Immune response of the urinary tract – bacterial infections and alpha1-oleate treatment of bladder cancer

Ahmadi, Shahram

2026

Document Version:
Publisher's PDF, also known as Version of record

Immune response of the urinary tract – bacterial infections and alpha1-oleate treatment of bladder cancer

SHAHRAM AHMADI
DEPARTMENT OF LABORATORY MEDICINE | FACULTY OF MEDICINE | LUND UNIVERSITY

Printed by Media-Tryck, Lund 2026 NORDIC SWAN ECOLABEL 3041 0903

SHAHRAM AHMADI has a Bachelor degree from the Medical University of Shiraz, his hometown, and a Master degree from the University Putra Malaysia. His PhD research focuses on immune responses during urinary tract infections and bladder cancer. The work integrates immunology and translational research to advance the understanding of host–pathogen interactions and cancer-related immune mechanisms.

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Laboratory Medicine

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2026:5
ISBN 978-91-8021-803-0
ISSN 1652-8220

Immune response of the urinary tract – bacterial infections and alpha1-oleate treatment of bladder cancer

Shahram Ahmadi

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 29th of January 2026 at 13.00 in Belfragesalen, Biomedical Centre, D15, Lund, Sweden

Faculty opponent
Professor Ann Stapleton
Department of Medicine, Division of Allergy & Infectious Diseases, University of Washington

Organization: LUND UNIVERSITY

Document name: DOCTORAL DISSERTATION **Date of issue**

Author(s): Shahram Ahmadi **Sponsoring organization:**

Title and subtitle: Immune response of the urinary tract – bacterial infections and alpha1-oleate treatment of bladder cancer

Abstract:

The immune response of the urinary tract is essential for the defense against infections (UTIs) and bladder cancer, yet immune hyper-activation can drive disease. The urine protein profile, defined by kidney filtration and local tissue release, offers a valuable source of information. In this study, proteomic analyses of clinical samples was combined with transcriptomics, and imaging technologies to study acute immune activation in febrile UTI and therapy-induced responses in non–muscle-invasive bladder cancer.

In infants with febrile UTI, urine and blood analyses detected a cytokine storm, particularly in patients with renal involvement, illustrating the severity of acute pyelonephritis, a condition associated with urosepsis and mortality.

The cytokine storm response was regulated by the MYC, IRF3, and IRF7 transcription factors, which were shown to control the disease responses in a murine UTI model. The cytokine storm and kidney injury in *Irf3*$^{-/-}$ mice, was prevented by treatments silencing IRF-7 with *Irf7* siRNA or MYC with the recombinant Lon protease, identifying these molecules as potential, non-antibiotic therapeutics for severe kidney infections.

In bladder cancer, intravesical alpha1-oleate treatment activated a rapid, predominantely innate immune respone (IL-1 family, chemokines, interferons) with an adaptive component (IL-12, Granzyme B, PD-L1) and a profile closely resembling the BCG-induced response. The results suggest that in addition to directly killing tumor cells and reducing tumor numbers and tumor size, alpha1 oleate activates an immune response that adds to its protective anti-tumor effects.

Key words:

Classification system and/or index terms (if any) Supplementary bibliographical information

Language **Number of pages**: 80

ISSN and key title: **1652-8220**

ISBN: 978-91-8021-803-0

Recipient's notes Price Security classification

Signature Date 2026-01-29

Immune response of the urinary tract – bacterial infections and alpha1-oleate treatment of bladder cancer

Shahram Ahmadi

LUND
UNIVERSITY

Coverphoto by Shahram Ahmadi

Faculty of Medicine
Department of Laboratory Medicine

ISBN 978-91-8021-803-0
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2025

To my father, Jahangir

Table of Contents

Abstract

The immune response of the urinary tract is essential for the defense against infections (UTIs) and bladder cancer, yet immune hyper-activation can drive disease. The urine protein profile, defined by kidney filtration and local tissue release, offers a valuable source of information. In this study, proteomic analyses of clinical samples was combined with transcriptomics, and imaging technologies to study acute immune activation in febrile UTI and therapy-induced responses in non–muscle-invasive bladder cancer.

In infants with febrile UTI, urine and blood analyses detected a cytokine storm, particularly in patients with renal involvement, illustrating the severity of acute pyelonephritis, a condition associated with urosepsis and mortality.

