



Molecular determinants of disease severity in urinary tract infection

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Abstract | The most common and lethal bacterial pathogens have co-evolved with the host. Pathogens are the aggressors, and the host immune system is responsible for the defence. However, immune responses can also become destructive, and excessive innate immune activation is a major cause of infection-associated morbidity, exemplified by symptomatic urinary tract infections (UTIs), which are caused, in part, by excessive innate immune activation. Severe kidney infections (acute pyelonephritis) are a major cause of morbidity and mortality, and painful infections of the urinary bladder (acute cystitis) can become debilitating in susceptible patients. Disease severity is controlled at specific innate immune checkpoints, and a detailed understanding of their functions is crucial for strategies to counter microbial aggression with novel treatment and prevention measures. One approach is the use of bacterial molecules that reprogramme the innate immune system, accelerating or inhibiting disease processes. A very different outcome is asymptomatic bacteriuria, defined by low host immune responsiveness to bacteria with attenuated virulence. This observation provides the rationale for immunomodulation as a new therapeutic tool to deliberately modify host susceptibility, control the host response and avoid severe disease. The power of innate immunity as an arbitrator of health and disease is also highly relevant for emerging pathogens, including the current COVID-19 pandemic.

Infections remain a major cause of mortality worldwide and the long-term consequences can be severe for those who survive. The cycle of infection is one of 'bad bugs', susceptible patients, acute disease and debilitating sequelae¹. Pathogens are the aggressors, and the host immune system is responsible for the defence. However, the host immune system can also become destructive, and excessive innate immune activation is a major cause of infection-associated morbidity².

Innate immunity provides an immediate antimicrobial defence to all classes of plant and animal life^{3–5}. Through specific recognition and activation strategies, the immune response targets and eliminates pathogens while sustaining symbiosis with the commensal flora; an impressive level of precision⁶. The defence is executed by a variety of mechanisms that also trigger inflammation and disease symptoms. Fortunately, this response is mostly transient and health is restored, but in susceptible hosts, a lack of molecular control can create exaggerated inflammatory states, severe disease, mortality and in some cases chronic inflammatory disorders.

Host susceptibility can be attributed, in part, to shortcomings of the innate immune system, which provides opportunities for the infecting pathogens^{7,8} (FIG. 1). Detailed molecular insights now reveal how a

subset of susceptible individuals develop severe infections, recurrences and tissue damage. The crucial role of innate immunity for host susceptibility is apparent in the urinary tract, where some of these defence mechanisms were first identified^{9,10}. Urinary tract infections (UTIs), are extremely common and increasingly difficult to treat owing to antimicrobial resistance^{11–13}. At least half of all women experience acute cystitis, an infection of the urinary bladder characterized by pain, dysuria and frequency of voiding^{14–17} (FIG. 2). A subset of these women develop recurrent cystitis, which seriously impairs quality of life. Men are substantially less affected than women (5–8 per 10,000)^{18,19}. Acute pyelonephritis (APN) is a serious, potentially life-threatening infection of the kidneys, accompanied by urosepsis in about 30% of adults^{20–23} (FIG. 2). In childhood, APN is at least as common as pneumonia, and can delay renal growth or cause focal renal tissue damage, increasing the risk of long-term morbidities such as hypertension and renal failure^{24–27}. At present, no reliable tools exist to predict the risk of recurrent APN, renal tissue damage or other detrimental effects of infection.

Fortunately, important insights into molecular determinants of UTI susceptibility offer new solutions to this long-standing dilemma^{6,28–34}. This Review highlights

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Key points

- Host resistance to infection is maintained by tight control of the immune response. If uncontrolled, excessive immune activation can lead to severe disease and inefficient microbial clearance.
- Urinary tract infections (UTIs) are a major cause of acute disease morbidity and mortality, as well as chronic sequelae. Uropathogens trigger innate immune responses in the kidneys (acute pyelonephritis) or the urinary bladder (acute cystitis).
- Disease severity is regulated at specific innate immune checkpoints, which control transcription and critical antimicrobial effector functions.
- Distinct susceptibility genes distinguish the two forms of symptomatic UTI through different mechanisms of immune hyperactivation.
- Bacteria with reduced virulence are carried asymptotically and enhance their fitness by actively inhibiting the host response.
- This level of molecular resolution in UTI offers an exciting potential for improved diagnosis and novel immunomodulatory therapies.

advances in our understanding of the molecular determinants of UTI pathogenesis, focusing on specific host susceptibility factors and their consequences. The molecular basis of disease is discussed, as well as the key events that define disease severity, with different characteristics in the kidneys and the bladder. By contrast, we also describe asymptomatic bacteriuria (ABU) and the molecular characteristics that explain this protective state. These novel findings have major implications for the precision of future diagnostic and therapeutic practices, especially the use of immunotherapy to reduce the excessive immune activation and restore a functional defence^{33–36}.

Asymptomatic bacteriuria

The urinary tract is a preferred niche for bacterial growth, similar to the intestinal and respiratory tracts, which harbour a rich microflora. Certain bacterial strains, attenuated for virulence, can establish a ‘symbiotic state’ in individual hosts and persist long term^{37,38}. No evidence exists suggesting that ABU increases the risk of symptomatic infection — quite the contrary, in fact^{15,39,40}. Epidemiological studies have demonstrated that ABU reduces the risk of symptomatic infection and recommendations from the Infectious Diseases Society of America (IDSA) and European Association of Urology (EAU) are to leave ABU untreated^{41,42}.

ABU was discovered when urine cultures were first performed in the general population and defined as growth of $>10^5$ colony-forming units (cfu) per millilitre of urine in an asymptomatic individual^{14,15}. Asymptomatic bacterial carriage was found in ~1% of schoolgirls, 2–10% of pregnant women and $>20\%$ of individuals >70 years of age^{21,43,44} (FIG. 2). Long-term follow-up studies demonstrated that asymptomatic carriage is protective, as the frequency of symptomatic UTI recurrences is reduced in patients with untreated ABU compared with patients treated with antibiotics^{39,45}. In a controlled prospective study of ABU, children treated with antibiotics were at an increased risk of symptomatic episodes compared with patients in whom ABU was left untreated⁴⁶. This observation is the rationale for deliberately establishing ABU in patients who are refractory to other therapies^{37,47–49}. Intravesical inoculation of ABU strains, such as *Escherichia coli* 83972, has been used successfully

and protection has been demonstrated in various studies, including a placebo-controlled clinical trial^{49–52}. After deliberate inoculation with *E. coli* 83972 the time to a symptomatic episode was prolonged and the likelihood was decreased (11.3 versus 5.7 months; 15 versus 73 recurrences after 12 months)⁵⁰. A friendly coexistence between bacteria and host, therefore, seems to be a highly desirable outcome^{53–55}.

Adaptation strategies of bacteria and host

ABU provides a very useful model for studying molecular determinants of asymptomatic carriage and symbiosis in a human ecological context. The asymptomatic carrier state allows bacteria to persist without evoking a destructive host response and at least four different adaptation strategies have been identified (FIG. 3). First, most ABU strains have a virulence-attenuated phenotype and successfully avoid triggering the innate immune response⁵⁶. Second, ABU strains actively suppress host gene expression, for example, by inhibiting RNA polymerase II, including genes crucial for the innate host defence against UTI^{36,57}. Third, attenuating Toll-like receptor 4 (*TLR4*) promoter polymorphisms reduce innate immune activation and downstream signalling in patients with ABU^{58,59}. Mutations or deletions affecting *Tlr4* abrogate innate immune activation by *E. coli* in mice and create an ABU-like state⁶⁰. Fourth, the mucosal TLR4 response is attenuated in the human urinary tract mucosa, owing to a lack of pattern recognition co-receptors for lipopolysaccharides (LPSs) such as CD14 (REFS^{61,62}).

Virulence attenuation. Early population-based studies revealed that most ABU strains lack the virulence factors that typically define APN strains, including P fimbriae^{63–65}. When cellular infection models were first established, most ABU strains were shown not to adhere to urothelial cells and to lack virulence-associated adherence factors such as P fimbriae^{63,66,67}. Two categories of ABU strains can now be distinguished: ‘primary ABU strains’, which resemble the commensal *E. coli* flora, and ‘attenuated pathogens’, which share overall sequence characteristics with uropathogenic strains but have lost virulence, presumably by reductive evolution leading to a sequential loss of virulence gene function, caused by deletions and point mutations^{53,56,65}. Loss of pathogenicity island (PAI) genes can result in a complete or partial loss of virulence function, further strengthening the argument that the strains have evolved away from the virulent phenotype⁵⁶. For example, attenuating mutations frequently affect fimbrial adhesins (P, type 1, S and F1C), α -haemolysin, aerobactin and other virulence genes^{54,67}. The loss of the pathogenicity island marker *malX*, as well as *iha* and *ompT*, is observed in ABU isolates⁶⁸. ABU strains produce substantially fewer siderophores such as aerobactin and enterobactin and less frequently express *hlyA* (which encodes α -haemolysin), *iha* and *fyuA* (a high-PAI marker gene) than virulent strains⁶⁷.

The ABU strain *E. coli* 83972 has been used as a molecular model to study this gradual loss of virulence in human carriers. *E. coli* 83972 is characterized by a

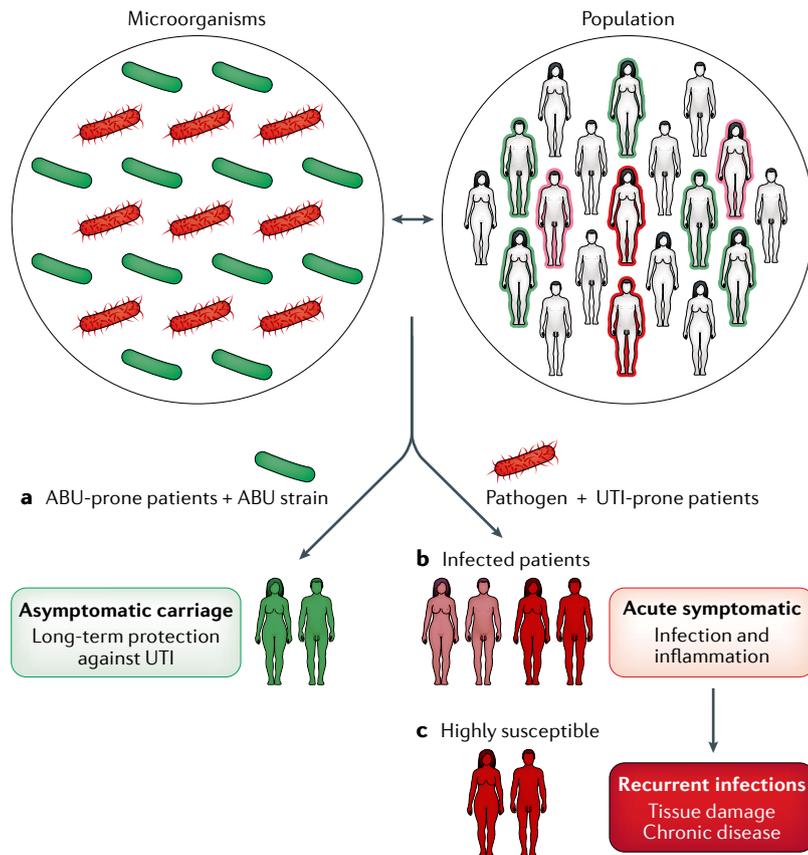


Fig. 1 | Susceptibility to urinary tract infection. Host susceptibility to urinary tract infection varies in the population, as does the virulence of bacteria that humans are exposed to. The majority of exposed individuals get rid of the bacteria without developing symptoms or bacteriuria. Three main outcomes of host–microorganism interaction can occur in the urinary tract. **a** | Asymptomatic bacteriuria (green) is a beneficial carrier state established by virulence-attenuated bacteria in active cross-talk with their hosts. **b** | Symptomatic infections occur in UTI-prone patients infected with pathogens. **c** | A subset of UTI-prone patients is highly susceptible and these individuals develop severe acute disease, recurrent infections and chronic disease states. ABU, asymptomatic bacteriuria; UTI, urinary tract infection.

smaller genome size than virulent strains of the same phylogroup and specific virulence genes are inactivated by deletions (such as *fimEAIC*), multiple point mutations (such as *papG* and *focD*) and a premature stop codon (such as *hlyA*)^{56,69}. Importantly, virulence attenuation has been shown to continue in human hosts carrying *E. coli* 83972 (REFS^{50,53,70}). During ABU, rapid and distinct bacterial genome alterations occur in a host-specific manner, involving functionally important genes such as regulators of gene expression, metabolism and virulence⁵³. Interestingly, the host-specific mutation pattern was reproduced in patients who received the same strain on several occasions, suggesting a strong, host-specific adaptation pattern of ABU strains.

