Immunomodulation therapy offers new molecular strategies to treat UTI

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Abstract | Innovative solutions are needed for the treatment of bacterial infections, and a range of antibacterial molecules have been explored as alternatives to antibiotics. A different approach is to investigate the immune system of the host for new ways of making the antibacterial defence more efficient. However, the immune system has a dual role as protector and cause of disease: in addition to being protective, increasing evidence shows that innate immune responses can become excessive and cause acute symptoms and tissue pathology during infection. This role of innate immunity in disease suggests that the immune system should be targeted therapeutically, to inhibit over-reactivity. The ultimate goal is to develop therapies that selectively attenuate destructive immune response cascades, while augmenting the protective antimicrobial defence but such treatment options have remained underexplored, owing to the molecular proximity of the protective and destructive effects of the immune response. The concept of innate immunomodulation therapy has been developed successfully in urinary tract infections, based on detailed studies of innate immune activation and disease pathogenesis. Effective, disease-specific, immunomodulatory strategies have been developed by targeting specific immune response regulators including key transcription factors. In acute pyelonephritis, targeting interferon regulatory factor 7 using small interfering RNA or treatment with antimicrobial peptide cathelicidin was protective and, in acute cystitis, targeting overactive effector molecules such as IL-1β, MMP7, COX2, cAMP and the pain-sensing receptor NK1R has been successful in vivo. Furthermore, other UTI treatment strategies, such as inhibiting bacterial adhesion and vaccination, have also shown promise.

Innate immunity provides a rapid and selective first line of defence[1–4], preventing pathogens from gaining access to host tissues while sustaining symbiosis with the commensal flora. This impressive level of precision is maintained by specific pathogen recognition mechanisms coupled with the immediate activation of the innate immune system[4–8] (FIG. 1). Health is rapidly restored when the innate immune response is efficient and transient, but an imbalanced response can create exaggerated inflammatory states and cause severe acute disease, mortality and chronic sequelae[9]. By targeting and correcting these weaknesses therapeutically, a functional innate immune response can be restored and the bacteria removed.

The potential of innate immunomodulation therapy is supported by successful studies of its use in urinary tract infection (UTI), in which this concept has been developed[10–13] (FIG. 2). The innate immune response controls the severity of acute pyelonephritis (APN) and acute cystitis (ACY), and genetic screens have identified important transcriptional checkpoints as disease determinants[10–19]. For example, transcriptional regulators interferon regulatory factor 3 (IRF3) and IRF7 control disease severity in infected kidneys[10,11,20] by regulating the defensive (IRF3) or destructive (IRF7) response cascades (FIG. 3). In the bladder, the inflammasome constituents apoptosis-associated speck-like protein containing a CARD (ASC) and NOD-, LRR- and pyrin domain-containing 3 (NLRP3) serve as transcriptional repressors of the protease matrix metalloproteinase 7 (MMP7) and pain sensor neurokinin 1 receptor (NK1R), controlling the level of inflammation by a non-canonical mechanism of pro-IL-1β processing[12,13,21–23] (FIG. 4). These findings illustrate the tight genetic control of innate immune activation and disease pathogenesis in UTI.

The adaptive immune response adds antigen specificity and longevity to the host defence (FIG. 1). Antigens on invading pathogens are recognized and memory is created to prevent recurrent infections[24]. An adaptive immune response is detected in patients with APN, but

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Key points

- Excessive innate immune responses to infection cause symptoms and pathology in acute pyelonephritis and acute cystitis.
- Innate immunomodulation therapy is, therefore, a realistic option for treating these conditions.
- Targeting excessive innate immune responses at the level of transcription has been successful in animal models.
- Innate immunomodulation therapy reduces excessive inflammation and tissue pathology and accelerates bacterial clearance from infected kidneys and bladders in mice.
- Innate immunomodulation therapy also accelerates the clearance of antibiotic-resistant bacterial strains.

the protective role of this immune response remains unclear[25–27]. In mice, gene deletions affecting adaptive immunity have not been found to drive disease development but to weaken the defence[28–31]. X-linked immunodeficient and RAG1-deficient mice, with attenuated B and T lymphocyte function, were not more susceptible to *E. coli*-induced UTIs than controls and had low bacterial counts after 24 h (REFS[15,32]). Additionally, αβ T cell-deficient and γδ T cell-deficient mice did not show increased susceptibility to kidney infections[28]. However, some studies have suggested that severe combined immunodeficiency mice have increased bacterial burden compared with wild-type mice during UTI[28,33]. Additionally, T helper 17 (T_H17) cell responses have been proposed to affect bacterial clearance, based on reduced neutrophil and macrophage infiltration in IL-17A-deficient mice[17]. Sentinel natural killer (NK) cells have been proposed to influence resistance to uropathogenic *Escherichia coli* (UPEC) infection through mechanisms involving the release of TNF; however, the underlying protective mechanisms of this cell population remain unknown and additional depletion studies will be essential to fully understand the role of NK cells in UTI[34]. In addition, resident macrophages in the bladder suppress T_H1-mediated immunity and macrophage depletion helps the host to clear the infection, but T cell depletion had no effect on bacterial clearance, confirming that myeloid cells rather than lymphocytes are essential for UTI pathogenesis[35].

The WHO has declared antimicrobial resistance 1 of the top 10 global public health threats facing humanity; thus, novel antimicrobial therapeutic strategies are urgently needed[36]. UTIs are among the most prevalent bacterial infectious diseases globally[37,38], most often caused by UPEC originating from the gut flora or perineal flora[39,40]. *E. coli* infections account for half of the estimated global burden of antibiotic resistance[36,41,42], with about 90% of *E. coli* strains being resistant to at least one antibiotic[43]. In Europe, UPEC isolates are resistant to third-generation cephalosporins and fluoroquinolones (11.8 % and 22.3% of UPEC strains, respectively)[44] and fluoroquinolone-resistant UPEC were 31.3% of the isolates in hospitalized patients with UTI[45,46].

In this Review, we discuss innate immune hyperactivation as a disease determinant in UTI and identify targets for innate immunomodulatory therapy. We envisage that this new approach might benefit large patient groups in the future. The use of innate immunomodulation therapy would be similar to antibiotics, aiming to reduce acute inflammation and infection but, in contrast to antibiotics, innate immunomodulation therapy might offer a solution for treating infections caused by antibiotic-resistant strains. Furthermore, efforts to develop UTI vaccines and other prophylactic measures are discussed, as well as investigations into broad anti-inflammatory therapies such as NSAIDs.

Disease determinants in acute pyelonephritis

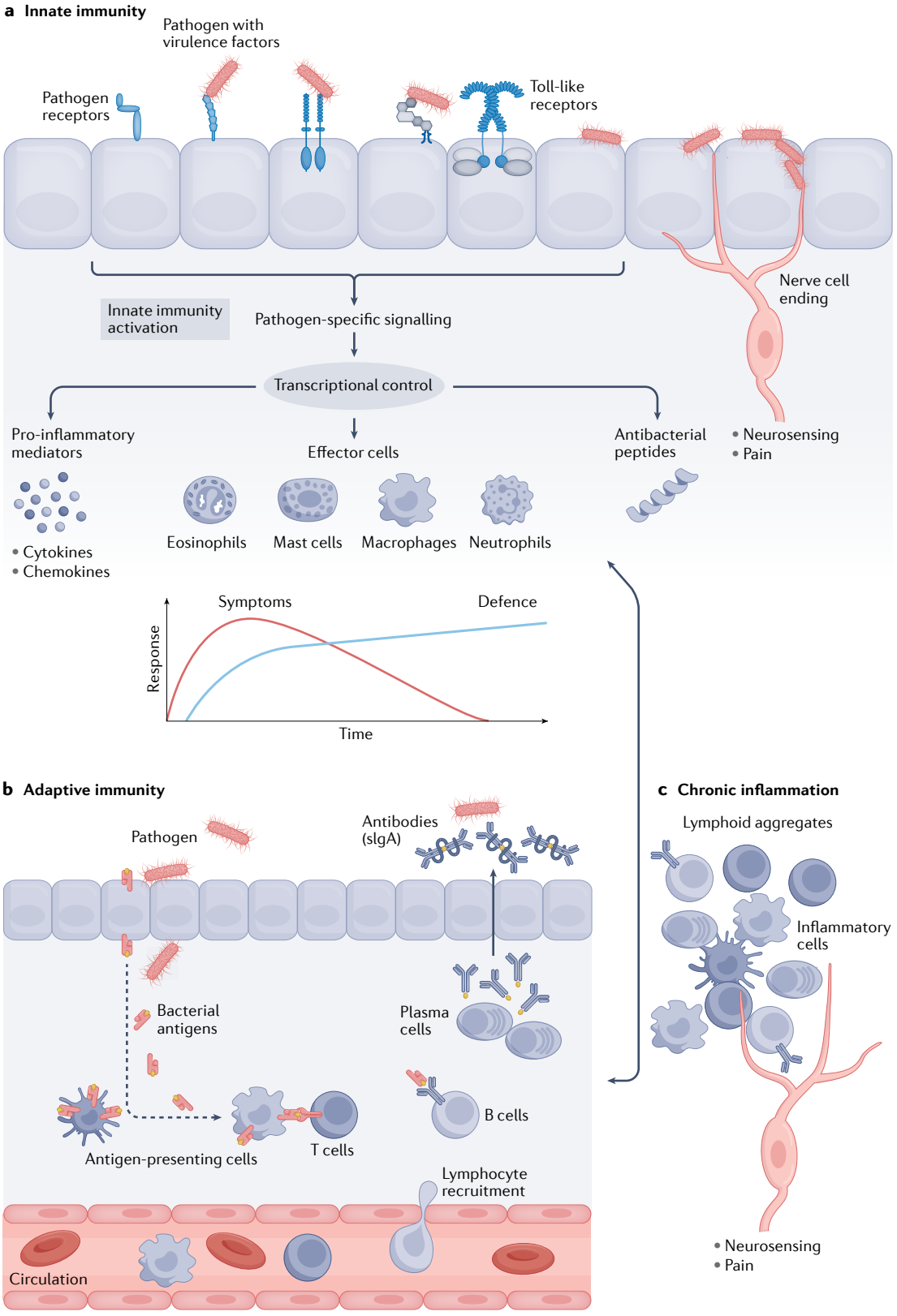
APN is a severe, sometimes life-threatening infection[37,47,48], initiated by UPEC strains that attack the renal pelvic mucosa and elicit a local innate immune response that is amplified and becomes systemic[49–52]. Local symptoms at the site of infection are triggered by excessive kidney inflammation and the systemic spread of inflammatory mediators generates fever and general malaise in the infected patient[53–55]. APN is accompanied by urosepsis in ~30% of adults, and urosepsis remains a major cause of mortality, especially in the elderly[56–59]. The mortality rate in APN was estimated to be 10–20% before the introduction of antibiotics, and a frequency of 7.4% was recorded