The cytokine storm response was regulated by the MYC, IRF3, and IRF7 transcription factors, which were shown to control the disease responses in a murine UTI model. The cytokine storm and kidney injury in *Irf3*$^{-/-}$ mice, was prevented by treatments silencing IRF-7 with *Irf7* siRNA or MYC with the recombinant Lon protease, identifying these molecules as potential, non-antibiotic therapeutics for severe kidney infections.

In bladder cancer, intravesical alpha1-oleate treatment activated a rapid, predominantely innate immune respone (IL-1 family, chemokines, interferons) with an adaptive component (IL-12, Granzyme B, PD-L1) and a profile closely resembling the BCG-induced response. The results suggest that in addition to directly killing tumor cells and reducing tumor numbers and tumor size, alpha1-oleate activates an immune response that adds to its protective anti-tumor effects.

Popular Science Summary

The host immune response plays a critical role for the urinary tract defense against urinary tract infections and bladder cancer. As excessive immune responses may lead to disease, molecular control is essential. Insights into the key disease control mechanisms can be gained from controlled clinical studies based on strict clinical protocols, protein and gene expression analysis and DNA sequencing technologies. This study investigated immune activation in febrile UTI and to therapies like intravesical alpha1-oleate in non-muscle invasive bladder cancer, using these technologies.

In children with severe kidney infections, known as acute pyelonephritis, proteomic analysis revealed potent local and systemic innate immune response characterized as a "cytokine storm". This storm was particularly strong in infants whose kidneys were affected, and closely resembled the immune overreaction seen in severe COVID-19 cases. The excessive immune activation explains why UTIs escalate into dangerous conditions like urosepsis, which is one of the most common causes of death world-wide.

Experiments in mice uncovered the genetic "switches" that regulate the immune response to kidney infection. Three transcription factors: MYC, IRF3, and IRF7 were found to control whether the immune response was balanced or spiraled out of control, into damaging overreaction. By blocking IRF7 or MYC with inhibitory drug candidates, we were able to regain balance of the immune response, prevent kidney damage and accelerate the clearance of infection, pointing to a new, non-antibiotic concept for treating UTIs.

Finally, in bladder cancer patients treated with alpha1-oleate, protein analysis identified the activation of multiple cytokines, activating both innate (e.g., IL-1 family, chemokines and interferons) and adaptive (e.g., IL-12, Granzyme B, CD-40, and PD-L1) immune responses. The findings highlight alpha1-oleate's ability to not only kill tumor cells, and reduce the tumor number and size, but also to activate a protective immune response. This cytokine response profile was similar to that reported in patients receiving BCG, a standard bladder cancer treatment, showing that alpha1-oleate stimulates the immune system to fight back.

List of papers

This thesis is based on the following articles:

I. **Shahram Ahmadi,** Therese Rosenblad, Samudra Sabari, Magnus Linden, Per Brandström, Sing Ming, Catharina Svanborg and Ines Ambite. Analysis of the Innate Immune Response to Febrile UTI in Infants: Evidence of an Acute Cytokine Storm. Pediatric Infectious Disease J. 2025 Aug 26. doi: 10.1097/INF.0000000000004914

II. Ines Ambite, Sing Ming Chao, Therese Rosenblad, Richard Hopkins, Petter Storm, Yong Hong Ng, Indra Ganesan, Magnus Lindén, Farhan Haq, Thi Hien Tran, **Shahram Ahmadi**, Bernett Lee, Swaine L Chen, Gabriela Godaly, Per Brandström, John E Connolly and Catharina Svanborg. Molecular analysis of acute pyelonephritis - excessive innate and attenuated adaptive immunity. Life Sci Alliance. 2024 Dec 20;8(3):e202402926.

III. Murphy Lam Yim Wan, **Shahram Ahmadi**, Ines Ambite, Thi Hien Tran, Farhan Haq, Sing Ming Chao and Catharina Svanborg. Targeting a transcriptional node formed by IRF3, MYC and IRF7 to treat bacterial infections. Submitted manuscript

IV. **Shahram Ahmadi**, Ines Ambite, Antonin Brisuda, Jaromir Hacek, Farhan Haq, Samudra Sabari, Kamala Vanarsa, Chandra Mohan, Marek Babjuk and Catharina Svanborg. Similar immune responses to alpha1-oleate and Bacillus Calmette-Guérin (BCG) treatment in patients with bladder cancer. Cancer Medicine.2024 Apr;13(7):e7091. doi: 10.1002/cam4.7091.