Inhibition of host gene expression by targeting RNA Pol II phosphorylation. ABU strains were further shown to actively modify the host environment in which they persist. Changes in gene expression were detected by studying RNA samples obtained from patients inoculated with *E. coli* 83972 (REF.⁵⁷). Interestingly, >60% of all genes were

inhibited after 24 h compared with the pre-inoculation sample in each patient, suggesting that bacteria actively modify the host response to promote their own survival.

Further investigation identified a mechanism used by ABU strains to actively suppress RNA polymerase II (Pol II)-dependent gene expression in the host by regulating crucial steps in the Pol II transcription cycle⁵⁷. The multi-protein Pol II complex catalyses the transcription of mRNA precursors and most snRNAs and microRNAs through a series of steps, called the RNA Pol II cycle^{71–73}. Phosphorylation of the C-terminal domain of the Pol II subunit RPB1 is required to initiate productive RNA elongation⁷². *E. coli* 83972 suppressed several transcriptional activators (including *FOSB*, *CTNNB1* and *TFAP2A*), activated transcriptional repressors (including *DACH1*, *BANP* and *DDX20*) and suppressed genes involved in transcription initiation and recognition of the TATA box-binding protein (TBP)⁵⁷. Specifically, the ABU strain regulated promoter release and productive elongation by inhibiting the RNA Pol II phosphorylation complex and small nuclear RNAs (*RN7SK*, *RNU6ATAC*, *RNA28S5*). The frequency of Pol II inhibition was higher among ABU strains and faecal strains than in APN strains, supporting an association with ABU and commensalism⁵⁷.

The bacterial *nlpD* gene was identified as a key regulator of bacterial RNA Pol II inhibition in the host by using a loss-of-function mutant from an inoculated patient³⁶. NlpD is a divisome-associated, outer membrane lipoprotein that activates the peptidoglycan hydrolase AmiC during bacterial cell separation^{74,75}. These results suggest that the bacterial NlpD protein directly targets constituents of the Pol II phosphorylation complex³⁶ (the Pol II subunit RPB1 and the phosphorylation complex adaptor, PAF1C; FIG. 4). NlpD was released by ABU strains followed by efficient transfer of the protein across host cell membranes³⁶. This mechanism is fascinating as it enables bacteria to act directly on host cells by releasing proteins that modify the transcription machinery. In the case of NlpD from ABU strains, the bacterial protein inhibits transcription and suppresses excessive host innate immune responses. NlpD treatment was shown to reduce inflammation and enhance bacterial clearance from infected bladders in vivo when recombinant NlpD was injected intraperitoneally before intravesical *E. coli* infection³⁶. The findings suggest major therapeutic potential for NlpD as a modifier of inflammation and illustrate the potential of molecules of bacterial origin as immunomodulatory agents.

Attenuated TLR4 activation and mucosal pathogen recognition. Targeting of host receptors by pathogens alerts the host to the presence of the bacteria. Their virulence factors act as ‘danger signals’ and the host responds by activating the innate immune response, potentially leading to disease. By contrast, in ABU, such responses are avoided and the relative inertia of the host mucosa has been associated with reduced innate immune recognition of the infecting bacteria.

Host recognition of Gram-negative bacteria has been extensively studied^{10,76,77}. TLR4 controls the innate host response to Gram-negative bacterial infection

generally, but with considerable complexity, depending on the pathogen and the site of infection⁷⁷ (FIG. 3). Early studies in a mouse UTI model showed that *Lps*, later redefined as *Tlr4* (REF.¹⁰), is a major determinant of the host response to UTI^{9,78}. However, the mechanism of TLR4 activation was shown to differ between epithelial cells in the mucosal lining of the urinary tract and monocytes, which had been used as a model⁶². Urothelial cells express TLR4, but not the co-receptors CD14 and MD2 (also referred to as LY96) and, as a result, LPS recognition is weak, unless soluble CD14 is present at the site of infection^{61,62}. This lack of reactivity explains why ABU strains persist without creating a state of chronic inflammation in the urinary tract mucosa (FIG. 3; TABLE 1). Importantly, bacteria that express TLR4

agonists, such as P fimbriae, can override this unresponsiveness by engaging different TLR4 coreceptors, such as glycosphingolipids⁷⁹. This mechanism provides mucosal membranes with a much needed, pathogen-specific solution for when to respond to bacteria in the lumen. If epithelial cells recognized LPS, the entire Gram-negative microflora would trigger TLR4 signalling and chronic inflammation in the intestine and at other mucosal sites.

Attenuating TLR4 promoter polymorphisms in patients with ABU. A protected, ABU-like phenotype was first observed in C3H/HeJ mice, which carry a point mutation in the Toll/interleukin-1 receptor (TIR) domain of TLR4 (REFS^{9,58,59}). *Tlr4*^{-/-} mice are protected from symptomatic UTI. Mice carrying single *Tlr4* adaptor gene deletions (*Trif*^{-/-}, *MyD88*^{-/-}) infected with

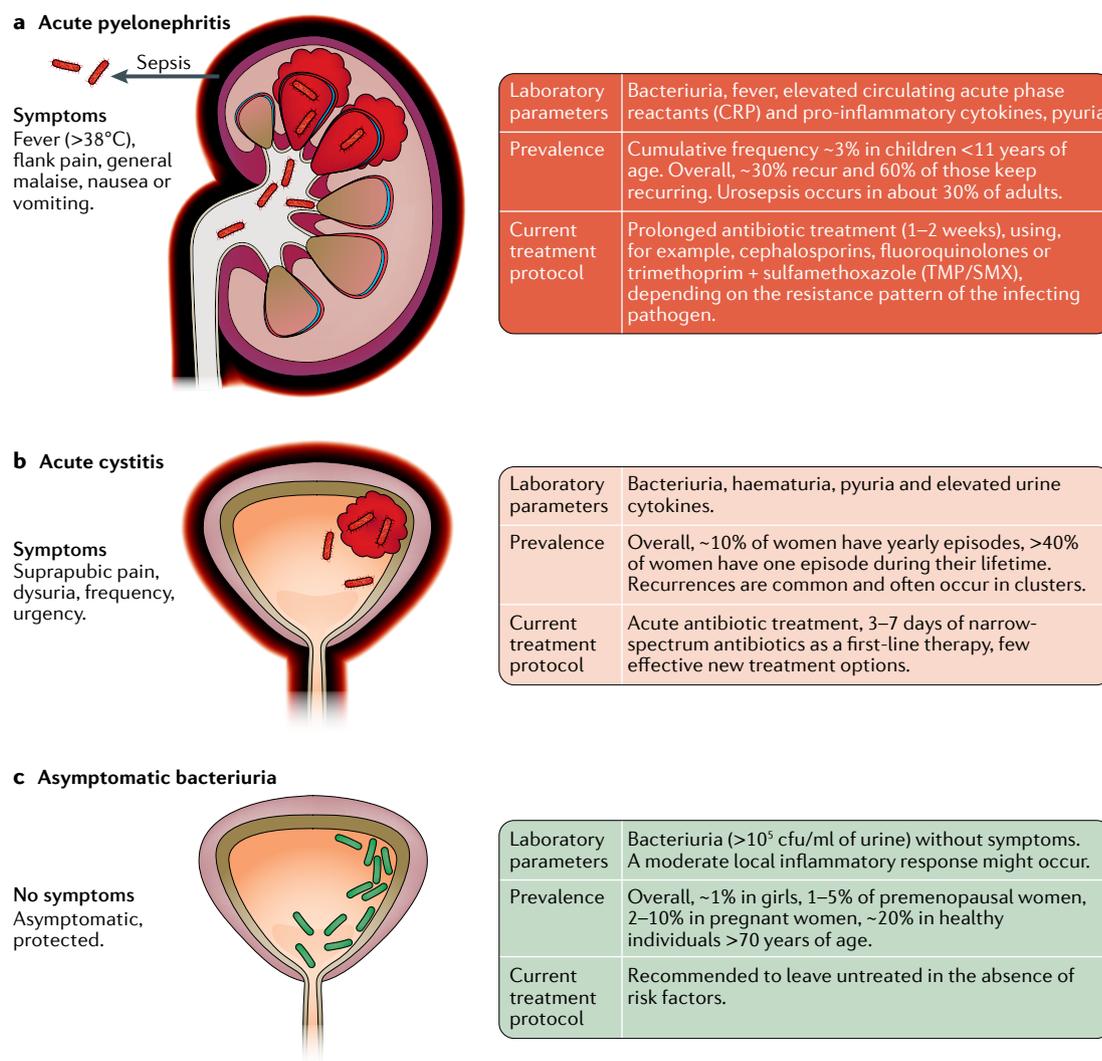


Fig. 2 | Symptomatic infections and asymptomatic bacteriuria. a | Acute pyelonephritis is a severe bacterial infection localized to the kidneys with systemic involvement and considerable mortality. Spread of bacteria to the blood stream (urosepsis), occurs in about 30% of adults^{20–23}. Infection gives rise to an innate immune response and local inflammation in the kidneys, as well as systemic spread of pro-inflammatory mediators, which cause fever, general malaise and activate the acute phase response. **b** | Acute cystitis is an infection of the urinary bladder and lower urinary tract. An often potent, inflammatory response causes bladder oedema, hyperaemia and pyuria, and patients experience pain, dysuria and increased frequency. **c** | However, bacteriuria is not always accompanied by inflammation and disease. In asymptomatic bacteriuria, the urinary tract is colonized by bacteria without evidence of disease. This condition is defined by repeated positive urine cultures in an asymptomatic patient. CRP, C-reactive protein.

uropathogenic *E. coli* develop an ABU-like state, owing to reduced inflammation and bacterial clearance^{9,32,60,80}. Consistent with these studies, *Cd14*^{-/-} mice show increased bacterial numbers in urine and bladder tissues compared with wild-type mice, suggesting that like *Tlr4*^{-/-} mice, *Cd14*^{-/-} mice could develop ABU⁶².

Clinical studies have provided evidence of TLR4 attenuation in patients with ABU. *TLR4* expression was reduced compared with age-matched healthy participants, and this phenotype was attributed to attenuating *TLR4* promoter polymorphisms not observed in symptomatic UTI^{58,81}. The identified promoter polymorphisms were shown to reduce *TLR4* expression in reporter assays and were further associated with reduced IL-8, IL-6 and neutrophil responses in patients inoculated with *E. coli* 83972 (REFS^{59,82,83}). However, genetic variants affecting *TRIF*, *TRAM* or *MYD88* expression were not detected in these patients, indicating that the receptor rather than the adaptor proteins influences ABU establishment.

These results show that ABU strains are sophisticated, actively adapting organisms that persist in the urinary tract, especially in hosts in whom *TLR4* expression is reduced. By actively suppressing host gene expression, ABU strains adapt further, potentially inhibiting destructive immune hyper-activation in the host.

From ABU to APN

An unexpected observation has suggested that an ABU strain might become virulent if equipped with a single ‘super-virulence factor’, specifically, the *papG* adhesin gene⁷⁹. These findings contradict the dogma of virulence necessarily being multifactorial and suggest that a single super-virulence factor might suffice to orchestrate a molecular transition from ABU to APN in the host⁷⁹.