Fig. 1 | **Innate and adaptive immune responses to UTI.** The mucosal immune system controls susceptibility to infection by regulating the defence against pathogenic microbes. Pathogen recognition mechanisms mobilize a rapid innate immune response and activate the antibacterial defence, resulting in efficient clearance of infection. A balanced response is essential, as loss of homeostasis might cause disease as mechanisms intended for protection to underperform or overreact. Innate immunotherapy is aimed at correcting such weaknesses and restoring the efficiency of the immune defence in the host. **a** | Schematic overview of the innate immune response to mucosal infection. Uropathogenic *E. coli* triggers Toll-like receptor 4 (TLR4) signalling in responding cells in the mucosal barrier and the release of pro-inflammatory mediators activates a rapid local innate immune response cascade, which might become systemic. Cytokines with paracrine activity stimulate cells in the mucosal environment, chemokines recruit neutrophils from the circulation and infected nerve cells participate in a neuroinflammatory loop involved in pain sensing. Bacteria are cleared by the direct effects of antibacterial molecules, such as defensins, as well as neutrophils, which phagocytose the bacteria and exit with their cargo across the mucosal barrier into the urine. A number of additional defence mechanisms may be activated as well. The response is mostly transient and infection is cleared but host defects that reduce the efficiency of bacterial clearance or increase the inflammatory response are associated with increased disease severity. **b** | Adaptive immunity is activated by infection when antigens from infecting bacteria reach antigen-presenting cells and activate local or distant lymphocyte populations. Plasma cells producing specific antibodies have been identified in the kidneys and bladder, especially in mice with a deficient neutrophil response. Infected patients secrete secretory IgA (sIgA) antibodies into the urine, which inhibit bacterial adherence[49]. Circulating antibodies are detected in patients, with acute pyelonephritis and plasma cells detected in infected kidneys of susceptible mice[79]. Lymphoid aggregates might form in chronically infected individuals and specific T cell populations regulate the efficiency of the host defence by affecting the crosstalk between innate and adaptive immunity[7,8].

in 2005, after the introduction of antibiotics[60]. In addition, APN is an important cause of renal growth retardation and permanent kidney damage in childhood, leading to chronic sequelae such as hypertonia, renal insufficiency or renal failure as well as premature delivery[61–64].

Molecular control of the innate immune response in APN

Mechanisms of APN pathogenesis and innate immune activation have been extensively studied and reviewed[4–6,29]. Briefly, pathogen-specific recognition mechanisms



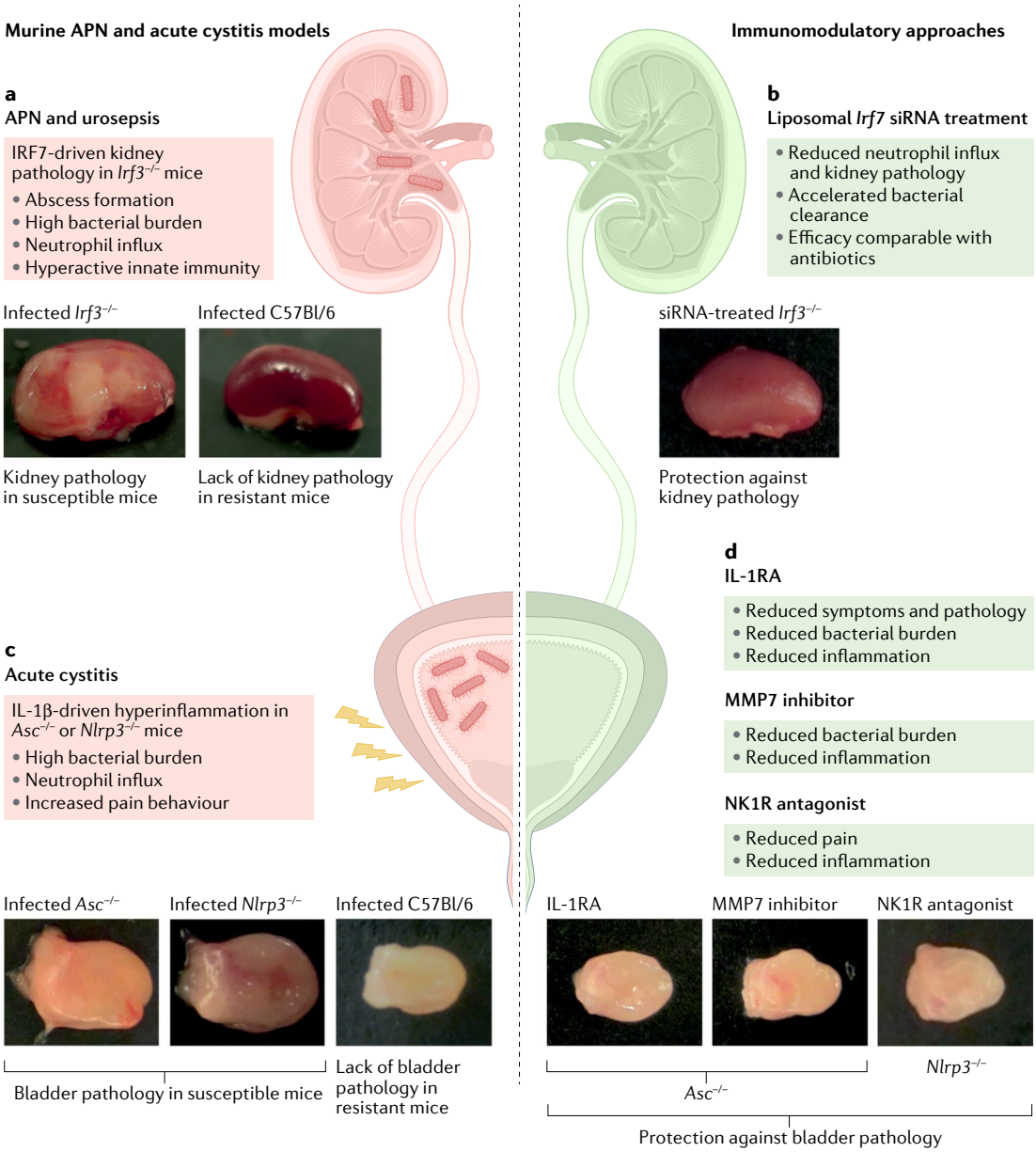


Fig. 2 | **Examples of innate immunomodulation therapy in UTI.** Genetic determinants of disease severity (left) and corresponding treatment approaches (right). **a** | Acute pyelonephritis (APN) is reproduced in infected *Irf3*$^{-/-}$ mice, in which a hyperactive innate immune response and exaggerated neutrophil recruitment drive tissue pathology. In parallel, bacterial clearance is impaired. The kidney images illustrate the severity of APN in *Irf3*$^{-/-}$ mice, with extensive abscess formation, compared with infected control mice with a balanced innate immune response (C57Bl/6 mice)[10,11]. The severe pathology in *Irf3*$^{-/-}$ mice is contrasted against protection in *Irf7*$^{-/-}$ mice[11], and *Irf7* overactivation has been identified as an essential disease mechanism. **b** | Innate immunomodulation therapy was achieved by targeting IRF7 in infected *Irf3*$^{-/-}$ mice[11]. Liposomal *Irf7* siRNA was used as an *Irf7*-silencing strategy and treatment substantially reduced kidney pathology and accelerated bacterial clearance compared with untreated mice. *Irf7* siRNA treatment had similar therapeutic efficacy to cefotaxime treatment at an intermediate dose. **c** | Severe acute cystitis is driven by IL-1β overactivation involving the MMP7 protease. Bladders become enlarged, hyperaemic and nerve cell activation triggers a pain response. The disease severity is illustrated by images from infected mice, showing enlarged, oedematous bladders with evidence of hyperaemia in *Asc*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice compared with C57Bl/6 mice[12]. **d** | This severe cystitis phenotype can be reversed by treatment with an IL-1 receptor antagonist (IL-1RA) or an MMP7 inhibitor, which inhibits the excessive IL-1 response, reduces inflammation and accelerates bacterial clearance[12]. Furthermore, blocking the pain response by targeting NK1R has been shown to reduce pain behaviour and inflammation in *Nlrp3*$^{-/-}$ mice[13]. Liposomal *IRF7* siRNA treatment and IL-1RA treatment were shown to have similar efficacy to antibiotics in reducing the disease severity, illustrating the potential of this interesting new immunomodulatory approach for treating UTIs. Part **c** adapted from REF.[12], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Part **d** adapted from REF.[13], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

activate a rapid innate immune response in infected tissues, leading to inflammation and the mobilization of an antibacterial defence[65]. Specific bacterial adherence strategies are essential, involving surface fimbriae or non-fimbrial adhesins and host cell receptors[16,54,66,67]. P fimbriae are expressed by 90–100% of UPEC strains in patients with uncomplicated APN[68], with a strong association with disease severity, and have been proposed to facilitate bacterial invasion, leading to urosepsis[49,65,69,70]. Adherence also facilitates tissue interactions of other virulence factors, including the endotoxin LPS, exotoxins (such as haemolysin and CNF), iron-binding proteins and capsular polysaccharides[5,71–74].

Mechanisms of innate immune activation and essential effector functions in APN have been mapped using cellular infection technology, animal models and clinical studies[4,10,11,14,16,18,19,32,75–80]. Key regulators of UTI severity have been evaluated using gene knockout technology and their relevance to human disease has been verified in clinical studies[10,11,29,78]. The Toll-like receptor (TLR) family acts as a shared upstream control node of the pathogen-specific innate immune response[16,32,81–84] and TLR4 responds to UPEC virulence factors by engaging different co-receptors, including glycosphingolipid receptors for P fimbriae recognizing Galα1-4-Galβ epitopes[65,85,86] or mannosylated glycoproteins recognized by type 1 fimbriae[87–92]. The adaptor proteins regulate the downstream response through phosphorylation cascades[10,16,32,93,94] and the activation of transcriptional regulators defines the quality and quantity of the inflammatory cascade[10,11], through mediators such as cytokines and chemokines and inflammatory cells recruited to the site of infection[95,96] (FIG. 1).

Different arms of the TLR4 signalling cascade can be engaged, depending on the fimbrial adhesins and virulence factors that the pathogens express[16,54,66,67,97–99]. P fimbriated UPEC strains mainly activate the adaptor proteins TIR-domain-containing adapter-inducing interferon-β (TRIF) and TRIF-related adaptor molecule (TRAM)[16,85,93,94] and the phosphorylation of mitogen activated protein (MAP) kinases, p38 and cyclic AMP-responsive element-binding protein (CREB) defines signalling cascades downstream of TLR4, which converge on specific transcription factors (including IRF3, IRF7, AP-1 and NF-κB)[10,93]. The resulting pro-inflammatory cascades include cytokines in the kidneys, such as type I interferons, IL-6 and TNF, and IL-1 in the bladder, as well as neuropeptides (substance P (SP) and galanin) and their receptors. Chemokines (IL-8 (also known as CXCL8), CCL3 (also known as MIP1α), CCL5 (also known as RANTES) and CCL2 (also known as MCP1))[12,13,76,95,100–105] create the inflammatory cell infiltrate as the recruitment and activation of inflammatory cells are essential for the antibacterial defence[14,106–108].

