.

Understanding innate immune responses to UTI will be crucial in defining susceptibility to rUTI. Mediators that are known to help control the rheostat for governing the extent of the innate immune response include interferon regulatory factor-3 (IRF-3) and IRF-7⁹, but neither of these genes were differentially expressed between sensitized and resolved mice. Instead, O'Brien et al⁵ identified 837 genes that were expressed differentially in these bladder transcriptomes including regulators of innate immunity such as IRF-9 and interleukin-10. The roles of these factors in remodeling innate immune responses to rUTI and in resolution versus chronicity of UTI are likely to be the subject of future research.

Beyond uroepithelial responses, how does cellular innate immune remodeling occur to facilitate recurrent infection? Traditional views of innate and adaptive immune responses hold that the former remains essentially unchanged upon exposure to a recurrent challenge, while secondary adaptive responses, comprising immune cells that can learn and remember, are qualitatively and/or quantitatively distinct from those elicited by an initial antigenic encounter. However, recent discoveries showing that innate immune cells can build immunological memory via epigenetic reprogramming after stimulation with microbial ligands challenge this idea¹⁰. "Memory" NK cells, for example, mediate what appears to be adaptive immunity at the axis of C-X-C chemokine receptor type 6 (CXCR6) and CXCL16¹¹. Interestingly, CXCL16 mediates macrophage phagocytosis of *E. coli*¹² and O'Brien et al. showed that CXCR6 and CXCL16 are more highly expressed in the bladders of sensitized mice⁵. Also, monocytes and macrophages can undergo epigenetic reprogramming to protect against certain infections independently of lymphocytes¹³, which may have a metabolic basis. This concept of innate immunity displaying epigenetic memory of an earlier infection is referred to as "trained immunity"¹⁴, which is neither innate nor adaptive and has

been described in the context of recurrent papillomavirus infection and repeat *Plasmodium* exposures. While antigen-specific responses can accelerate UPEC clearance in the bladder¹⁵, this work is the first to associate the concept of trained immunity with rUTI.

Overall, the study by O'Brien et al. reveals a new paradigm for how a history of UTI can radically alter innate immune responses in ways that impact future infection, suggesting the existence of pathogen-driven mechanisms that exploit a heightened state of host susceptibility to benefit the pathogen's lifestyle. As successful bacterial pathogens associated with recurrent infections deploy multiple strategies to avoid innate and adaptive defense mechanisms, future studies of rUTI might thus explore the role of UPEC virulence factors that may specifically 'remodel' the bladder innate immune response.

Figure 1. Features of UPEC colonization and the host response in rUTI. (A) Infection by uropathogenic *E. coli* (UPEC) in adult naïve bladder involves bacterial adherence, invasion and intracellular replication within umbrella cells to form an intracellular bacterial community (IBC). Infection leads to rapid neutrophil influx, COX-2 expression, inflammation and exfoliation of superficial umbrella cells. **(B)** Resolved mice spontaneously clear infection or are treated with antibiotics early on in infection. The resolved uroepithelium exhibit smaller umbrella cells and altered transcriptional and proteomic profiles compared to naïve mice, including reduced Cox-2 expression and inflammation. These mice have increased resistance to subsequent UTIs. **(C)** UTI in the sensitized bladder develops chronically upon initial infection. Even after antibiotic treatment, the superficial umbrella cells are smaller and more immature (mononucleated) than those of naïve mice and the intermediate and basal cell layers are expanded. Subsequent UPEC infection of these mice did not lead to invasion, but bacterial attachment to the remodeled bladder epithelium resulted in elevated neutrophil chemokines and myeloid cell development factors, enhanced COX-2 expression and severe inflammation. These mice had increased susceptibility to rUTI, but this could be reduced through use of Cox-2 inhibitors or vaccination with FimH adhesin protein.

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