Divergent effects of fimbriae

The observation of the potential of a single super-virulence factor was made in patients with recurrent UTIs who were inoculated with the ABU strain *E. coli*

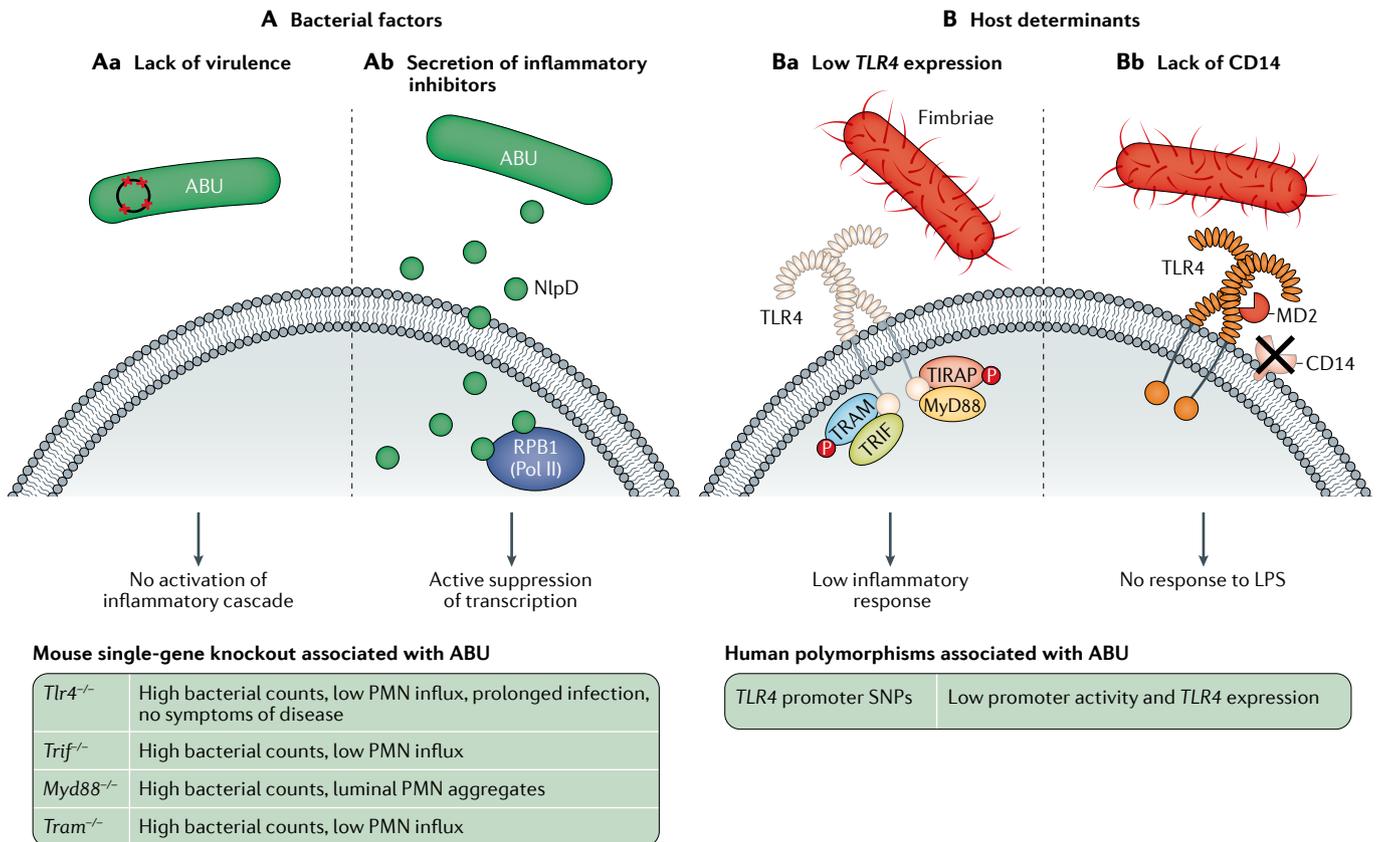


Fig. 3 | Molecular determinants of asymptomatic bacteriuria. Asymptomatic bacteriuria (ABU) is explained by both bacterial factors and host determinants that facilitate symbiosis. **A** | Bacterial factors: ABU strains coexist with their hosts without triggering symptoms. Several mechanisms contribute to this effect. **Aa** | ABU strains do not provoke a potent innate immune response in the host owing to a lack functional virulence genes. **Ab** | ABU strains modify the host environment by actively inhibiting host gene expression. They secrete inhibitors of host RNA polymerase II (Pol II) such as NlpD, which targets the Pol II phosphorylation complex and inhibits gene expression, including pro-inflammatory genes. **B** | Host determinants: Toll-like receptor 4 (TLR4) signalling controls many aspects of the innate response to urinary tract infection. TLR4 signalling is attenuated in patients with ABU. At least

two mechanisms contribute to this effect. **Ba** | Attenuating *TLR4* promoter polymorphisms are prevalent in patients with ABU. These polymorphisms reduce *TLR4* expression and the inflammatory response to bacteriuria. **Bb** | Optimal TLR4 activation by lipopolysaccharide (LPS) requires co-receptors, such as CD14 and lymphocyte antigen 96 (MD2). As the bladder epithelium does not express surface CD14, the cells remain refractory to bacterial LPS alone and ABU strains do not efficiently activate TLR4 signalling. Virulence factors such as P fimbriae enable pathogens to overcome the lack of CD14 and trigger a TLR4-mediated inflammatory cascade. MyD88, myeloid differentiation primary response protein 88; PMN, polymorphonuclear neutrophil; TRAM, TRIF-related adaptor molecule; TRIF, Toll/interleukin 1 receptor (TIR)-domain containing adapter-inducing interferon-β.

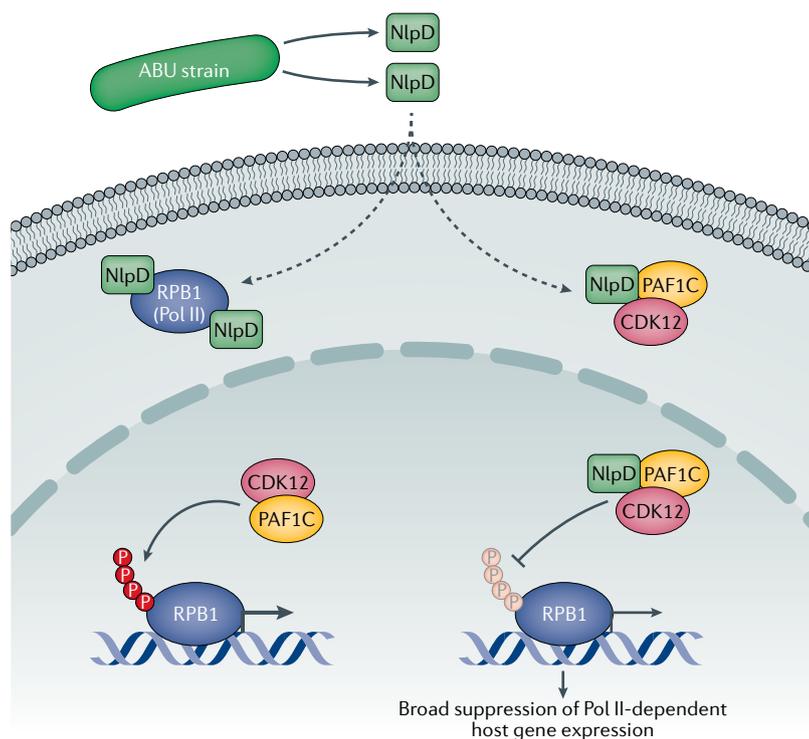


Fig. 4 | Mechanism of RNA polymerase II inhibition by asymptomatic bacteriuria strains. Asymptomatic bacteriuria (ABU) strains establish a symbiotic relationship with their host, in part by inhibiting RNA polymerase II (Pol II)-dependent gene expression. The NlpD protein, released by the bacteria, is internalized by host cells, where it targets DNA-directed RNA polymerase II subunit RPB1/POLR2A (RPB1) and the phosphorylation complex constituent polymerase-associated factor 1 complex (PAF1C), which recruits the cyclin-dependent kinase 12 (CDK12) to the complex. As a result, Pol II phosphorylation by CDK12 is inhibited and host gene expression is suppressed. Protective effects of NlpD in mice infected with the uropathogenic *Escherichia coli* (UPEC) strains CFT073 or CY17 have also been associated with reduced Pol II phosphorylation. P, phosphate group.

83972, a safe and protective approach to treating UTI, especially in patients with residual urine⁵⁰. To further develop the *E. coli* 83972 inoculation therapy for patient groups with increased diversity, fimbrial gene clusters that are either mutated or not expressed in *E. coli* 83972 have been repaired. The *pap* gene cluster was repaired in *E. coli* 83972 by reconstituting the *papG* adhesin gene, promoting bacterial adherence to the mucosal surface of the bladder and potentially enhancing the efficiency of *E. coli* 83972 inoculation as a therapeutic tool in patients without residual urine⁷⁹. The *pap* gene cluster was chromosomally reconstituted using homologous recombination, replacing the mutated *papG* with functional *papGX* genes from the uropathogenic *E. coli* (UPEC) strain *E. coli* CFT073 (REF.⁸⁴). The resulting *E. coli* 83972*pap* expressed functional P fimbriae, shown by P blood group-specific agglutination of human erythrocytes and attachment to human kidney cells. Separately, the entire *fim* operon from *E. coli* CFT073 was introduced into *E. coli* 83972 to repair the internal 4,253-bp *fim* deletion. The reconstituted *E. coli* 83972*fim* strain expressed functional type 1 fimbriae, shown by α -D-methyl-mannose reversible agglutination of human and guinea pig erythrocytes and reversible adherence to human kidney cells⁷⁹.

The reconstituted strains were used for human inoculation and five patients inoculated with *E. coli* 83972 or *E. coli* 83972*fim* on different occasions, developed ABU⁷⁹. Unexpectedly, two of the patients who received *E. coli* 83972*pap* became symptomatic. The patients were treated with antibiotics, their symptoms resolved, and the study was discontinued. The molecular basis of the symptomatic response was investigated using genome-wide transcriptomic analysis technology⁷⁹ (FIG. 5). *E. coli* 83972*pap* was found to reprogramme host gene expression, including genes in the interferon pathway and the pattern receptor recognition (PRR) pathway, which were activated³². At the time of symptoms, regulated genes also included *IRF7*, *OAS3*, *OAS1* and *OAS2*, complement components, *IFIH1* (which encodes MDA5), *DDX58* (which encodes RIG-1), *IL1B*, *NLRC4* (which encodes IPAF), *TNF*, *RIPK2* (which encodes RIP2), *IL6* and *NOD2*, several of which have been highly implicated in UTI pathogenesis. A strong *IRF7* response was detected in samples taken close to the symptomatic episodes^{34,79}, confirming observations in the mouse UTI model, in which *IRF7* has been identified as a major regulator of the disease response during APN. Other identified transcriptional nodes included *IRF3* and *STAT1*, which regulate the protective, type I interferon-dependent response³². The *PapG* adhesin protein was further shown to enter host cells and to regulate *IRF7* expression by direct promoter binding, affecting the *IRF7* transcription complex assembly, together with *IRF3*, *MYC* and *IFN β* ⁷⁹ (FIG. 6). These findings provide a mechanism for how classical transcriptional regulators might contribute to the pathophysiology of APN and be the link to *IRF7*-driven disease³⁴.

By contrast, the *fim* reconstituted strain *E. coli* 83972*fim* was shown to attenuate host gene expression, inhibiting genes involved in RNA processing, post-transcriptional regulation and ribosome biogenesis⁷⁹ (FIG. 5). No evidence of inflammation was observed and pro-inflammatory gene sets were not significantly regulated (cut-off of $P < 0.05$ compared with the pre-inoculation sample in each patient). Furthermore, effects on gene expression did not include *IRF7* or *IRF*-dependent genes.

The results suggest that type 1 fimbriae enhance the inhibitory effects of *E. coli* 83972 on host gene expression by affecting the post-transcriptional environment in infected hosts (FIG. 5). The analysis also revealed moderate activation of potassium channels, ion anti-porters, voltage-gated cation channels and substrate-specific ion channels⁷⁹. Activated genes also affected neuropeptide signalling, sensory perception of pain, neurotransmitter receptors and nervous system development^{35,79}. These gene profiles are interesting as type 1 fimbriae have been associated with acute cystitis^{85–88}, in which ion channel activation can lead to neuropeptide activation, causing pain, urgency and frequency.