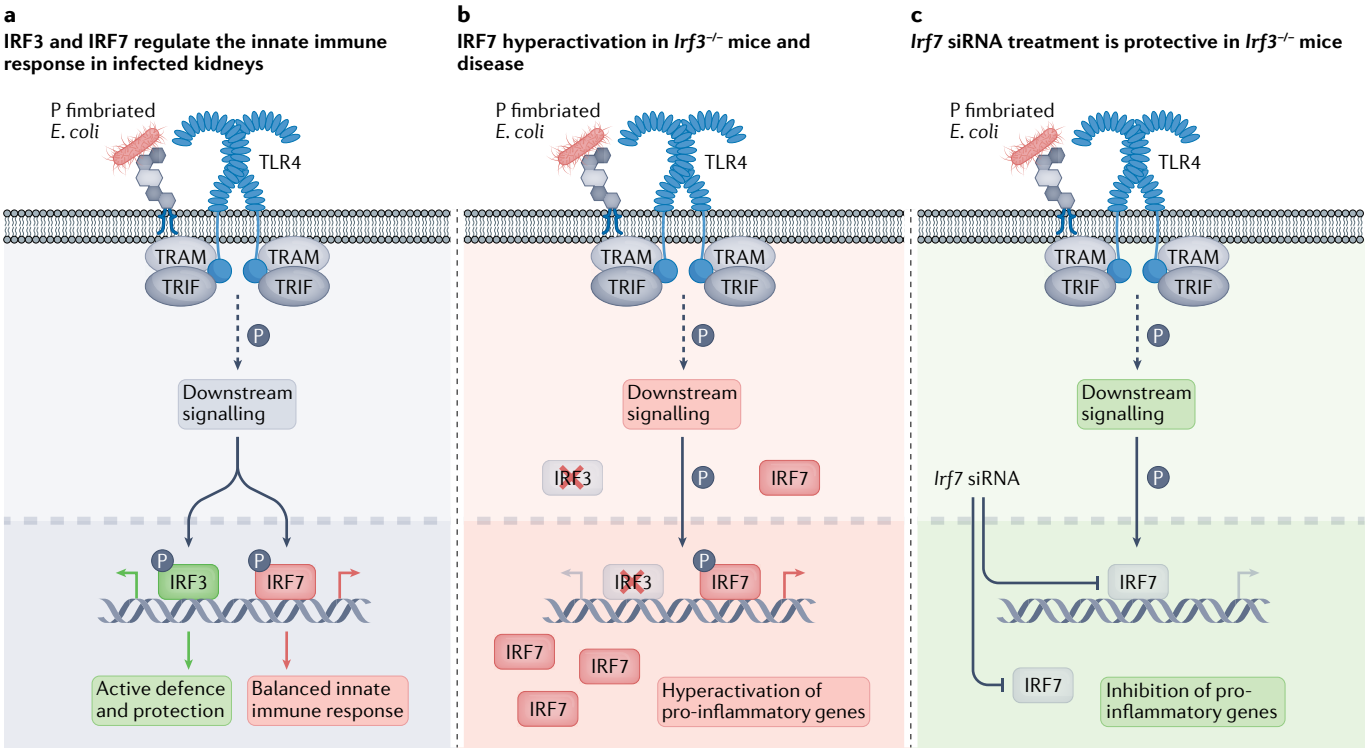


Fig. 3 | **Molecular basis of *Irf7* siRNA-based therapy for acute pyelonephritis. a** | Uropathogenic, P fimbriated *Escherichia coli* activates a TLR4-dependent signalling cascade in the renal pelvic epithelium involving the TRIF–TRAM adaptor proteins, ultimately leading to transcription factor activation and an active defence. The balance between transcription factors IRF3 and IRF7 is essential to control the quality of the defence and the outcome of infection[10,11]. The IRF3 response is protective, limiting inflammation and promoting bacterial clearance. **b** | By contrast, an overactive IRF7 response is destructive and leads to acute pyelonephritis, with potentially severe consequences for renal health. **c** | *Irf7*-specific siRNA treatment was shown to inhibit the excessive IRF7 response and to effectively reduce kidney pathology in *Irf3*$^{-/-}$ mice[11]. siRNA treatment also accelerates bacterial clearance, in a manner similar to antibiotics.

Other TLRs affecting UTI pathogenesis include TLR1, TLR2 and TLR5, which mainly activate the MyD88 arm of the TLR cascade[83,109–113].

The essential role of TLR4 signalling in UTI was first observed in *Tlr4*$^{-/-}$ mice[82]. UPEC-infected *Tlr4*$^{-/-}$ mice do not develop APN or ACY or an inflammatory

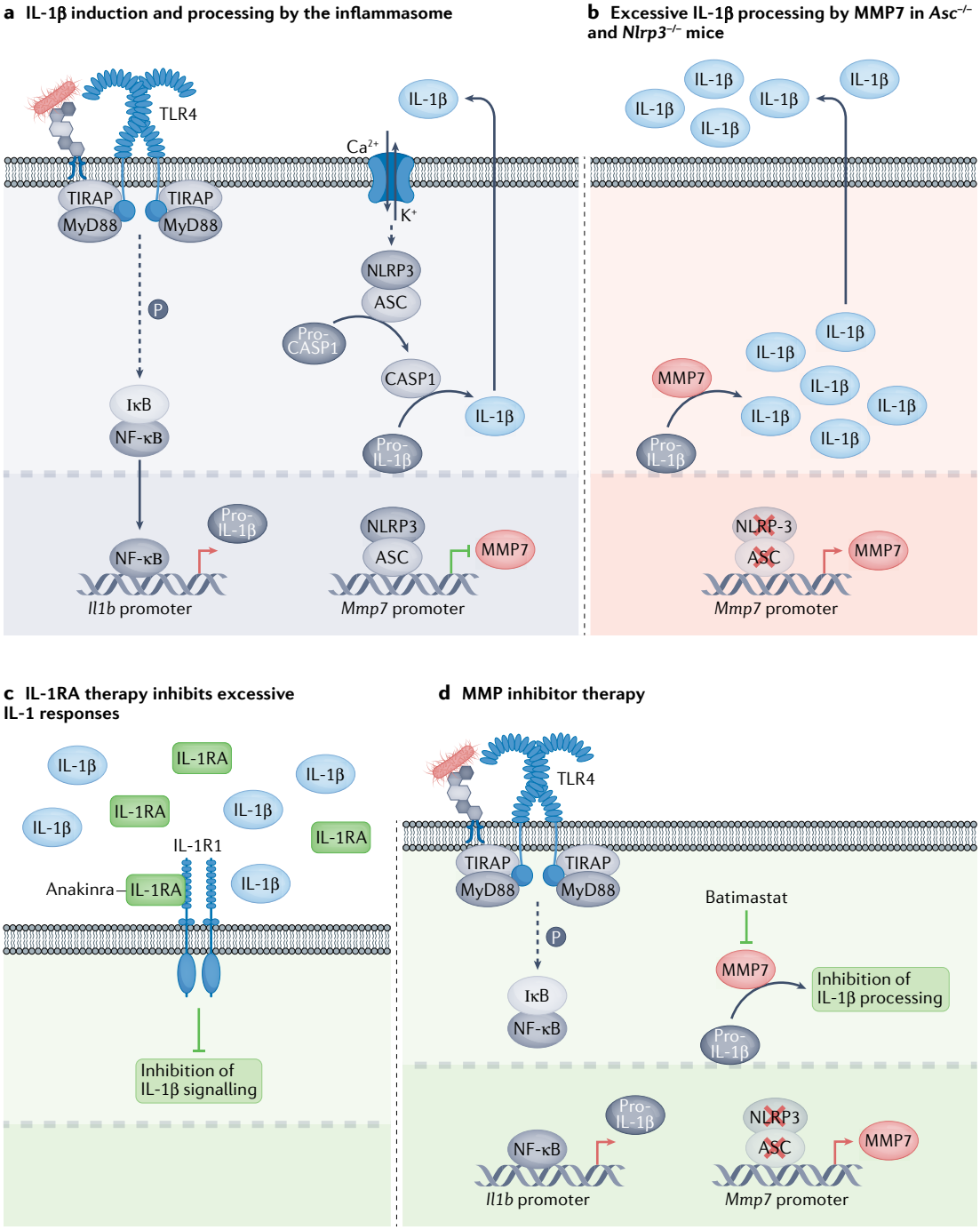


Fig. 4 | **Molecular basis of excessive IL-1 signalling in acute cystitis and immunomodulatory treatment approaches.** Uropathogens trigger the TIRAP–MyD88 arm of TLR4 signalling and activate NF-κB, which transcribes pro-inflammatory cytokines, most prominently IL-1β, a key mediator of bladder pathology. **a** | In wild-type mice, IL-1β is activated by NLRP3-inflammasome processing, leading to a transient, self-healing, inflammatory response peaking at 3 days. The ASC and NLRP3 proteins are essential inflammasome constituents, which facilitate the processing of pro-caspase 1 and cleavage of pro-IL-1β. **b** | Surprisingly, *Asc*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice develop an excessive IL-1β response, increased pyuria, bacteriuria and severe bladder pathology. The overactive IL-1β response is explained by a non-canonical mechanism of IL-1β processing by matrix metalloproteinase 7 (MMP7)[12]. **c** | Innate immunomodulation therapy approaches to inhibiting IL-1β signalling. IL-1 receptor antagonist (IL-1RA)-based therapy (anakinra) considerably reduced acute cystitis severity in *Asc*$^{-/-}$ mice and accelerated bacterial clearance[12]. **d** | Blocking MMP7 using batimastat (an MMP inhibitor) inhibited IL-1β processing, reducing disease severity with a decrease in gross pathology and bacterial counts compared with untreated mice[12].

response[32,106]. To identify the TLR4-dependent mechanisms of disease, single genes in the TLR4 pathways have been systematically deleted and the resulting phenotypes have been characterized in UPEC-infected mice[10]. Specific gene deletions have been shown to create an APN or ACY disease phenotype, providing essential insights into the mechanisms of disease pathogenesis. *Irf3*$^{-/-}$ mice lack the protective IRF3-dependent arm of innate immunity and develop severe APN, characterized by hyperinflammation, urosepsis and massive renal abscess formation[10,11]. IFNβ is activated downstream of IRF3, and *Ifnb1*$^{-/-}$ mice showed a similar severe APN disease phenotype to *Irf3*$^{-/-}$ mice[10].

A strong APN phenotype is also observed in *Cxcr2*$^{-/-}$ mice, deficient in the chemokine receptor CXCR1, which regulates neutrophil activation and neutrophil exit from infected kidneys into the urine[14,79,114]. The *Cxcr2*$^{-/-}$ mice develop severe APN with urosepsis and acute mortality, accompanied by renal abscesses[14,115]. Pathology is caused by massive neutrophil retention in the kidneys, as recruited neutrophils fail to exit into the urine and to scavenge and kill the bacteria[14,115,116]. The *Cxcr2*$^{-/-}$ mice also develop renal damage resembling renal scarring in children with APN[14,79,116]. Neutrophil recruitment is rapid, regulated by the urothelial chemokine response to infection[100,117] and release of high levels of TNF from epithelial cells and resident mast cells, which support the neutrophil response[54,89,118]. Cells in the subepithelial compartment, including dendritic cells, are affected through increased IL-1β and IL-1R expression[119] and infiltrating neutrophils have high COX2 expression and release of prostaglandin E_2 (PGE_2) affects inflammation[120]. The chemokine receptors are essential determinants of the disease response, by controlling neutrophil activation and migration[121].

C5aR1 has been identified as a determinant of APN disease progression in mice using decreased bacterial counts, lymphocyte infiltration and kidney pathology in the kidneys in *C5ar1*$^{-/-}$ mice compared with wild-type controls[122]. Furthermore, bacterial burden and neutrophil and macrophage infiltration was decreased in *P2x7*$^{-/-}$ mice, suggesting a role of the purinoreceptor $P2X_7$ in APN and associated renal fibrosis[123]. Finally, increased bacterial burden in the kidneys of *Car2*$^{-/-}$ mice suggested that carbonic anhydrase 2 in intercalated cells in the kidneys might promote renal bacterial clearance[124].

Human relevance of the genetic screens

The human relevance of these findings has been supported by clinical studies[10,78,125]. *IRF3* promoter sequence variants were detected in two APN-prone patient populations[10]. The homozygous A/A–C/C genotype (nucleotide positions −925 and −776) was prevalent the APN-prone patients (79%), whereas the co-segregating heterozygous single-nucleotide polymorphisms (SNPs) were more common in those with asymptomatic bacteriuria (ABU; 69%)[10]. The APN haplotype was shown to decrease *IRF3* expression in reporter assays, suggesting that IRF3 needs to be fully functional to avoid APN[10]. *CXCR1* mRNA levels are low in children with APN and CXCR1 expression is reduced[78]. APN-prone patients carry heterozygous *CXCR1* polymorphisms affecting receptor expression and the presence of *CXCR1* variants has been confirmed in several APN-prone patient groups[126,127]. *CXCL8* SNPs were detected in infected patients with renal involvement confirmed by positive dimercapto succinic acid (DMSA) scans[125,127–129].

In addition, *PTX3* is polymorphic in APN-prone children and in adults who suffer from recurrent cystitis[18]. *IL10* polymorphisms were identified in APN-prone patients[80] as well as SNPs in *CCL5*, encoding the eosinophil and T cell chemoattractant RANTES[130], supporting human disease relevance.

The information obtained from these studies is essential, as it provides a framework for distinguishing protective from destructive molecular determinants of the innate immune response and for targeting the cause of disease therapeutically.

IRF7 siRNA innate immunotherapy in APN

The transcription factor IRF7 takes over the innate immune response and drives disease pathology in *Irf3*$^{-/-}$ mice (FIG. 3), in which IRF7-dependent gene networks are strongly upregulated, including *Tlr4, Stat3* and *Il6* as well as downstream genes involved in the acute phase response[11]. Upregulation of *IRF7* expression and IRF7-dependent gene networks is also observed in patients with febrile UTI, supporting the human relevance[11,99]. Direct binding of IRF-7 to promoter DNA fragments (*OAS1*, *CCL5* and *INFB1*) was demonstrated, supporting the role of IRF7 as a transcriptional regulator, especially in hosts with reduced IRF3 expression[99]. Further evidence supporting the role of IRF7 was obtained in infected *Irf7*$^{-/-}$ mice, which were protected from infection and showed no evidence of kidney pathology[11]. *IRF7* was further identified as an important transcriptional mediator during group b streptococcus-induced UTI[131].

The protected phenotype in *Irf7*$^{-/-}$ mice and overactivation of *Irf7* in disease-prone *Irf3*$^{-/-}$ mice identified IRF7 as a potential therapeutic target. Using an short interfering RNA (siRNA)-based strategy of *Irf7* inhibition[11], siRNA treatment of *Irf3*$^{-/-}$ mice was shown to inhibit the excessive innate immune response in *Irf3*$^{-/-}$ mice and to improve bacterial clearance, resulting in resolution of infection by day 7 (FIG. 3). siRNA therapy compared favourably with antibiotic treatment and *Irf7* siRNA treatment considerably reduced the disease score[11]. Importantly, *Irf7* silencing immunomodulation therapy accelerated bacterial clearance to the same extent as cefotaxim[11]. Furthermore, recombinant IFNγ treatment was shown to increase survival, reduce bacterial burden and reduce kidney pathology in a rabbit model of APN, compared with untreated rabbits[132].

The IL-6 response to UTI was first described in the 1980s[53,133]; IL-8, IL-1 and TNF were also detected. The IL-6–STAT3 pathway is activated in the kidneys of infected *Irf3*$^{-/-}$ mice, shown by transcriptomic analysis and tissue staining[11]. The IL-6–STAT3 response is IRF7-dependent, and this pathway is inhibited in mice treated with liposomal *Irf7* siRNA. The role of IL-6 in an APN mouse model has been investigated[19]. In *Il6*-knockout mice with experimental UTI, STAT3 phosphorylation was significantly reduced but total STAT3 expression was unchanged. Furthermore, levels of

STAT3 transcriptional targets were reduced. Inhibition of IL-6, using an IL-6 neutralizing antibody in *Il6*-intact mice, resulted in reduced IL-6 levels and decreased bladder and kidney STAT3 phosphorylation in response to UPEC infection, increased bacterial burden, as well as abscess formation, supporting a role for this cytokine in the host defence[19].

The IL-6–STAT3 pathway is also activated in patients infected with P fimbriated bacteria[99], and IRF7 has been identified as a key transcription factor driving the response in these patients. IL-6 and other cytokines were subsequently measured in patient urine in a number of studies, in which the levels of these cytokines correlated positively with bacterial virulence and disease severity, fever and CRP[75,100,134]. A strong effect of P fimbriation, a feature that defines this virulence factor as a host response inducer[66,67], was also observed in these studies. The early studies led to further detailed analysis of TLR4 and the TLR4-dependent signalling pathway that activates IL-6–STAT3[10,53,81].

These results highlight how individual transcription factors regulate beneficial or destructive effects of innate immunity and identify IRF7 as a target for immunoregulation therapy in APN.

Clinical trials support the feasibility of siRNA-based therapies for indications including familial amyloidosis, haemophilia and hepatitis C[135–138]. The use of siRNA-based therapies in humans is still developing, and the challenges of off-target effects, pharmacokinetics and immune activation need to be addressed for this approach to be a clinical reality[139,140]. However, positive effects of siRNA therapy have been observed in human experimental trials[141,142], and the successful development of RNA-based vaccines suggests that such technologies should be clinically feasible. The *Irf7* siRNA treatment approach is intended to be acute, with similar treatment protocols to those used for antibiotics, which is essential in order to avoid long-term suppression of the immune system and effects on the susceptibility to other infections, including those of viral origin.

Anti-inflammatory therapies in APN

APN is caused by exaggerated inflammation in infected kidneys. A number of general anti-inflammatory agents have been tested in animal models of APN, with limited benefits[120,143,144]. Early studies showed that glucocorticoids attenuate inflammation, but they also impaired bacterial clearance[145]. NSAIDs inhibited the inflammatory response but the infection was not cleared in treated mice[145]. These results indicate that broad inhibition of the inflammatory response does not equal protection against disease and support the conclusion that drugs with increased specificity are needed to achieve an anti-inflammatory and antibacterial effect.

Antimicrobial peptides

Antimicrobial peptides (AMPs) are effectors of the innate immune response and resemble antibiotics, in that they mainly target the bacteria rather than the immune system per se[146,147]. AMPs, including defensins, have been investigated as therapeutics owing to their direct antibacterial effects[18,146–148]. AMPs (including β-defensins, ribonucleases and cathelicidin) are potent effectors of the renal defence against infection[147,149–153]. RNase7 and cathelicidins act by disrupting the phospholipid membranes of various microorganisms and are constitutively synthesized by the kidney[147,151,152]. Lipocalin 2 (LCN2; also known as NGAL) is a specific antibacterial protein secreted both in the urine and systemically after Gram-negative infection, kidney injury or urosepsis. LCN2 binds the bacterial siderophore enterochelin, preventing iron transfer and sequestration by the bacteria[154]. The humoral pattern recognition molecule pentraxin 3 (PTX3) serves as an opsonin and promotes bacterial uptake by neutrophils[18].

The cathelicidin AMP (CAMP) LL-37, which is primarily released by macrophages and neutrophils, targets UPEC strains in vitro[147]. *Cramp*$^{-/-}$ mice (CRAMP is an LL-37 homologue) showed reduced pathology compared with wild-type controls after infection with the *E. coli* cystitis strain UTI89 (REF.[155]), and *Cramp*$^{-/-}$ mice infected with the pyelonephritis strain *E. coli* CFT073 were protected[147], supporting a role in the antimicrobial defence, similar to antibiotics[147,155]. Although positive results have been observed in vitro and in knockout mice in vivo, further studies are required to address if purified AMPs can be administered as a therapeutic in disease models.

Immunomodulation in acute cystitis

ACY is mainly caused by bacterial infections of the urinary bladder[37,68]. The patients experience pain, dysuria and frequency of urination, and the diagnosis is supported by the presence of bacteriuria and pyuria[37,156,157]. A subset of susceptible patients develop severe ACY with an excessive innate immune response, severe symptoms and pathology[158]. Recurrent infections are common, and chronic inflammation can lead to sequelae such as interstitial cystitis/bladder pain syndrome (IC/BPS)[159,160].

Type 1 fimbriae act as virulence factors in the mouse urinary tract[161–163], mediating bacterial adherence to the bladder mucosa[88–91]. The FimH adhesin binds several mannosylated host cell glycoconjugates and has been proposed to facilitate bacterial invasion of mucosal cells[92]. TLRs control the innate immune response to *E. coli* infection in the bladder, and *Tlr4*$^{-/-}$ mice are protected from disease[16,32,106,111]. Type 1 fimbriated strains preferentially activate the MyD88 and TIRAP adaptors and NF-κB-dependent effector functions[88,162–165]. A pro-inflammatory effect of type 1 fimbriae has been observed in a mouse model, but not in human studies using isogenic strains differing in fimbrial expression[99]. In contrast to P fimbriae, which triggered an IRF7-driven inflammatory response in the patients, direct effects of type 1 fimbriae on innate immunity were not detected in patients[99]. Type 1 fimbriae were shown to inhibit basic cellular functions such as RNA translation and effects on neurosensing and solute carriers suggested a potential link to the host reponse[99].