These results suggest that P fimbriae hijack the transcriptional machinery of the host, creating a fimbriae-specific gene expression profile. P fimbriae are expressed by 95–100% of APN isolates in patients without underlying disorders⁸⁹, suggesting that expression of these adhesive ligands is essential for the

pathogenesis of APN. Thus, loss of P fimbriae would be a first step towards virulence attenuation and adaptation to long-term persistence in the urinary tract. This possibility is supported by a high frequency of inactivating papG mutations in ABU isolates⁵⁶. These findings suggest that virulence gene attenuation could be a new therapeutic alternative to antibiotics for treating UTI, focusing on super-virulence gene attenuation

rather than indiscriminate removal of the bacteria. The results further suggest that ABU strains without functional P fimbriae would be preferred for use for human inoculation.

These findings further illustrate the remarkably divergent effects of P fimbriae and type 1 fimbriae, in infected hosts. Specifically, P fimbriae act as agonists of IRF7 and of downstream genes associated with APN. The response to *E. coli* 83972fim suggests novel cellular targets that need further study.

Table 1 | Mouse single-gene knockout models

Mouse strain	Outcome of infection	Refs
Asymptomatic bacteriuria		
<i>Tlr4</i> ^{-/-}	High bacterial counts, low PMN influx, prolonged infection, no symptoms of disease	60
<i>Trif</i> ^{-/-}	High bacterial counts, low PMN influx	60
<i>Tram</i> ^{-/-}	High bacterial counts, low PMN influx	60
<i>Myd88</i> ^{-/-}	High bacterial counts, luminal PMN aggregates	60
Cystitis, recurrent UTI		
<i>Nlrp3</i> ^{-/-}	High bacterial counts, influx of leukocytes	33
<i>Asc</i> ^{-/-}	High bacterial counts, influx of leukocytes	33
<i>Tlr5</i> ^{-/-}	Prolonged infection and inflammation at later time points	139
<i>Tlr11</i> ^{-/-}	High bacterial counts, influx of leukocytes	182
<i>Cox2</i> ^{-/-}	High bacterial counts in bladder	189
<i>Cd14</i> ^{-/-}	High bacterial counts in bladder	241
<i>Ptx3</i> ^{-/-}	Increased susceptibility, high bacterial counts in bladder and kidneys	179
<i>Il17a</i> ^{-/-}	Increased susceptibility, fewer PMNs	205
<i>Tcrd</i> ^{-/-} , <i>Ifnb1</i> ^{-/-} , <i>Il4</i> ^{-/-}	Increased susceptibility	242
Acute pyelonephritis		
<i>Irf3</i> ^{-/-}	Severe APN, acute mortality, sepsis, high bacterial counts, large renal abscesses	32,34
<i>Ifnb1</i> ^{-/-}	Severe APN, acute mortality, sepsis, high bacterial counts, large renal abscesses and pathology	32
<i>Cxcr2</i> ^{-/-}	Severe APN, acute mortality, sepsis, high bacterial counts, large renal abscesses and pathology	31,164,169
<i>Il6</i> ^{-/-}	Severe APN, high bacterial counts	156
<i>cAMP</i> ^{-/-}	High bacterial counts, pathology and severe symptoms	174
<i>Thp</i> ^{-/-}	High bacterial counts	188
<i>Nod2</i> ^{-/-}	Kidney abscesses	243
<i>Thrombomodulin</i> ^{-/-}	Elevated bacterial loads	244
<i>P2x7</i> ^{-/-} , <i>P2x4</i> ^{-/-}	Increased sensitivity to sepsis	245
Resistant phenotype		
<i>Irf7</i> ^{-/-}	Highly resistant to UTI	34
<i>Il1b</i> ^{-/-}	Highly resistant to UTI	33,80
<i>Atg16l1</i> ^{-/-}	Increased bladder clearance, protection	246
<i>C5ar1</i> ^{-/-}	Protected against kidney infection	247
<i>Nos2</i> ^{-/-} , <i>Il10</i> ^{-/-}	No increased susceptibility	242
<i>S100a9</i> ^{-/-}	Intact response, clearance	248
<i>Tlr2</i> ^{-/-}	Intact response, clearance	60
<i>Casp1</i> ^{-/-} , <i>Mmp7</i> ^{-/-}	Intact response, clearance	33

APN, acute pyelonephritis; PMN, polymorphonuclear neutrophil; UTI, urinary tract infection.

Acute pyelonephritis

APN is a worst-case scenario and the opposite of ABU. UPEC strains attack the renal pelvic mucosa and can invade the renal parenchyma and reach the blood stream, causing urosepsis^{90–92}. Systemic involvement gives rise to fever (>38 °C), elevated C-reactive protein (CRP) in blood and general malaise, owing to the spread of innate immune mediators, such as IL-6, from the site of infection^{93,94} (FIG. 2). In addition to the acute morbidity and mortality associated with APN, chronic tissue damage is common and renal tissue damage can cause focal scar formation, growth retardation and loss of function^{25,95–97}.

Triggering the host response in APN

APN is caused by excessive innate immune activation in infected kidneys⁶, which is controlled by TLR interactions, and by the activation state of downstream transcriptional switches, which regulate cytokine and chemokine cascades, the recruitment and activation of inflammatory cells and the effector functions required to clear bacteria from infected tissue sites⁹⁸. Early studies showed that UPEC strains activate epithelial IL-6, IL-1, TNF and IL-8 production, providing a link between microbial recognition by epithelial cells and neutrophil-dependent inflammation^{93,94}. Subsequent studies and proteomic screens have extended the repertoire of inflammatory mediators and responding host cells to include nerve cells, mast cells, lymphocytes and NK cells, among others⁹⁹. The mechanism of neutrophil recruitment involves specific chemokines, such as CXCL8, KC and MCP1, and the CXCR1 receptor is required for neutrophil activation, bacterial clearance and neutrophil exit from infected tissues into the urine, a prerequisite for avoiding tissue damage³¹. Extended gene expression profiling of infected cells and tissues has been used to characterize key transcriptional regulators of the innate immune response in the kidneys, including IRF3 and IRF7, MYC and NF-κB, and gene deletion studies in mice have shown IRF3, IRF7 and CXCR1 to be disease regulators^{31,32,34}. Innate immune hyper-activation is rapid and pronounced in *Irf3*^{-/-} mice, owing to *Irf7* hyper-activation and a gene network comprising IL-6, TLR4, FOS and STAT3 (REF.³⁴) (FIG. 5).

Virulence determinants in APN strains

APN strains are efficient pathogens defined by co-ordinately regulated virulence genes^{100,101}, which engage in multiple parallel mechanisms of tissue attack^{94,102,103}. Efficient bacterial adherence characterizes the disease isolates⁶³, providing a mechanism for

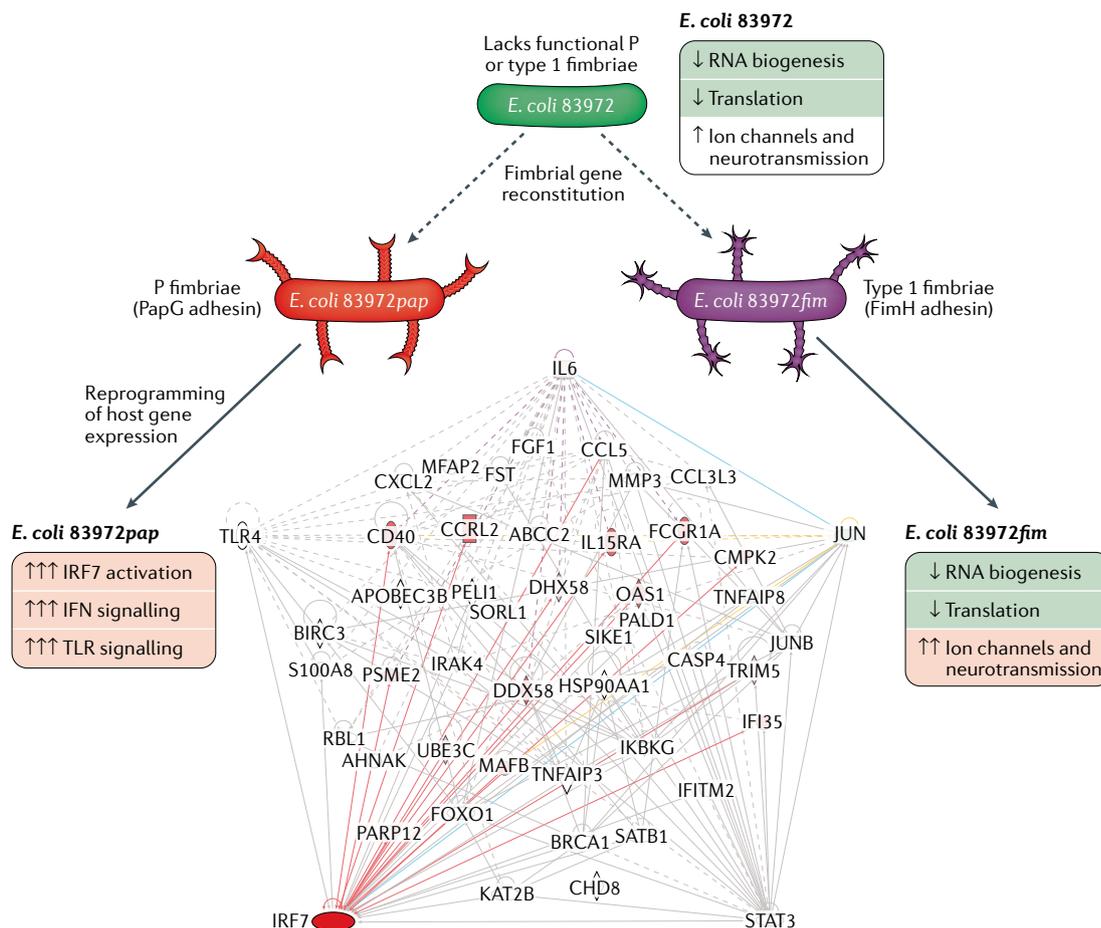
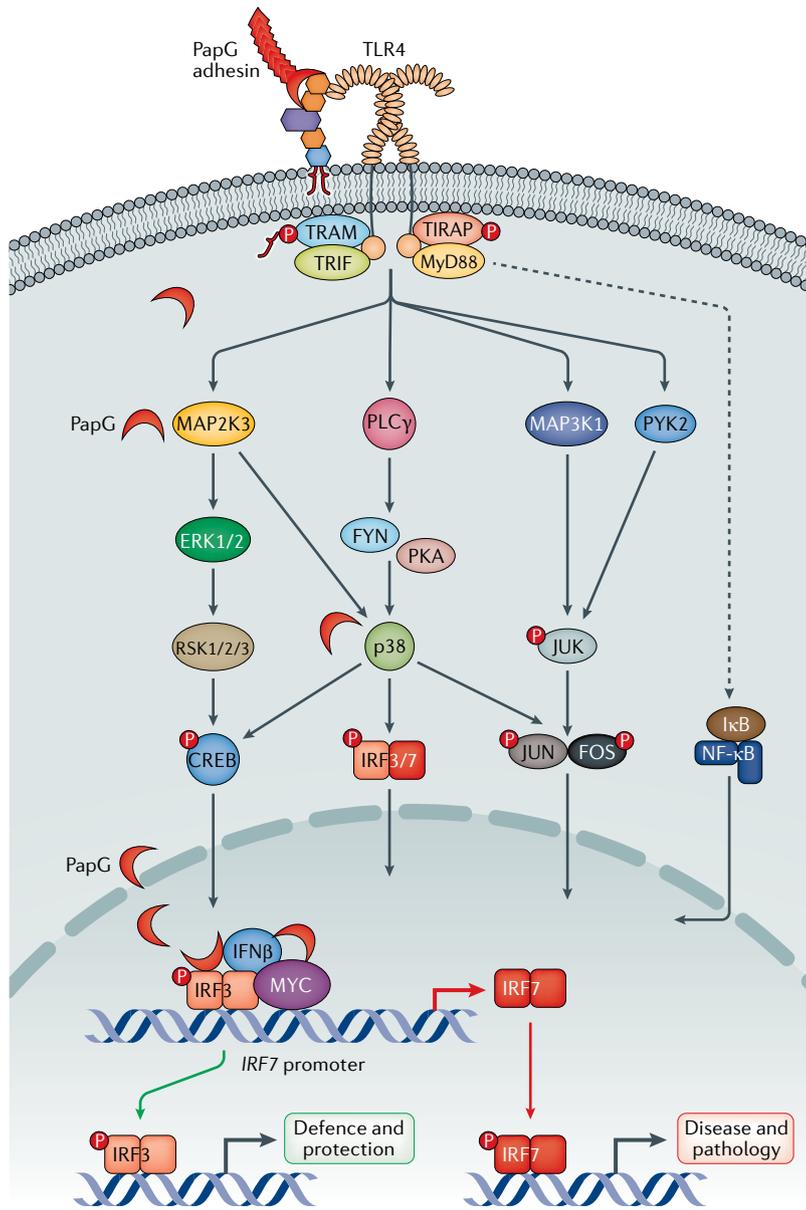