IL-1 hyperactivation as a cause of acute cystitis

IL-1β is a potent pro-inflammatory cytokine that amplifies innate immune responses in several chronic infection models, including cystic fibrosis, tuberculosis

and inflammatory bowel disease[9,166–168]. ACY has been identified as an IL-1β hyperactivation disorder triggered by *E. coli* infection of the bladder mucosa in a mouse model[12] (FIG. 4). Clinical ACY isolates activated pro-IL-1β expression in human 5637 bladder epithelial cells in vitro, and the processing and release of mature IL-1β were accelerated[12]. The IL-1 response to *E. coli* infection is controlled by TLR4 and transcription factors, including ERK, p38 and NF-κB[169,170]. In addition to IL-1β, a cascade of IL-1-dependent genes is activated, and effectors of the host response include IL-8 and PGE_2 as well as SP and NK1R[12,13].

Genetic screens further identified a non-canonical mechanism of IL-1β hyperactivation that creates severe ACY[12,13] (FIG. 4). The inflammasome controls pro-IL-1β processing in many models; therefore, inactivating gene deletions affecting the inflammasome would be expected to be protective. Instead, a severe ACY disease phenotype was observed in infected mice carrying single-gene deletions of *Nlrp3* or *Asc*[12]. The excessive, disease-associated IL-1β response in $Asc^{-/-}$ mice was further mapped to the protease MMP7 and the pain sensors NK1R and SP, which were overexpressed[12,13]. Direct molecular interaction studies attributed the excessive IL-1 response to pro-IL-1β processing by MMP7 (FIG. 4), and included a cascade of IL-1β-dependent, downstream genes[12]. Importantly, $Il1b^{-/-}$ mice were protected against infection and inflammation, further supporting the importance of IL-1β in this disease[12,94]. The human relevance of these findings was supported by elevated urine IL-1β levels in patients with ACY, compared with patients with ABU[12].

IL-1 receptor antagonist treatment of acute cystitis

IL-1β and its receptor are potential therapeutic targets for immunomodulation therapy in ACY (FIG. 4). The recombinant human IL-1 receptor antagonist (IL-1RA) protein binds to IL-1R1 with similar affinity to IL-1α and IL-1β, inhibiting their binding and the dimerization of IL-1R1 and the IL-1 receptor accessory protein, as well as downstream signalling[9]. The IL-1RA anakinra was, therefore, investigated as an inhibitor of IL-1β hyperactivation in ACY (FIG. 2). $Asc^{-/-}$ mice, which are susceptible to ACY, were treated with daily injections of anakinra for 7 days and disease severity and bacterial clearance were quantified at sacrifice[12]. Anakinra treatment reduced tissue pathology by 75–80% compared with untreated mice and the inflammatory response to infection was markedly attenuated, as shown by reduced neutrophil numbers in urine and bladder tissue as well as reduced IL-1-dependent gene expression. Furthermore, bacterial clearance was accelerated, suggesting that correcting the immune imbalance empowers the innate immune response of the host. The efficacy of anakinra treatment was verified in C57Bl/6 wild-type mice with intact inflammasome function[13], which exhibit a milder, more transient disease phenotype than $Asc^{-/-}$ mice.

Anti-inflammatory effects of IL-1RA have been demonstrated in several hyper-inflammatory diseases such as rheumatoid arthritis, gout, cryopyrin-associated periodic syndrome[171–173] and in bacterial and viral infection models including cystic fibrosis[175] and COVID-19 (REFS[174,176]), supporting the feasibility of exploring the beneficial effects of IL-1RA as a therapeutic in patients with ACY[175,177,178].

The efficiency of IL-1RA therapy was compared with that of antibiotic therapy in $Asc^{-/-}$ mice infected with fully virulent *E. coli* strains. IL-1RA therapy showed similar efficacy to cefotaxime and both treatments accelerated bacterial clearance[179]; however, IL-1RA therapy inhibited the hyper-inflammatory response in infected bladders more effectively than cefotaxime, indicating an added benefit. In addition, IL-1RA therapy accelerated the clearance of extended-spectrum β-lactamase (ESBL)-producing *E. coli* strains against which cefotaxime was inefficient[179].

The results of innate immunomodulation therapy in a mouse model of ACY provides a rationale for initiating clinical trials of IL-1RA therapy in ACY.

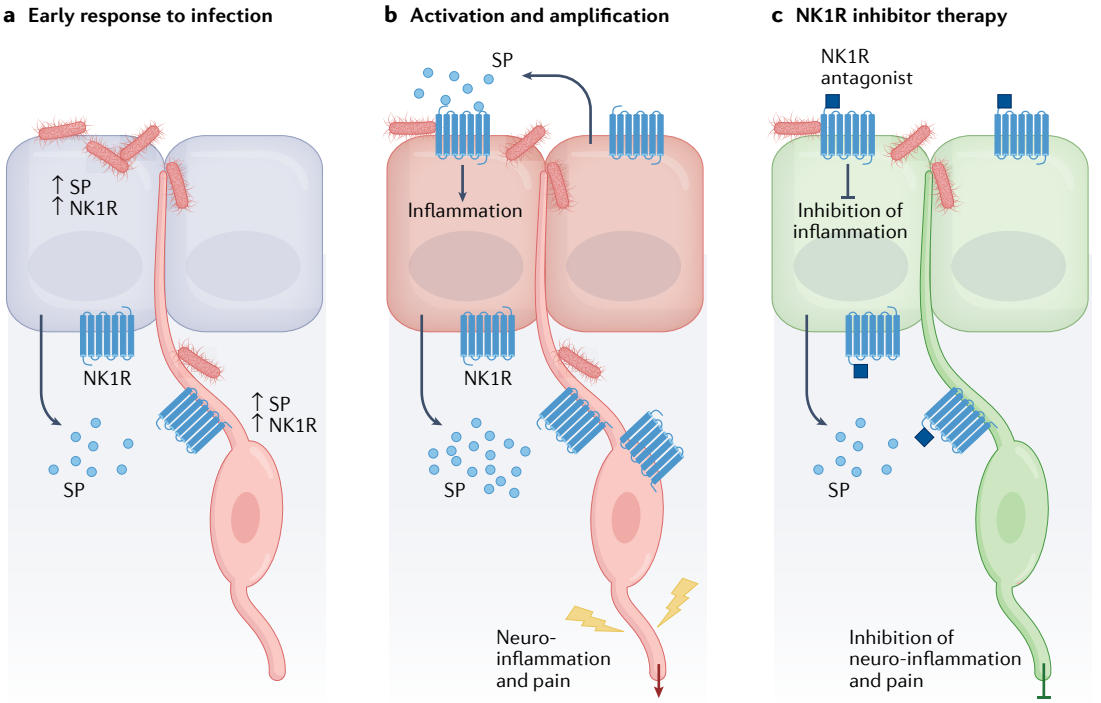
Therapeutic effects of MMP inhibition in acute cystitis

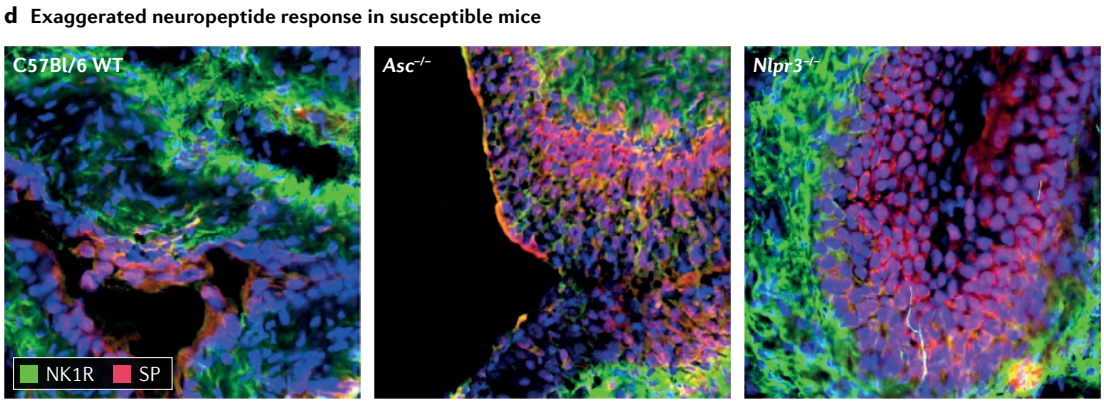
Mmp7 expression was strongly upregulated in mice that developed severe ACY, as shown by genome-wide transcriptomic analysis of whole-bladder mRNA[12]. MMP7 was also shown to cleave pro-IL-1β to its mature, active form (FIG. 4). Based on these findings, the broad MMP inhibitor batimastat (also known as BB-94) was tested for therapeutic efficacy using the same protocol as for IL-1RA treatment[12] (FIG. 2). The severity of ACY was markedly attenuated in the treated mice, as shown by reduced urine and tissue neutrophil levels and a substantial decrease in pathology scores (FIGS 2,4). Furthermore, treatment accelerated bacterial clearance but to a lower extent than IL-1RA treatment[12]. These results indicate that batimastat or related compounds might be of interest to explore as a potential treatment alternative in ACY.

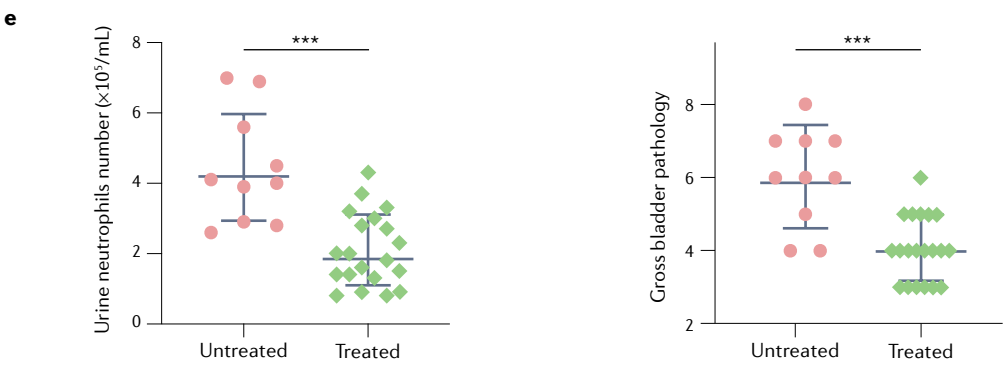
Pain attenuation in acute cystitis

Pain is a hallmark of ACY and symptom relief is a key result of ACY therapy. Pain is commonly regarded as secondary to inflammation and is defined as one of its hallmarks[180]. Pro-inflammatory cytokines promote pain by sensitizing nerves and activating transcription and release of pain molecules such as nerve growth factor (NGF), PGE_2, MMP9 and the neuropeptide SP, which is an effector molecule of inflammatory pain[181,182]. SP is released by several cell types and engages neurokinin receptors, particularly NK1R[183] (FIG. 5). The interaction between SP and NK1R helps to propagate peripheral pain signals from local afferent nerves to the dorsal roots of the central nervous system[184].

SP is spontaneously released within the bladder wall[185] and binds to NK1R, triggering the peripheral pain response[184]. LPS was proposed to cause the pain response during UPEC infection via a non-inflammatory TLR4-dependent mechanism, but the effector mechanisms of this response were not defined[186]. A direct effect of infection on the nervous system has been detected owing to elevated SP and NK1R levels in UPEC-infected nerve cells in vitro[13]. Increased levels of SP and NK1R were also detected in the bladder mucosa of UPEC-infected mice and accompanied by increased pain behaviour. The SP–NK1R response was shown to be hyperactivated in $Asc^{-/-}$ and $Nlrp3^{-/-}$ mice through







a neuroinflammatory loop controlled by IL-1β and NK1R[13] (FIG. 5). Urine SP levels were also found to be elevated in patients with ACY, suggesting the human relevance of this mechanism and a potential for use of SP as a biomarker of symptomatic lower UTIs and ACY[13]. Interestingly, *Nlrp3* and *Asc* were shown to control the expression of NK1R and SP and the processing of IL-1β, linking inflammation and pain in mice susceptible to ACY. Owing to the strong connection between SP and NK1R expression and pain in experimental ACY, an NK1R antagonist (SR140333) was investigated for therapeutic efficacy in *Nlrp3*$^{-/-}$ mice[13] (FIG. 5). NK1R antagonist treatment reduced NK1R staining in bladder tissue sections as well as *Nk1r* and *Ppt-A* mRNA levels

Fig. 5 | **Inhibition of neuroinflammation in the bladder. a** | Overview of the mucosal pain response to infection. Acute cystitis is accompanied by pain, owing in part to direct bacterial infection of the nerve cells and increased expression of the mucosal pain sensors substance P (SP) and NK1R in epithelial cells and nerve fibres. SP binds to NK1R, initiating a pain signal, which is propagated to the central nervous system. **b** | The pain response is further amplified by an IL-1β-driven inflammatory loop. **c** | Inhibition of pain and disease response by treatment with IL-1 receptor antagonist (anakinra) or the NK1R inhibitor SR140333 (REF.[13]). **d** | Staining of tissue sections from the bladder of infected mice, showing increased expression of Substance P and NK1R in *Asc*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice compared with C57Bl/6 wild-type (WT) mice[13]. **e** | The SP and NK1R response was inhibited, using a selective NK1R antagonist, which also reduced bacterial counts and tissue pathology. Part **d** adapted from REF.[13], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Part **e** adapted from REF.[13], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

in whole-bladder mRNA[13]. A marked decrease in tissue pathology (oedema, hyperaemia and mucosal integrity) was detected, and treatment inhibited IL-1 superfamily gene expression, inflammation, cytokine production and adaptive immunity[13]. These results suggest that pain from the urinary tract during ACY involves SP and NK1R signalling and that treatment with an NK1R antagonist can reduce symptoms and inflammation in UPEC-infected mice (FIGS 2,5).

Human relevance of innate immunomodulation therapy in the urinary tract

The extent to which these successful treatment strategies in mice can be translated to human ACY needs to be investigated in controlled clinical studies; however, insights into the human relevance of innate immunomodulation therapy have been gained in patients with IC/BPS[187]. These patients experience severe and debilitating pain during bladder filling, resulting in extreme urgency and frequency[188,189]. IC/BPS has a prevalence of about 0.1% and affects all aspects of life, as even morphine and morphine analogues fail to provide adequate symptom relief[188–190]. Numerous therapeutic approaches to IC/BPS have been tested in preclinical studies including NK1R antagonists and various chemicals[185,191,192], such as protamine sulphate, to induce urothelial cell shedding and facilitate bacterial clearance from the urinary tract[193]; however, the results of these studies have not been sufficiently convincing for human use[194]. More specific therapies have been lacking owing to poor understanding of the disease mechanisms.

The IL-1β-dependent and NK1R-dependent pain response in the mouse ACY model suggested that IL-1RA therapy might be an option for this patient group. Patients with severe IC/BPS ($n = 17$) were, therefore, offered off-label IL-1RA treatment[187], and ~70% of the patients showed an initial treatment response, characterized by a reduction in pain and micturition frequency and an increased quality of life, quantified by O'Leary's symptom score (7.2 versus 17.4)[187]. Clinical improvement was accompanied by a reduction in urine SP levels and gene expression analysis revealed considerable effects on neuroinflammatory and inflammatory gene sets, including IL-1, IL-6 and IL-8 signalling pathways, which were inhibited[187]. After the initial treatment cycle, 13 of the patients chose to continue IL-1RA treatment and individual treatment protocols have proven efficient in the long term (>1 year)[187].

These results provide clinical evidence that IL-1RA therapy might be useful and effective in patients with IC/BPS, but controlled clinical trials should be performed to validate these effects. In addition, studies of IL-1RA therapy might be of interest to treat IL-1β-induced symptoms and pathology in ACY, but clinical data are not yet available.

Additional approaches to inhibiting innate immunity in acute cystitis

A variety of anti-inflammatory regimens have been tested as therapeutics in ACY[47]. Numerous studies have shown that broad anti-inflammatory agents such as corticosteroids and NSAIDs reduce inflammation in UTI models[145,195,196], but have adverse effects on bacterial clearance.

Controlled clinical studies have compared diclofenac or ibuprofen with antibiotics, but no significant benefits were detected for bacterial clearance or symptom relief[144,197,198]. In a study of 181 women with ACY, comparing a 3-day ibuprofen course with pivmecillinam, approximately half of the patients who received ibuprofen had persisting or worsening symptoms during the 4-week follow-up period compared with 10% of patients treated with the antibiotic[198]. In studies comparing ibuprofen with fosfomycin treatment, symptom burden was increased in 34% in the ibuprofen group[143,144]. In one study, NSAID treatment with diclofenac was associated with an increased risk of APN[197,198].

Another mechanism by which ACY, specifically recurrent cystitis, is mediated in mice is through cyclooxygenase 2 (COX2), which catalyses the rate-limiting step in the conversion of omega-6 arachidonic acid to prostanoids and is involved in the development of acute inflammation. In mice sensitized to develop recurrent cystitis, *Ptgs2*, which encodes COX2, showed a 50-fold increased expression in bladder tissues from UPEC-infected mice, and immunofluorescent antibody staining of bladder sections showed robust expression of COX2 by urothelial cells in bladders exhibiting severe inflammation[120].

Specific inhibition of COX2, using a selective COX2 inhibitor (SC-236), was shown to reduce bladder inflammation and bacterial load in a mouse model of ACY, consistent with the role of inflammation in the disease process[120]; by contrast, a COX1 inhibitor did not significantly affect inflammation or bacterial counts[120]. Furthermore, SC-236-treated mice had lower bladder bacterial titres 24 h after infection than those treated with the COX1 inhibitor, suggesting that bacterial clearance was facilitated by the blockade of COX2.

Forskolin regulates exocytosis of* E. coli *in bladder epithelial cells. In a mouse model in which mice were catheterized and intravesically instilled with the type 1 fimbriated UPEC strain CI5, bacterial invasion into bladder epithelial cells was found to be mediated through fusiform vesicles[199]. Further in vitro investigations showed that *E. coli* infection of 5637 bladder epithelial cells initiated bacterial incorporation into secretory lysosomes and release of the secretory lysosomes. Secretory lysosomes are stimulated through

intracellular Ca^{2+} levels and cyclic AMP (cAMP) flux, and discharge their contents in response to fluctuations in these factors. *E. coli* infection was observed to induce Ca^{2+}-sensitive and cAMP-sensitive exocytosis of secretory lysosomes from bladder epithelial cells. Further investigation showed *E. coli* inside the exocytosed lysosomes[199].

Forskolin is a labdane diterpene that stimulates adenylate cyclase and increases intracellular levels of cAMP. Using forskolin in combination with gentamicin in BALB/c mice, a reduction in intracellular bacterial numbers was detected in bladder tissue, and urine IL-6 levels were lowered[199].

These results suggest that broad anti-inflammatory agents lack specificity as they affect both the protective and the destructive arms of innate immunity.

Immunomodulation by molecules of bacterial origin

Bacteria are an interesting source of molecules that regulate innate immunity in the host. In contrast to virulence factors, which can have detrimental effects, bacterial molecules have been shown to target the transcriptional machinery or TLR signalling and inhibit the innate immune response, resulting in a protective effect. These molecules have an interesting potential as candidates for innate immunomodulation therapy.

Bacterial NlpD inhibits Pol II-dependent gene expression

In early experiments, ABU strains were shown to inhibit host gene expression by targeting the RNA Pol II phosphorylation machinery[200,201] (FIG. 6). The RNA Pol II cycle controls RNA synthesis through numerous precisely regulated steps[202]. Productive RNA elongation requires phosphorylation of the RPB1 subunit, and this step was inhibited by most ABU strains[200]. The bacterial protein NlpD has been identified as an active inhibitor released by ABU and faecal *E. coli* isolates under normal growth conditions[201,203,204]. NlpD is internalized by host cells and inhibits gene expression broadly, including effects on several pro-inflammatory mediators.

NlpD was further shown to act as an innate immune inhibitor in UPEC-infected mice treated with the recombinant NlpD protein. Inflammation was suppressed and bacterial clearance accelerated following intraperitoneal NlpD administration[201,204] (FIG. 6). The results identify NlpD as an efficient immunomodulator with therapeutic efficacy in a mouse UTI model. Further studies are required to determine the suitability of NlpD for human trials.

Bacterial TIR domain homologues inhibit innate immunity

The evolution of mechanisms that specifically interfere with TLR-mediated immune responses in bacteria is not surprising given the central role of the TLRs in host defence[16,82–84]. TIR domain homologues (TIR-containing proteins (TCPs)) have been detected in bacteria and viruses[205–207], and ~20% of clinical urinary tract isolates belonging to the B2 clade express the TIR-containing protein TcpC[94,206,208]. TcpC attenuates signalling cascades defined by the TIR domain of molecules such as MYD88, TIRAP, TRIF, TRAM and IL-1R, inhibiting TLR signalling, IL-1 expression and the STAT–IL-6 signalling pathway[94,207,209]. TcpC-deletion mutants showed reduced virulence in a mouse UTI model[206], suggesting that bacteria are capable of attenuating the TLR-dependent host defence, but the therapeutic potential of TIR domain homologues has not been further explored.