Fig. 5 | Transcriptional regulation by fimbriated bacteria in infected hosts. Human inoculation studies have been performed to prevent recurrent urinary tract infection in patients requiring alternative treatments against recurrences. The asymptomatic bacteriuria strain *Escherichia coli* 83972, which is attenuated for virulence, has been used successfully and protection has been demonstrated in placebo-controlled studies^{49–52}. To extend the therapeutic use, fimbriae expression was reconstituted in *E. coli* 83972 and *E. coli* 83972pap (P fimbriated), *E. coli* 83972fim (type 1 fimbriated) or *E. coli* 83972 wild type were used to inoculate the patients⁷⁹. Gene expression analysis of patient samples showed that the P fimbriated strain reprogrammed host gene expression, activating interferon and pattern recognition signalling pathways. Disease symptoms in two of the inoculated patients were accompanied by interferon regulatory factor 7 (IRF7) activation. By contrast, the type 1 fimbriated strain did not induce symptoms but inhibited RNA biogenesis and translation. Furthermore, *E. coli* 83972fim activated the expression of ion channels and neuropeptide-related genes in the host. TLR, Toll-like receptor.

the pathogen to engage with the host. Extensive studies have subsequently defined a number of bacterial virulence factors^{104,105} and increased understanding of virulence and the niche-specific adaptation to the urinary tract environment¹⁰⁶. The presence of large chromosomal islands distinguishes pathogenic strains from non-pathogenic *E. coli* K-12 strains¹⁰⁷. PAI-CFT073-*serU* and PAI-CFT073-*pheU*, harbouring P fimbriae and *TcpC* genes, are more strongly associated with APN and urosepsis than with ABU or cystitis¹⁰⁸. PAI deletions reduce virulence¹⁰⁹, despite fully functional virulence genes on other PAIs, suggesting a regulatory role¹¹⁰.

Adhesins and other virulence factors, expressed by uropathogenic *E. coli*. The interplay between fimbrial adhesins and host receptors provides molecular keys to innate immune activation and tissue access of additional virulence factors^{60,111,112}. P fimbriae provide a

direct disease-associated mechanism of tissue attack, by enhancing adherence, activating Tlr4 signalling and activating the expression of the APN-associated transcription factor IRF7 (REFS^{34,79}) (FIG. 6). P-fimbriated *E. coli* activate ceramide release by binding to receptor motifs present in the extracellular oligosaccharide domains of glycosphingolipid receptors, overriding the mucosal inertia to LPS^{113–115}. Downstream signalling involves the TLR4 adaptor proteins TIR domain-containing adapter molecule 2 or TICAM2 (TRAM) and TIR domain-containing adapter molecule 1 or TICAM1 (TRIF) (FIG. 6), the phosphorylation of mitogen-activated protein (MAP) kinases, phospholipase C_γ, p38, activating JUN N-terminal kinases (JNKs), CREB (cyclic AMP response element-binding) and IRF-dependent and AP-1-dependent transcription of cytokines and chemokines, as well as type I interferons including IFN β ^{32,116}. P fimbriae are expressed by up to 100% of



Mouse single-gene knockout

<i>lrf3</i> ^{-/-}	Acute mortality, sepsis, high bacterial counts, large renal abscesses and pathology
<i>lrf7</i> ^{-/-}	Highly resistant to UTI
<i>lfnb1</i> ^{-/-}	Acute mortality, sepsis, high bacterial counts, large renal abscesses and pathology

Human polymorphisms

<i>IRF3</i> promoter SNPs	Low promoter activity	APN
<i>IRF7</i> promoter SNP	Low expression	ABU

APN and urosepsis strains but are rare in ABU strains, unlike type 1 fimbriae^{89,104}.

Other virulence-associated adhesins in APN strains include the Dr adhesin family of fimbrial and afimbrial components and S fimbriae that bind to the mucosa and type IV collagen on basement membranes^{117,118}, facilitating invasion¹¹⁹. Type 1 fimbriae contribute to the pathogenesis of APN in the mouse UTI model but are not directly associated with virulence in epidemiological studies, suggesting a different role^{120,121}.

Fig. 6 | TLR4 signalling and transcriptional control of the innate immune response in acute pyelonephritis.

P fimbriated uropathogenic *Escherichia coli* (UPEC) strains activate a Toll-like receptor 4 (TLR4) signalling cascade by phosphorylation of the TLR4 adaptor proteins (TRIF, TRAM, MyD88 and TIRAP). The PapG adhesin binds to glycosphingolipid receptors, releasing ceramide, which activates TLR4 signalling. Downstream MAP kinase signalling converges on p38, which controls the phosphorylation and activation of interferon regulatory factor 3 (IRF3), as well as NF-κB and activator protein 1 (AP-1); a heterodimer of FOS and JUN. IRF7 activation requires de novo synthesis followed by phosphorylation. IRF3 and IRF7 control disease severity by regulating different aspects of the antimicrobial defence and the inflammatory response of mice infected with UPEC strains. IRF3 is essential for the protective response and mice lacking IRF3 (*lrf3*^{-/-} mice) develop severe acute infection with sepsis and mortality, followed by renal damage^{32,34}. Downstream mediators such as IFNβ are of crucial importance for the *lrf3*-dependent defence to occur and *lfnb1*^{-/-} mice develop a similar disease phenotype when infected with UPEC strains³². By contrast, IRF7 drives disease and tissue pathology and mice lacking IRF7 (*lrf7*^{-/-} mice) are protected from acute pyelonephritis³⁴. In addition, PapG is internalized and engages the *IRF7* promoter complex together with IRF3, IFNβ and MYC. The PapG adhesin acts as a transcriptional *IRF7* agonist, affecting the repertoire of genes expressed during acute pyelonephritis. Human promoter polymorphisms predicted to affect the susceptibility to acute pyelonephritis by reducing the expression of *IRF3* have been detected in about 70% of patients with acute pyelonephritis³². Attenuating *IRF7* polymorphisms have been detected in asymptomatic bacteriuria³⁴. ABU, asymptomatic bacteriuria; APN, acute pyelonephritis; CREB, cyclic AMP response element-binding; P, phosphate group; SNP, single-nucleotide polymorphism; UTI, urinary tract infection. Adapted from REF.⁶, Springer Nature Limited, and from REF.⁷⁹, CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>).

Endotoxins and exotoxins are important for bacterial tissue attack and act either directly as cytotoxic agents or indirectly by triggering inflammation and tissue damage¹²². Uropathogenic strains produce haemolysin, which is toxic to kidney cells and causes urothelial damage and haematuria^{123,124}. The precise mechanism of action of the *E. coli* haemolysin and the pore formation in human cells is still debated, as most studies of haemolysin have been performed using α-haemolysin isolated from other bacterial species such as *Staphylococcus aureus*^{125,126}. Currently, two theories exist that suggest that haemolysin either directly engages the lipid bilayer of the plasma membrane or the membrane-bound protein ADAM10, which then further facilitates membrane access¹²⁷. Furthermore, the cytotoxic necrotizing factor 1 (CNF1) and Rho-GTPase-activating toxins secreted by UPEC strains are associated with adherence and invasion, inducing actin cytoskeleton reorganization in host cells and initiating apoptosis¹²⁸.

Moreover, bacterial iron acquisition systems are activated in the urinary tract¹²⁹ and several siderophores expressed by UPEC strains are essential in vivo, where they contribute to bacterial fitness during infection¹³⁰⁻¹³⁴. The siderophore aerobactin, shows a

virulence-associated expression pattern and promotes persistence in the urinary tract¹³⁵. Lipocalin 2 binds and sequesters enterobactin, a siderophore implicated in iron acquisition¹³³. UPEC strains can also escape the host defence by glycosylation of enterobactin, and the product known as salmochelin is not recognized by its ligand lipocalin 2 (REFS^{133,134}).

Flagella, capsular polysaccharides and immune evasion proteins all contribute to the disease process by supporting the ascent of bacteria to the upper urinary tract¹³⁶ and conferring resistance to antibacterial effectors of inflammation. Flagella are essential for bacterial motility and to enable bacteria to ascend from the infected bladder into the renal pelvis. Flagella have been shown to engage TLR5 and activate innate immune signalling¹³⁷. The uptake of UPEC strains by renal cells is supported by opsonization of bacteria with the complement component C3 and the presence of epithelial cell membrane C3 receptors¹³⁸.

Studies of individual virulence factors clearly support their effects in cellular systems and animal models, but further studies are required to evaluate their specific contributions to disease alone or in combination.

TIR domain proteins in UPEC strains suppress the innate immune response to virulent strains. Given the central role of the TLRs in the host defence^{60,77,139,140}, unsurprisingly, bacteria have evolved mechanisms to specifically interfere with TLR-mediated immune responses. TIR domain homologues have been detected in plants, mediating pathogen detection and defence responses¹⁴¹, and in bacteria, viruses and archaea, illustrating the importance of this protein family^{142–144}. The ancestral prokaryotic TIR domains have been found to act as NADase enzymes, cleaving NAD⁺ into nicotinamide and ADP-ribose, therefore regulating oxidoreduction processes and cellular metabolism¹⁴⁵. In bacteria, TIR-containing proteins (Tcps) suppress innate immunity in mice with UTI¹⁴³. A TIR homologue is present in the genome of UPEC CFT073 and the bacteria secrete inhibitory TIR domain homologues that interfere with TLR signalling by competitive binding to MyD88 *in vitro*^{80,144}. TcpC is most commonly expressed in virulent UPEC strains and has been shown to promote bacterial survival and kidney pathology in a mouse UTI model¹⁴³. These observations suggest that pathogens are capable of directly modifying the TLR-dependent host defence by using TIR domain homologues.

UPEC strains produce inhibitors of the MYC oncogene. The bacteria–host interaction model helps in the discovery of molecules with biologically relevant effects in diseases other than UTI. In addition to producing molecules that directly affect virulence, UPEC strains were shown to inhibit and degrade the MYC oncogene protein¹⁴⁶. Cancer cells are fast growing, outcompete non-malignant cells and spread to distant sites, where they cause metastasis¹⁴⁷. Understanding what makes cancer cells so efficient and threatening is crucially important, and stopping them has always been the goal of cancer research. Early studies identified oncogenes; genes that normally control cell growth but when

mutated can be responsible for oncogenic transformation and explain the competitive advantage of cancer cells¹⁴⁸.

The pleiotropic transcription factor MYC has been named ‘the quintessential oncogene’ and is hyperactive in the majority of human cancers¹⁴⁹. Targeting MYC is, therefore, highly desirable. *Myc* inhibition clearly arrests cancer progression and restores tissue integrity in transgenic mouse models^{149,150} and a vast number of technologies have been applied in attempts to target MYC and its different co-factors in cancer cells. Still, finding MYC inhibitors for therapeutic use has been problematic and MYC itself has long been viewed as undruggable¹⁵¹.