The bacterial Lon protease is a MYC inhibitor

Transcriptional control of the innate immune response is essential, and targeting upstream transcriptional regulators such as IRF7 has a therapeutic potential. The pleiotropic transcription factor MYC controls the expression of about 60–70% of all genes, affecting metabolism, cell growth and survival and inflammatory networks[210–212] (FIG. 7). MYC is essential for renal development, guiding the fusion of ectoderm and endoderm and regulating renal growth[213,214]. Infants and children suffering from APN in childhood often show renal growth retardation, consistent with MYC inhibition by infection[215] (FIG. 7).

Virulent UPEC strains have been shown to trigger MYC protein degradation and to inhibit the expression of *MYC* and MYC-related genes in a wide range of human cells[216]. MYC degradation was shown to be executed by the bacterial Lon protease, which enters human cells and animal tissues[216]. Treatment with recombinant Lon protease delayed cancer progression in models of bladder and colon cancer and increased long-term survival (FIG. 7), seemingly without any toxic response[216]. Furthermore, MYC regulated the renal response to infection by affecting the IRF3 and IRF7 transcription factors, suggesting that the protective potential of the MYC inhibitor in APN should be investigated[216].

Competitive adherence inhibitors

Fimbriae-specific adherence to host cell receptors is competitively inhibited by soluble receptor analogues. Oligosaccharides that competitively inhibit adherence have shown protective effects in rodent UTI models[217,218]. Soluble glycolipid receptor antagonists have been shown to inhibit UPEC adhesion by occupying the P fimbrial adhesin PapG and have been shown to reduce bacterial numbers in the mouse UTI model[219,220]. Using a high-affinity inhibitory mannoside in C3H mice, a reduction in intestinal colonization of FimH and F17 fimbriated UPEC strains was shown while simultaneously protecting against UTIs[221]. No adverse effects on the intestinal microbiota were observed, suggesting a novel therapeutic approach to treating UTIs while leaving the microbiota unchanged. In early studies, α-methyl-D-mannose was shown to inhibit type 1 fimbrial binding to the bladder mucosa and affect cell shedding[161,217,222]. Inhibition of the FimH type 1 pilus lectin, was proposed to affect bacterial adherence, immune cell activation and the formation of intracellular bacterial communities in the mouse bladder epithelium[223]. An earlier study in a rat UTI model showed that low molecular-weight mannosides inhibit the bacterial adherence and persistence[218]. Oral treatment with active FimH inhibitors was non-inferior to trimethoprim-sulphamen toxazol in mediating bacterial

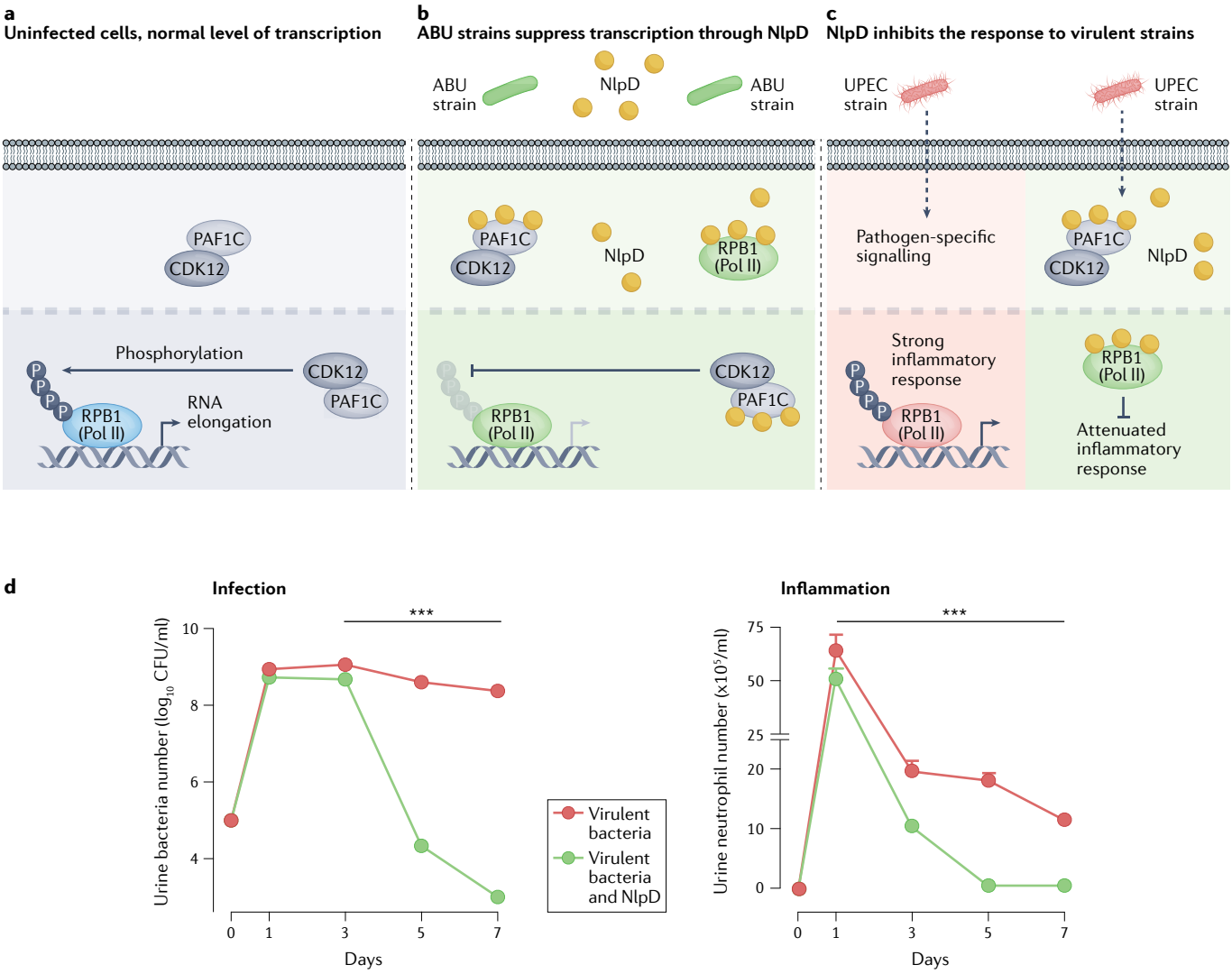


Fig. 6 | **The bacterial NlpD protein inhibits bladder inflammation and accelerates bacterial clearance.** Asymptomatic bacteriuria (ABU) strains have been shown to actively modify the host environment in the urinary tract using different molecular methods[200,201]. **a** | RNA polymerase II (Pol II) is phosphorylated to initiate productive RNA elongation during the transcription cycle. **b** | The bacterial NlpD protein is released by ABU strains, enters urothelial cells and binds to the RNA Pol II phosphorylation complex (PAF1C and CDK12), inhibiting Pol II Ser2 phosphorylation. **c** | The mechanism by which NlpD inhibits RNA Pol II Ser2 phosphorylation in the host. Treatment with NlpD inhibits the response to virulent strains by inhibiting Pol II Ser2 phosphorylation. **d** | Therapeutic effect defined by a marked reduction in bacterial counts and neutrophil infiltration in mice treated intraperitoneally with recombinant NlpD, compared with untreated mice[201,204]. ***$P < 0.001$. CFU, colony-forming unit; UPEC, uropathogenic *Escherichia coli*. Part **d** adapted with permission from REF.[204], American Society for Clinical Investigation.

clearance from bladders in an infected UTI mouse model using C3H/HeN mice[224]. Thus, blocking bacterial adhesion might provide an interesting therapeutic strategy. Nearly all clinical cystitis isolates are FimH positive, but translation to clinical treatment has not been reported.

Vaccination strategies

A number of antibacterial vaccination strategies have been successfully implemented, demonstrating that adaptive immunity can prevent infection by highly virulent organisms[225]. Vaccines prevent tetanus and diphtheria, as well as infections caused by *Haemophilus influenzae* and *Streptococcus pneumoniae*[225]. The awareness of vaccines and their crucial role for public health has increased dramatically, inspiring further attempts to prevent or treat infections by boosting adaptive immunity. Vaccination strategies are mostly designed to prevent infection, in contrast to innate immunomodulation therapy, which has been examined for therapeutic use.

Vaccination studies in UTI have a long history with varying results[226–229]. Early UTI vaccine studies targeted bacterial O-antigens expressed on LPS by the most virulent UPEC strains[226,230,231]. Clinical studies detected antibodies to a limited number of O-antigens in patients with APN but despite extensive studies in animal models, strong protective effects of vaccination were not observed[230]. Capsular polysaccharides were also tested

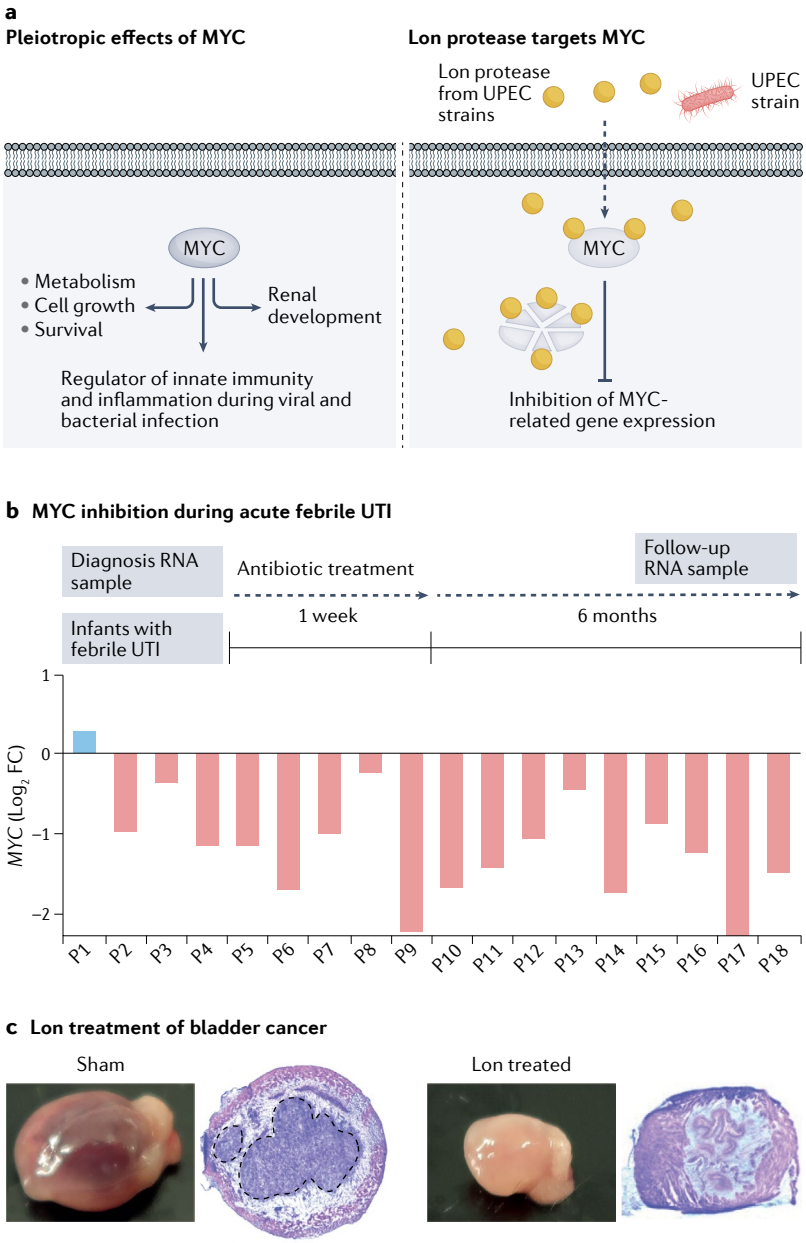


Fig. 7 | **A bacterial MYC inhibitor and effects of febrile UTI on *MYC* RNA levels.** Similar to asymptomatic bacteriuria (ABU) strains, uropathogenic *Escherichia coli* (UPEC) strains produce molecules that modify the host environment. UPEC strains have been shown to inhibit MYC, which is a major transcriptional regulator that also affects innate immunity. Infection reduced MYC levels and a bacterial protease degraded the MYC protein in infected cells. **a** | Schematic of MYC homeostasis and suggested effects of the bacterial Lon protease on the MYC protein and MYC-related gene expression. **b** | Reduction in *MYC* RNA levels during acute febrile urinary tract infection (UTI), compared with the 6-month follow-up point[216]. Paired samples were examined. **c** | Protective effect of recombinant Lon protease in a mouse bladder cancer model[217]. FC, fold change. Parts **b** and **c** reprinted from REF.[216], Springer Nature Limited.

as vaccine antigens, in analogy to the *Haemophilus influenzae* vaccine approach, but substantial protective effects were not achieved[231]. Antigenic heterogeneity and poor immunogenicity of UPEC capsular polysaccharides complicated vaccine design. Additional vaccine antigens that have been tested in animal models of UTI include the FimH adhesin[228], the PapDG protein[232], α-haemolysin[227] and iron acquisition molecules[229,233–235] as antigens, but no licensed UTI vaccines are available for use in the USA.

Overall, four vaccines have been tested in human clinical trials, Uro-Vaxom, Urovac, ExPEC4V and Uromune. Uro-Vaxom (OM-89), which contains lyophilized UPEC lysates and is administered as a daily oral tablet, was first approved in Switzerland in 1988 for the prevention of recurrent cystitis[236]. Urovac (StroVac) is an intramuscular injection containing heat-killed uropathogenic bacteria, including *E. coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Morganella morganii* and *Enterococcus faecalis*, and is approved for human use in Europe[237]. ExPEC4V consists of four conjugated O-antigens from *E. coli* serotypes O1A, O2, O6A and O25B, common to UPEC strains[238]. Uromune (MV140) consists of a sublingual preparation of inactivated strains of *E. coli*, *P. vulgaris*, *K. pneumoniae* and *E. faecalis*[239]. Retrospective observational studies showed a reduced number of recurrences[240], but no randomized controlled trial results have been reported yet. In a study comparing the efficacies of Uro-Vaxom, Urovac and ExPEC4V vaccines in adults with recurrent UTI, Uro-Vaxom but not ExPEC4V reduced the UTI recurrence rate[241]. However, the daily regimen and toxic effects have limited the widespread use of Uro-Vaxom[242].

Interesting vaccine studies have identified siderophores and their receptors as vaccine antigens that trigger a mucosal and systemic immune response and show promising results in mouse UTI models[229,234,243]. Siderophores are essential iron-acquisition molecules in UPEC strains and have an important role in UTI pathogenesis, enhancing bacterial virulence[71,154,244]. The potential of siderophores as vaccine antigens was investigated by adding the siderophores Ybt or Aer to the carrier protein cBSA[229]. A robust adaptive immune response was observed, with protection against bladder and kidney infection in the mouse[229].

Potential vaccine candidates have further been selected from a pool of bacterial cell surface proteins expressed during growth in human urine, mouse infection models and confirmed in human infections[245,246]. Using an immunoproteomics approach, 23 outer-membrane proteins were shown to be immunoreactive[247], including four that were prevalent among UPEC isolates[248,249]. Intranasal immunization with Hma, IutA, FyuA, or IreA, conjugated to cholera toxin, considerably reduced the bacterial burden in the bladder or kidneys after transurethral challenge with UPEC[234]. As cholera toxin is unsuitable for human use[250], other mucosal adjuvants were tested for efficacy, including a double mutant heat-labile *E. coli* enterotoxin, dmLT[251]. Intranasal immunization with dmLT-Hma and dmLT-IutA induced antigen-specific antibody production and provided robust protection in immunized mice following transurethral challenge with UPEC[243].

The recognition of bacterial adherence as a virulence factor, and secretory IgA antibodies as potent anti-adhesives in patient urine, suggested that mucosal vaccination could be feasible, using fimbriae as antigens[27,87,252]. Not limited by antigen recognition, secretory IgA possesses broad antibacterial function through

carbohydrate receptors for the mannose-specific lectin of type 1-fimbriated *E. coli* and resulting in agglutination of the bacteria and inhibition of attachment to epithelial cells[87]. Urinary secretory IgA levels are elevated during symptomatic UTIs and are low in children susceptible to recurrent UTIs in the absence of infection, making low urinary sIgA values a possible marker for recurrent symptomatic infections[27,252].

The vaccine concept has been advanced by using the FimH adhesin as the antigen[253]. In an open-label, dose-escalation phase I trial, 67 women with or without history of recurrent UTIs received intramuscular injections of FimH adhesin on four occasions. The drug was well tolerated with no serious adverse events reported and women with a history of UTIs had a 150-fold increase in FimH antibodies[254]. These preliminary positive data have led to FDA permission for compassionate use of the vaccine in patients with UTIs caused by multi drug-resistant *E. coli*[6]; however, no data on therapeutic efficacy have been disclosed.

Classical questions and obstacles in triggering an adaptive immune response are the antigenic variation of the infecting bacterial strains, problems relating to defining pathogen-specific vaccine antigens, avoiding detrimental effects on the normal flora and identifying target populations suitable for different vaccine candidates. Most vaccination studies focus on creating a successful antibody response, and T cell responses in the bladder have previously received less attention[7]. However, macrophage depletion has been shown to promote T_H1-mediated responses and subsequent bacterial clearance while not affecting T_H2 responses[35]. These observations are supported by the results of a study that showed that bladder infection triggers a robust T_H2 cell response, causing re-epithelialization with a limited capacity to clear infection[255]. Furthermore, immunization with UPEC antigens combined with the T_H1-skewing adjuvant CpG was observed to protect mice from developing both single-episode and recurrent UTIs compared with untreated mice[256]. Intravesical vaccination provided a substantially better response than subcutaneous vaccination and was accompanied by an increase in local T_H1 cells. These results suggest that boosting T_H1 responses in the urinary tract might enhance protection. Controlled clinical trials are needed to define the protective potential of the different vaccination strategies.

Conclusions

This Review highlights the importance of innate immune control in UTI and the potential of innate immunomodulation therapy to target 'bad inflammation' as a cause of disease. This approach differs from the use of broad anti-inflammatory compounds, in that it seeks to correct specific innate immune defects in susceptible hosts. Potent therapeutic effects have been achieved in animal models, and patients with IC/BPS have responded favourably to off-label treatment with an IL-1RA[187], but further studies are needed to understand the clinical potential of this approach. Controlled clinical trials are being initiated to improve understanding of the potential of IL-1RA treatment for ACY and important future target populations include patients with infections caused by antibiotic-resistant strains, in whom the need for new therapeutic options is immediately obvious.

Most previous studies of UTI pathogenesis and therapy have been conducted in animals without a clear disease phenotype or established human relevance. Genetic screens of mice with single gene deletions have now established that APN and ACY can be recreated, using single-gene deletions affecting innate immunity. Different innate immune defects distinguish APN from ACY, illustrating the molecular specificity for each organ system. Specific innate immunomodulatory therapeutic approaches have been designed to correct these defects and restore the defence. The disease phenotypes in mice were shown to share important features with human disease, suggesting that the translation of some of these findings into the clinic is of interest.

The finding of accelerated bacterial clearance in the kidneys of mice receiving *Irf7* siRNA interference therapy is intriguing and partly unexpected. A similar effect was seen after IL-1RA treatment of ACY, suggesting that reducing inflammation restores immune balance and the antibacterial defence. The mechanisms are not entirely clear, but the findings suggest that the defence is impaired or overwhelmed by the excessive inflammatory response that accompanies disease. Despite a massive neutrophil infiltrate and hyperactive IRF7 or IL-1-dependent genes, the functionality of the defence seems to be lost. This effect was first observed in *mCxcr2*$^{-/-}$ mice, in which neutrophil activation is impaired and defective exit across the mucosa into the urine creates massive neutrophil retention in the kidneys, leading to massive tissue damage[14,79,116]. The results also suggest that in addition to restored homeostasis, as yet undefined mechanisms of bacterial clearance might contribute to the resistance to infection in *Irf7*$^{-/-}$ or *Il1b*$^{-/-}$ mice and immediate bacterial clearance from their tissues, a fascinating topic for further studies.

The strong effects of innate immunotherapy are promising, as they suggest new potential therapeutic solutions for the treatment of UTI and other bacterial infections. The early work on bacterial adherence and the identification of specific host cell receptors resulted in the realization that mucosal cells respond to infection and a number of host response parameters were identified, establishing the importance of innate immunity in UTI. Further characterization of signalling pathways and transcriptional regulators of the innate immune response led to the identification of specific defence dysfunctions and pinpointed exaggerated inflammation as the cause of acute, severe disease and tissue pathology. The control of these processes at the level of transcription and the involvement of individual transcription factors as arbitrators of protection or disease adds a new perspective. Adding the perspective that these mechanisms can be targeted to treat infection has provided convincing evidence that controlling the innate immune responses can be a potent alternative to antibiotics.

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

Competing interests
Patents have been filed with the scientists as inventors for the therapeutic use of the NlpD protein, *IRF7* siRNA, IL-1R antagonists, MMP and NK1R inhibitors for treating urinary tract infections. The rights to develop these patents are held by SelectImmune Pharma, where the scientists hold shares. Patents for the MYC inhibitor have also been filed for treating cancer and infections.

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