Surprisingly, UPEC strains deplete MYC protein from infected cells and tissues as a result of accelerated MYC protein degradation and attenuated *MYC* expression¹⁴⁶. This bacterial strategy was translated to cancer therapy, demonstrating that Lon protease treatment is efficacious in slowing cancer progression and increasing survival¹⁴⁶. The bacterial Lon protease is shown to rapidly degrade MYC without affecting MAX and other upstream regulators such as AKT, CDK9 or CPEB1, suggesting a high degree of specificity for MYC¹⁴⁶.

The Lon protease is a conserved, ATP-dependent serine protease that maintains homeostasis in prokaryotes by processing short-lived regulatory proteins¹⁵². The Lon protease targets MYC in a variety of cancer cells¹⁴⁶, including Burkitt lymphoma cells, in which MYC was first discovered¹⁵³. Gene expression profiles in Lon-treated tissues showed a strong inhibitory effect on cancer-related genes, but no major effects on other gene categories, also supporting the potential of this therapeutic approach¹⁴⁶.

These results suggest that bacteria have evolved strategies to control MYC tissue levels in the host. As MYC drives renal development¹⁵⁴, and APN is a substantial cause of renal growth retardation¹⁵⁵, *MYC* overexpression might have evolved to protect local host environments against detrimental effects of pathogenic bacteria. Speculatively, by inhibiting mucosal MYC expression bacteria might inadvertently provide a defence against oncogenic transformation.

Genetic control of APN susceptibility

Infections constitute a major evolutionary driving force, selecting resistance traits in infected hosts and bacterial genome alterations that enhance persistence. UTIs are an excellent example, as the most severe infections cause considerable mortality and impaired renal health, and, in women specifically, reduced fertility and premature delivery, starting from childhood. Host susceptibility is now being associated with inherited genetic traits and bacteria shown to evolve by loss of virulence, facilitating symbiosis and long-term persistence.

Genes that control immune activation and essential effector functions in APN have been identified using cellular infection models, animal models and clinical studies^{31,32,34,156–158}. Specific genes have been shown to control APN severity by affecting initial disease activation, transcriptional regulators of inflammatory cascades, cellular antibacterial effector functions and mechanisms of tissue repair^{32,34} (FIG. 6; TABLE 2). Further

Table 2 | Gene-association studies in patients

Genetic variant	Biological effect	Disease association	Refs
TLR4 promoter SNPs	Reduced promoter activity, low TLR4 expression	ABU	58,249
TLR2 coding region SNP	NR	ABU and recurrent UTI	250
TLR1, TLR2, TLR4, CXCR1, CXCR2 SNPs	NR	ABU	81
IRF7 promoter SNP	Low IRF7 expression	ABU	34
CXCR1 intronic SNP	Reduced promoter activity, low CXCR1 expression	APN	157,171
CXCR1 3' UTR SNP	Increased 3'-mRNA processing	APN	157,171, 251
IRF3 promoter SNPs	Low promoter activity	APN	32
CXCL8 promoter and 3' UTR SNP	NR	APN	158, 251–254
CCL5 promoter SNP	NR	APN	181
IL10 promoter SNP	NR	Recurrent APN	180
NLRP3 intronic SNP	NR	Negative association with APN	255
LTA coding region, TNF promoter SNP	NR	Recurrent UTI	256
HSPA1B coding region SNP	NR	Recurrent UTI	257
TLR1, TLR4, TLR5 coding region SNP	NR	Recurrent UTI	83
DEFA1A3 copy number	Low copy number	Recurrent UTI	258
TGFB1, VEGFA promoter SNP	NR	UTI	259
TLR4 coding region SNP	Low monocyte and/or neutrophil expression	UTI	257, 260–263
PTX3 intronic SNP	NR	UTI	179
VDR (vitamin D receptor) coding region and intronic SNP	NR	UTI susceptibility	264
DNA copy number variations	Enrichment in innate immunity and development genes	UTI susceptibility in patients with VUR	97

ABU, asymptomatic bacteriuria; APN, acute pyelonephritis; NR, not reported; SNP, single-nucleotide polymorphism; TLR4, Toll-like receptor 4; UTI, urinary tract infection; UTR, untranslated region; VUR, vesicoureteral reflux.

evidence that APN susceptibility is genetically controlled was obtained in a three-generation family study. Inheritance of APN susceptibility was documented as an increase in APN frequency in both male and female children from families diagnosed with APN compared with children from families without UTI¹⁵⁷. Cystitis morbidity was not inherited in these families, supporting the notion that different mechanisms control the susceptibility to APN and cystitis^{6,29} (TABLE 2).

Genetic control of transcriptional regulation. Gene knockout studies have been used to identify key activation steps of the innate immune response to infection. Inactivating TLR4-*tir* domain mutations or *Tlr4* deletions inhibit innate immune activation in mice, resulting in a phenotype that resembles ABU^{9,60,80}. Signalling cascades downstream of TLR4 converge on specific transcription factors (including IRF3, IRF7, AP1,

NF-κB) and their role as regulators of UTI severity has been evaluated using gene knockout technology and in clinical studies^{32,34} (TABLE 1; FIG. 6). The closely related transcription factors IRF3 and IRF7 form heterodimers that regulate the activation of type I interferons and interferon-dependent genes, especially during viral infections^{159,160}. However, in APN, these transcription factors have opposite functions. After UPEC infection, mice lacking the transcription factor *Irf3* develop severe disease, accompanied by urosepsis and massive renal abscess formation³². IFNβ is activated downstream of IRF3, and *Ifnb1*^{-/-} mice showed a similar phenotype to *Irf3*^{-/-} mice, in the same infection model.

By contrast, *Irf7*^{-/-} mice were protected from acute renal inflammation and showed no evidence of kidney pathology³⁴, suggesting that IRF7 might drive kidney pathology^{34,79}. IRF7-dependent gene networks were strongly upregulated in *Irf3*^{-/-} mice infected with the UPEC strain CFT073, including an *Irf7*-dependent gene network comprising *Stat3*, *Tlr4* and *Il6* and downstream genes involved in the acute phase response. Binding of IRF7 to promoter DNA fragments (*OAS1*, *CCL5* and *INF1*) was demonstrated, confirming the role of IRF7 as a transcriptional regulator. *IRF7* was further identified as an important transcriptional mediator during group B streptococcus-induced UTI in mice, suggesting a role during infections with Gram-positive pathogens as well¹⁶¹.

The human relevance of these findings was supported by the detection of an *IRF3* promoter sequence variants in two APN-prone patient populations³². The homozygous A/A-C/C genotype (nucleotide positions -925 and -776) was prevalent in patients prone to APN (79%), whereas the co-segregating heterozygous SNPs were more common in patients with ABU (69%). The APN haplotype was shown to decrease *IRF3* expression in reporter assays, suggesting that IRF3 needs to be fully functional to avoid APN³².

The protective phenotype in *Irf7*^{-/-} mice and overactivation of *Irf7* in disease-prone *Irf3*^{-/-} mice identified IRF7 as a potential therapeutic target. Support for this hypothesis was obtained using an siRNA-based strategy of *Irf7* inhibition³⁴. siRNA treatment of *Irf3*^{-/-} mice infected with the UPEC strain CFT073 was shown to inhibit the excessive and destructive innate immune response and to improve bacterial clearance, resulting in resolution of infection by day 7. siRNA therapy compared favourably with antibiotic treatment (cefotaxime treatment, 100 mg/kg)³⁴ and *Irf7* siRNA treatment was able to significantly reduce the disease score, including the number of kidney abscesses, by 95% compared with untreated mice ($P < 0.01$).

Prospective studies of genetic predisposition in childhood. The extent of genetic predisposition to APN and renal scarring has been investigated in two population-based studies. Children with a first febrile UTI episode were investigated and renal involvement was defined using dimercaptosuccinic acid (DMSA) scans. Inclusion criteria were matched, and the age range of the patients was 1 month to 2 years in population I and 3–5 months in population II. Molecular

susceptibility determinants were identified using exome genotyping, and effects of infection and host genotype on gene expression were analysed in detail. According to preliminary findings, APN is a disease with a strong genetic component, affecting acute renal involvement and disease severity (C.S., unpublished work).

Genetic determinants of neutrophil infiltration and pathology. APN is characterized by excessive neutrophil recruitment, neutrophil accumulation and inefficient bacterial clearance from infected kidneys¹⁶². Early studies detected a rapid TLR4-dependent urine neutrophil response in the mouse model of UTI and the IL-8 family of chemokines (CXCL8) was shown to be activated during APN in mice and patients^{78,163}. The importance of neutrophils in the antimicrobial defence of the kidneys has been documented using genetic studies, anti-inflammatory agents, bone-marrow transplants and functional assays^{31,164}.

Gene knockout studies revealed that control of the neutrophil response is essential to clear the infection and maintain tissue integrity^{163,165–167}. The defective neutrophil recruitment, in, for example, *Tlr4*^{-/-} mice, protected the tissues from acute pathology but bacterial clearance was not achieved, creating an ABU-like state⁶⁰. Mice lacking a key receptor for the IL-8 chemokine family (*Cxcr2*^{-/-} mice), developed severe APN with urosepsis and acute mortality. Surviving mice developed renal scarring, with pathology similar to human pyelonephritis. This outcome was explained by a neutrophil migration deficiency, resulting in neutrophil accumulation in infected kidneys owing to an inability to cross the pelvic epithelium into the urine and to scavenge and kill the bacteria^{31,168,169}. The trapped neutrophils with their content of bacteria and debris became highly toxic for the renal parenchyma, resulting in renal scar formation. These results illustrate the importance of chemokines and their receptors for the control of neutrophil recruitment and bacterial clearance from infected kidneys^{31,164,170}.

In patients with APN, susceptibility is accompanied by low CXCR1 expression and *CXCR1* mRNA levels^{157,171}. Patients prone to APN carry heterozygous *CXCR1* polymorphisms attenuating receptor mRNA levels and protein expression. The presence of *CXCR1* variants has been confirmed in several APN-prone patient groups^{158,172}. Furthermore, SNPs affecting IL-8 expression (*CXCL8*) were detected in patients with renal involvement confirmed by positive DMSA scans, supporting the relevance in human UTI.

Other effector functions of the innate immune response. Antibacterial peptides from infected tissues and inflammatory cells are potent effectors of the renal defence against infection (such as β -defensins, ribonucleases and cathelicidin)^{173–178}. RNase 7 and cathelicidins act by disrupting the phospholipid membranes of various microorganisms and are constitutively synthesized by the kidneys^{174,176}. Lipocalin 2 (LCN2 or NGAL) is an antibacterial protein secreted both in the urine and systemically after Gram-negative infection, kidney injury or urosepsis. LCN2 binds the bacterial siderophore

enterobactin, preventing iron transfer and sequestration by the bacteria¹³³. The humoral pattern recognition molecule pentraxin 3 (PTX3) serves as an opsonin and promotes bacterial uptake by neutrophils¹⁷⁹. *PTX3* is polymorphic in UTI-prone children and adults¹⁷⁹ (TABLE 2). Polymorphisms affecting *IL10* were identified in APN-prone patients¹⁸⁰ as well as SNPs in the *CCL5* gene encoding the eosinophil product and T cell chemoattractant RANTES¹⁸¹. In mice, a loss-of-function mutation affecting *Tlr11* increased bacterial burden and leukocyte infiltration compared with wild-type mice in a UTI model. TLR11 is strongly expressed in mice but the human *TLR11* gene is defective and human relevance remains unclear¹⁸².

Taken together, these observations suggest that a combined effect of bacterial ‘super virulence factors’ and host susceptibility converge to cause severe disease in patients with APN. Starting from bacterial adherence and TLR4-mediated signalling, a blatant host response takes place with increased severity depending on the host genetics.

Acute cystitis

Acute cystitis is characterized by a pronounced mucosal inflammatory response in the lower urinary tract¹⁵. Patients experience frequency, dysuria and suprapubic pain, often accompanied by haematuria and pyuria¹⁸³. A systemic host response is not typically seen, and unlike APN, acute cystitis is not accompanied by fever and general malaise¹⁸⁴. However, recurrent episodes of acute cystitis are common and can become debilitating, especially in subsets of susceptible patients who develop interstitial cystitis and/or bladder pain syndrome as a result of prolonged mucosal inflammation and nerve excitation^{185,186}.

Genetic control of severity

The molecular determinants of disease severity in acute cystitis have been unclear; however, mechanisms of innate immune hyper-activation have now been identified using a combination of cellular infection technology, animal models and clinical studies^{30,33,35}. The results show that acute cystitis is an IL-1 β -driven, hyperinflammatory disorder of the infected urinary bladder driven by atypical IL-1 β processing through matrix metalloproteinase 7 (MMP7)³³ (FIG. 7). The results also suggest a genetic susceptibility mechanism of severe cystitis through mutations affecting *IL1* as well as *ASC* and *NLRP3* expression.

Pro-IL-1 β is normally processed by the NLRP3 inflammasome, leading to transient, self-limiting inflammation in the bladders of C57BL/6 wild-type mice with an intact inflammasome. *Il1b*^{-/-} mice were protected and the effect was similar to that previously observed in *Tlr4*^{-/-} mice, suggesting that IL-1 β is essential to drive the inflammatory response to bladder infection. Paradoxically, *Asc*^{-/-} and *Nlrp3*^{-/-} mice carrying inflammasome gene deletions developed severe, progressive bladder disease with large, hyperaemic bladders, loss of bladder tissue integrity and elevated bacteria counts, accompanied by IL-1 β hyper-activation³³. An alternative mechanism of pro-IL-1 β processing was detected in these severely ill mice, using gene expression analysis

of bladder tissue. The metalloproteinase *Mmp7* was transcriptionally hyperactivated (about 200-fold) and was shown to cleave pro-IL-1 β , generating excessive amounts of active IL-1 β in mice and human bladder cells¹⁸⁷. ASC and/or NLRP3 were further identified as transcriptional

repressors of the *MMP7* promoter, providing an explanation for the observed MMP7 over-activation in *Asc*^{-/-} and *Nlrp3*^{-/-} mice³³.

Further experiments have indicated that *Tlr5*, *Thp* and *Cox2* also modify innate immunity to UTI and that

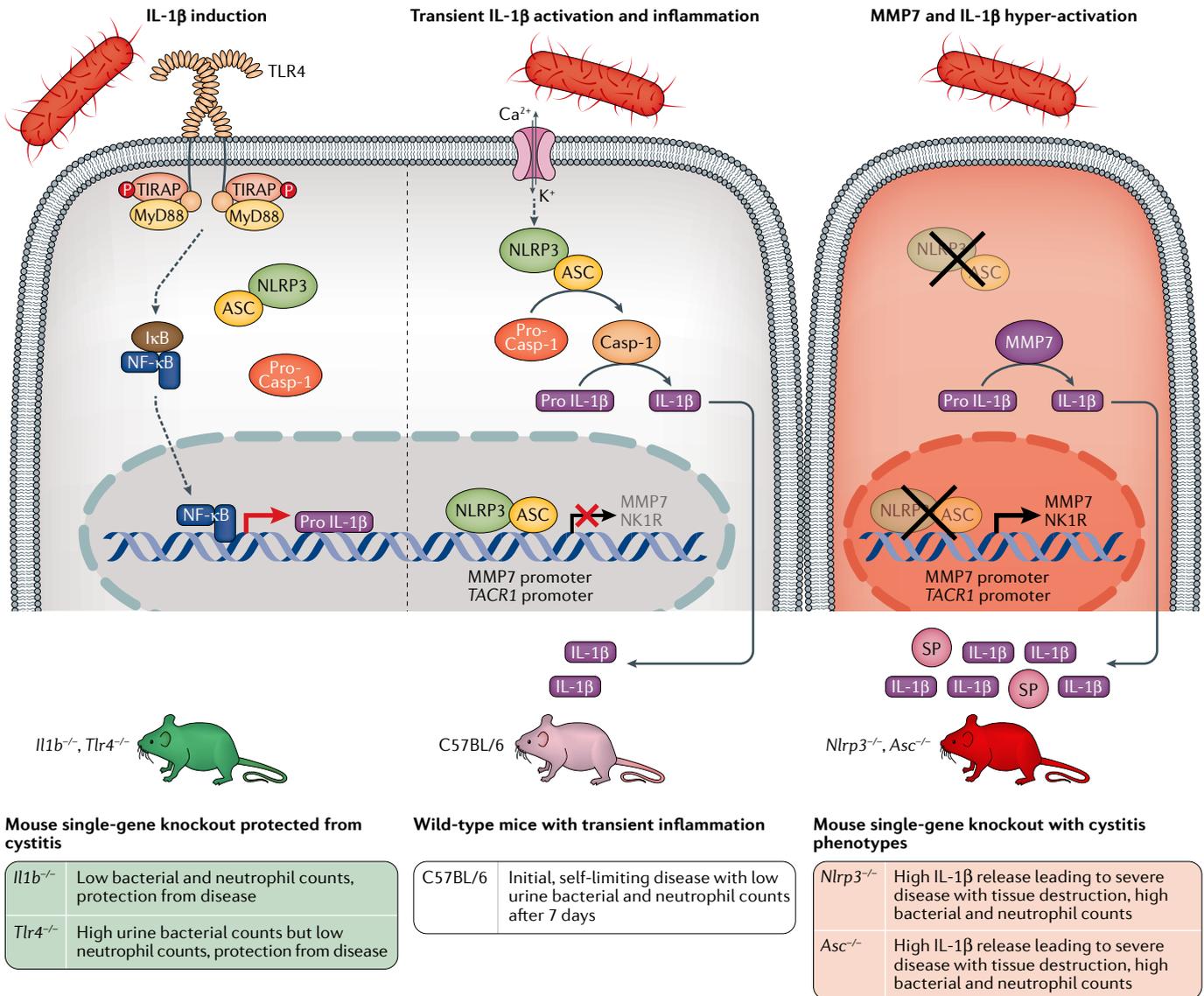


Fig. 7 | Host determinants of disease severity in acute cystitis; mechanism of IL-1 β and SP hyperactivation. Acute cystitis is a disease caused by excessive inflammation in the urinary bladder, accompanied by pain, frequency of micturition and urgency. The molecular basis of disease is illustrated here, focusing on the host determinants of disease and especially the IL-1 β response. Left panel: bacteria engage urothelial cell receptors and trigger the expression of pro-IL-1 β by activating Toll-like receptor 4 (TLR4) signalling through the MyD88 adaptor pathway and the transcription factor NF- κ B. IL-1 β is essential for disease pathogenesis, as shown by the protection from acute cystitis in *Il1b*^{-/-} and *Tlr4*^{-/-} mice, which lack the pro-IL-1 β response to infection with uropathogenic *Escherichia coli* (UPEC) strains. Middle panel: bacteria activate the NLRP3 inflammasome via a second signal involving mechanisms such as ion fluxes. Activated caspase 1 (Casp-1) then cleaves pro-IL-1 β to its mature and active form. The release of IL-1 β triggers inflammation in the bladder and creates transient, mild disease in C57BL/6 wild-type mice. Right panel: in inflammasome-deficient mice an alternative IL-1 β -processing mechanism takes over. Mice

lacking the NLRP3 inflammasome owing to single gene deletions (*Asc*^{-/-} or *Nlrp3*^{-/-} mice) develop an IL-1 β hyper-activation syndrome explained by excessive processing by matrix metalloproteinase 7 (MMP7)³³. In addition, ASC and NLRP3 regulate the expression of the pain receptor neurokinin 1 receptor (NK1R; encoded by *TACR1*), by acting as transcriptional repressors of both *MMP7* and *TACR1*. ASC and NLRP3, therefore, act as molecular gatekeepers, defining the level of transcription of the pro-inflammatory response driven by pro-IL-1 β , and the pain response, driven by NK1R and substance P (SP). In *Asc*^{-/-} or *Nlrp3*^{-/-} deficient mice, in which this gatekeeping function is lost, UPEC-infected mice develop severe acute cystitis, mediated by excessive MMP7 cleavage of pro-IL-1 β and by over-production of NK1R^{33,35}. ASC, apoptosis-associated speck-like protein containing a CARD; I κ B, inhibitor of κ B; MyD88, myeloid differentiation primary response protein 88; NF- κ B, nuclear factor- κ B; P, phosphate group; TIRAP, Toll/interleukin 1 receptor-domain-containing adaptor protein. Adapted from REF.³³, CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>).

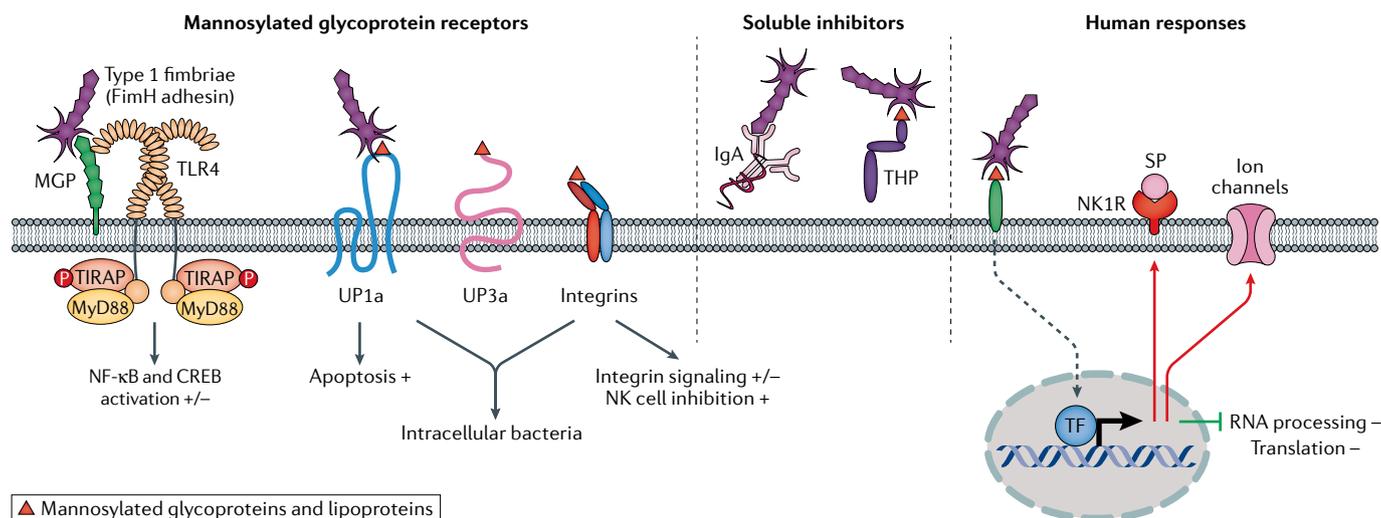


Fig. 8 | Host recognition and response parameters associated with type 1 fimbriae. Left panel: examples of receptors for type 1 fimbriae. The FimH adhesin binds mannosylated host cell glycoconjugate receptors and activates Toll-like receptor 4 (TLR4) signalling, mainly involving the myeloid differentiation primary response protein 88 (MyD88) adaptor protein arm of this signalling pathway⁶⁰, resulting in pro-inflammatory effects in mice. Binding to uroplakin particles (UP1a, UP1b, UP2 and UP3a) promotes bacterial internalization by mammalian cells; β 1 and α 3 integrins modulate F-actin dynamics and CD48 receptors trigger TNF responses in mucosal mast cells. Type 1 fimbriae have also been proposed to trigger apoptosis²²³. Middle panel: soluble receptors act as binding antagonists. Soluble IgA immunoglobulins or Tamm–Horsfall protein (THP) (or uromodulin) that carry mannose residues bind FimH and prevent the bacteria from engaging

cellular receptors in the urinary tract. Right panel: response of patients to type 1 fimbriated bacteria. The transcriptional response to type 1 fimbriae has been characterized in patients carrying *Escherichia coli* 83972 *fim*⁷⁹. *E. coli* 83972 *fim* acts as a broad inhibitor of RNA processing and translation in human cells. In addition, type 1 fimbriae potentiate ion channel activation and solute carrier expression in the patients. In human cells, FimH triggers increased neurokinin receptor (NK1R) expression. CREB, cAMP response element-binding protein; MGP, mannosylated glycoprotein; NF- κ B, nuclear factor- κ B; NK, natural killer; P, phosphate group; SP, substance; TF, transcription factor; TIRAP, Toll/interleukin 1 receptor-domain-containing adaptor protein. Adapted from REF.⁶, Springer Nature Limited, and from REF.⁷⁹, CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>).

mice carrying homozygous deletions have increased susceptibility to acute cystitis^{139,188,189} (TABLE 1). The bladders of UPEC-infected *Tlr5*^{-/-} mice showed prominent sub-mucosal oedema with leukocyte infiltration, as well as focal microabscesses and accumulation of leukocyte-rich exudates on the bladder surface¹³⁹. In infected *Tlr5*^{-/-} mice, bacteria were predominantly observed on the surface of the urothelium, with no evidence of intracellular bacterial communities. *TLR5* polymorphisms have also been observed in patients prone to recurrent UTI⁸³ but genetic variation affecting *Thp* and *Cox2* has not been reported.

Molecular basis of pain

Mechanisms of infection-associated pain have been studied in several infection models, including *S. aureus*-mediated pain and *Clostridium difficile*-induced colitis^{190,191}. Pain is a hallmark of acute cystitis; however, the mechanism by which infection triggers pain sensing in the urinary tract has been unclear. Acute cystitis strains directly activate nerve cells in vitro and in the urinary bladder³⁵ (FIG. 8). The pain-sensing machinery involves the neurokinin 1 receptor (NK1R) and substance P (SP), which are activated in isolated nerve cells and in the urinary bladder mucosa. This response was potentiated by IL-1 β in vivo in infected mice and was inhibited by IL-1R antagonists, demonstrating an interdependence between the epithelial and nerve cell response circuits³⁵. These results suggest that NK1R and SP influence the severity of acute cystitis through a

neuroepithelial activation loop that controls pain and mucosal inflammation.

NK1R and SP have been identified as novel targets for immunomodulatory therapy as well as biomarkers of acute cystitis. IL-1 receptor antagonists and NK1R inhibitors have been used successfully to inhibit infection-induced pain in mice with acute cystitis³³. Pain behaviour, defined by locomotion, hunching and rearing behaviour, was markedly attenuated, in parallel with inflammation and pathology.

Clinical relevance was supported by off-label treatment of patients with bladder pain using an IL-1 receptor antagonist. A rapid reduction in pain and increase in the quality of life were recorded, suggesting considerable improvement after treatment.

Innate immune mediators

Early studies demonstrated that bladder epithelial cells produce cytokines in response to infection; a novel concept at the time^{93,165,192,193}. Since then, numerous cytokines and chemokines have been detected in the urinary tract that mediate different aspects of the disease response^{30,194–198}. The pro-inflammatory cytokine TNF, is rapidly and transiently expressed during the first days of infection^{165,195} and supports neutrophil recruitment and bacterial clearance in a mouse model of UTI¹⁹⁹. The granulocyte colony-stimulating factor (G-CSF; also known as CSF3) is secreted during infection, and depletion using neutralizing antibodies decreased neutrophil recruitment into the bladder and increased bacterial clearance

in infected mice¹⁹⁵. G-CSF induces the maturation of progenitor cells into neutrophils and promotes their systemic circulation^{200–202}. The chemokine CXCL12 is expressed in mice within hours of *E. coli* infection and promotes NK and T cell infiltration into the bladder²⁰³. IL-17 influences disease severity and is differentially expressed between female and male mice following bladder infection²⁰⁴. IL-17-depleted mice have reduced early urine neutrophils and increased bacterial burdens²⁰⁵ and IL-17 neutralization at the onset of infection leads to chronic UTI in female mice²⁰⁴. Mechanistic studies further suggest that IL-17 might be crucial in restoring mucosal integrity after infection, also affecting chronicity^{205,206}.

Type 1 fimbriae

Why acute cystitis strains mainly infect the bladder and elicit a host response with molecular characteristics different from those seen in the kidneys remains unclear⁸⁹. Epidemiological studies have detected a high frequency of type 1 fimbriae among acute cystitis strains, but type 1 fimbrial expression does not show a clear disease association^{64,89}. Gene expression studies in infected mice suggest that type 1 fimbriae are expressed in the urinary tract, but the fimbriae were undetectable in acute isolates from women with UTI²⁰⁷. Haemolysin and the *prsG*₁₉₆ type of P fimbriae have been proposed to occur more often in acute cystitis strains than in other *E. coli*, but this association varies between study populations^{208,209}.

Type 1 fimbriae act as virulence factors in the mouse urinary tract and tissue interaction mechanisms have been extensively investigated^{120,121,210,211}. Type 1 fimbriae improve bacterial attachment to the bladder mucosa, where several mannosylated host cell glycoconjugates act as receptors^{212–217} (FIG. 8). The FimH adhesin binds to the Tamm–Horsfall protein (THP; also known as uromodulin)²¹⁵, secretory IgA²¹², uroplakins (UPs)²¹⁶ and CD48 on mucosal mast cells²¹⁴, as well as integrins β 1 and α 3 (REF.²¹⁷). Type 1 fimbriae have been proposed to facilitate the invasion of mucosal cells and bacterial persistence and cystitis in mice^{218,219}. In humans, early findings of intracellular bacterial communities²²⁰ have not been confirmed.

Binding of type 1 fimbriae to UP1a stimulates the exfoliation of bladder epithelial cells, thereby shedding adherent bacteria and increasing access to underlying tissues^{86,221}. In response to FimH–FimC binding, UP3a phosphorylation activates casein kinase II and triggers calcium fluxes^{222,223} and cyclic AMP regulates the incorporation of *E. coli* into fusiform vesicles and exocytosis of *E. coli* from bladder epithelial cells. Intraperitoneal forskolin treatment, which elevates intracellular levels of cyclic AMP, was reported to eliminate >99% of intracellular *E. coli* by exocytosis without affecting bacterial viability²²⁴.

The relevance of these mechanisms to disease severity in acute cystitis remains unclear. Clear disease phenotypes have not been observed and consequences of these molecular effects for disease have not been clearly defined.

Mast cells in acute cystitis

Mast cell activation is important in the bladder mucosa²²⁵ and known effects of histamine and other mast-cell mediators correlate with the neuroinflammatory

response and symptoms of acute cystitis²²⁶. Pre-stored TNF-containing granules within mast cells have been shown to burst and participate in the recruitment and activation of neutrophils²²⁵, and TNF activation and neutrophil recruitment was reduced in mast cell-deficient mice²²⁵. Mast cells are activated by epithelial release of IL-1 β , ATP, IL-33 and β -defensins²⁸, and the histamine response magnitude correlated positively with the number of adherent type 1 fimbriated bacteria. Type 1 fimbriae interactions with mast cell glycosyl-phosphatidylinositol receptors (CD48) were shown to affect the uptake of adherent bacteria as well as mast cell degranulation²²⁷. The role of mast cells and their link to type 1 fimbriae are interesting and potentially important, but their role in host resistance and disease severity requires further study.

The shedding of superficial bladder epithelium during UTI is well known and UPEC infection was shown to trigger rapid desquamation in the 1980s²²⁸. In later studies, cell shedding was attributed to granule-releasing mast cells as a mechanism to reduce the tissue-associated bacterial load^{229,230}. In a mouse model of induced recurrent cystitis, a lack of superficial terminal differentiation markers in umbrella cells was interpreted to indicate a lack of tissue repair²³¹. Susceptibility to recurrent UTI was positively correlated with cyclooxygenase 2 (COX2) expression, as would be expected from effects on innate immunity²³², but the effects of bacterial clearance varied, suggesting additional variables²³³. Moreover, a strong T_H2 response was proposed to aid in repairing the superficial bladder epithelium following infection-triggered exfoliation of this barrier²⁰⁶.

Taken together, these studies highlight the importance of host susceptibility for disease in the urinary bladder and identify IL-1 hyperactivation as a potent disease determinant with immediate human relevance. In addition, pro-inflammatory loops involving pain sensors provide an explanation for the pain response, especially in acute cystitis. Clinical studies provide support for these mechanisms, as IL-1 and the neuropeptide substance P both show elevated levels in acute cystitis patients, compared with individuals with ABU.

Thus, these findings indicate that the use of immune response biomarkers should be explored to distinguish different forms of UTI and evaluate the level of immune activation in the patient.

Parallels with COVID-19 infection

The protective role of innate immunity and detrimental effects of excessive immune activation have become very obvious during the COVID-19 pandemic. The strategies of innate immune activation are conserved and the innate immune response to UTI shares several characteristics with the response seen in patients with COVID-19. For example, viral and bacterial ligands guide TLR4 activation^{234,235}. TLR4 has shown strong protein–protein interaction with the spike glycoprotein of SARS-CoV-2, leading to excessive TLR4 activation²³⁶. Furthermore, SARS-CoV-2 has an affinity for linoleic acid²³⁷, suggesting a potential parallel to P fimbriae, which bind to glycosphingolipid receptors and trigger the release of ceramide, consisting of sphingosine and

fatty acids, which acts as a signalling intermediate in TLR4 activation¹¹⁴. Severely ill patients with COVID-19 have excessive production of pro-inflammatory cytokines and chemokines including IL-6 (REF.²³⁸), which is associated with disease severity and outcome in APN and urosepsis as well²³⁹. Finally, IL-1 β levels are dramatically increased in patients with COVID-19 and MMP levels are elevated²⁴⁰, possibly indicating that the non-canonical processing mechanism of pro-IL-1 β by MMP7, which is seen in acute cystitis, might contribute to this excessive response.

Conclusions

UTIs are a common cause of morbidity and mortality and impair the quality of life for large numbers of individuals. Every other woman is affected by acute cystitis at least once and recurrences and chronicity are extremely common. APN in childhood is a major cause of renal scar formation and renal growth retardation, increasing the risk of hypertension and chronic renal disease requirement for dialysis or transplantation in adulthood and also premature birth in women. Antibiotics have provided an essential therapeutic option, but owing to the rapid increase in antibiotic resistance, detailed molecular questions need answers in order to design effective novel interventions with sufficient precision.

Specific molecular insights can, for the first time, provide appropriate tools to evaluate disease severity, susceptibility and future patient risk.

In this Review, we show how an overactive innate immune response can become a susceptibility factor in UTI, with debilitating consequences for the patients. The data also distinguish APN from acute cystitis, at the molecular level^{32–34}. APN susceptibility is enhanced by a transcription factor imbalance and by immunodeficiencies affecting neutrophil-dependent bacterial clearance³⁴. By contrast, acute cystitis is caused by IL-1 β hyperactivation owing to mutations that inactivate the inflammasome constituents ASC and NLRP3. A compensatory route of pro-IL-1 processing by the MMP7 protease, is shown to result in excessive cleavage of pro-IL-1 β , causing severe disease³³. Thus, even when mice are infected with the same *E. coli* strain, the host decides the outcome.

The findings emphasize how weaknesses of the host create innate immune imbalances and immune hyper-activation disorders leading to disease. The results also provide the rationale for immunomodulation as a new therapeutic tool to deliberately modify host susceptibility, control the host response and avoid severe disease.

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Author contributions

I.A., D.B., M.L.Y.W., T.R. and C.S. researched data for the article and wrote the manuscript. I.A., D.B., M.L.Y.W., C.S., T.H.T., S.M.C. and C.S. made substantial contributions to discussions of the content and I.A., D.B., M.L.Y.W. and C.S. reviewed and edited the manuscript before submission.

Competing interests

Patents have been filed for the use of the NlpD protein and IRF7 siRNA as immune modulators and for the use of IL-1R antagonists in the treatment of cystitis. The rights to develop these patents are held by SelectImmune Pharma, where the scientists I.A., D.B., T.H.T., M.L.Y.W. and C.S. hold shares. T.R. and S.M.C. declare no competing interests.

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