Abbreviations

HAMLET	Human Alpha-lactalbumin Made LEthal to Tumor
BCG	Bacillus Calmette-Guérin
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
hCFHrp	human Complement Factor H related protein
IL	Interleukin
NMIBC	Non-muscle Invasive Bladder Cancer
NMP22	Nuclear matrix protein 22
PBS	Phosphate Buffered Saline
fUTI	Febrile urinary tract infection
DMSA	Dimercaptosuccinic Acid
APN	Acute pyelonephritis
VUR	Vesico-Ureteric Reflux
NF-κB	Nuclear Factor kappa B
rLon	recombinant Lon
IRF	Interferon Regulatory Factors
TLRs	Toll-Like Receptors
NK Cell	Natural killer cell
APCs	Antigen-presenting cells

Introduction

Background

The immune system is essential for tissue homeostasis, to distinguish self from non-self and to recognize external danger. The rapid innate immune response must be mobilized on demand, and adaptive immunity recruited to fill defensive gaps. For the organism the immune response can be a double-edged sword, however. A well-balanced immune response is crucial for protection, but when dysregulated, the immune responses can cause susceptibility, disease and pathology,[1] as seen several clinical contexts, including infectious diseases, cancer and autoimmunity.[1,2] This thesis investigated the immune response to acute bacterial infection and the response to cancer treatment using the alpha1-oleate complex.

Immune response to infection

Infections remain a major cause of acute morbidity and mortality and long-term health consequences. Bacterial pathogens are aggressors that initiate tissue attack at sites of infection, and the host immune system is responsible for the defense. At mucosal surfaces, immune surveillance regulates susceptibility to infection by mounting rapid responses against pathogenic microbes.[3] The mucosal barrier distinguishes pathogens from commensals and is exquisitely equipped to orchestrate the local defenses needed to eliminate pathogenic microbes without disturbing the normal flora.[4,5] Innate immunity serves as a rapid and highly selective first line of defense that restricts pathogen access to host tissues while preserving interactions with commensal microbiota.[6,7] This specificity is mediated by pathogen recognition receptors and immediate activation of innate immune pathways.[8]

Pathogen clearance is mediated by multiple mechanisms: antimicrobial peptides such as defensins exert direct bactericidal effects, while neutrophils phagocytose bacteria and may traverse the epithelial barrier, exiting into the urinary lumen along with the ingested microbes.[1] Additional innate effector mechanisms may be activated depending on the context. While these responses are generally transient and effective, impairments in bacterial clearance or excessive inflammatory responses are linked to increased disease severity and complications. When properly

balanced, the innate immune response clears the infection, but a loss of control may result in excessive immune activation and lead to severe acute disease and chronic infection.[9-12] Maintaining immune homeostasis is therefore critical, as dysregulated responses whether hypoactive or excessive can contribute to tissue damage and disease. Innate immunotherapy targets these dysfunctions by enhancing or rebalancing innate immune mechanisms to restore effective host defense.[3]

The systemic arm of the innate immune response is activated when the mucosal defense fails and is less selective, aiming to eradicate any microbial intruder from the tissues.[1] The response is activated by pattern recognition and receptors such as Toll-like receptors (TLRs) that detect conserved molecular patterns and initiate immune signaling.[1,6] Key innate effector cells include neutrophils, macrophages, and natural killer cells and type I interferons and the inflammasome play vital roles in amplifying anti-bacterial and anti-viral responses.[6]

The innate immune system is transient, and if the infection persists, adaptive immunity is engaged. Involvement of the adaptive immune system is essential for long-term immunity.[1] Bacterial antigens are processed by antigen-presenting cells, which activate T and B lymphocytes locally or in secondary lymphoid organs. Chronic infection can lead to the formation of tertiary lymphoid structures, where localized immune responses are organized. Specific T cell subsets modulate the interplay between innate and adaptive immunity, influencing the magnitude and quality of the host's antibacterial response.[13] B cells control the antibody-mediated immune response, resulting in plasma cell maturation, enhancing pathogen neutralization and opsonization. Plasma cells producing pathogen-specific antibodies have been identified in the kidneys and bladders and infected individuals secrete secretory IgA, which prevents bacterial adhesion to epithelial surfaces.[14] Adaptive immunity also generates immunological memory, ensuring rapid and robust responses upon re-exposure to the same pathogen. The importance of adaptive immunity is illustrated by vaccination and the successful development of anti-bacterial and anti-viral vaccines, as well as recent therapeutic advances using immunotherapy.

Urinary tract infection

Urinary tract infections are common, encountered at all levels of healthcare. About 50% of all women experience an episode of acute cystitis at least once, and recurrences are common.[15] Acute pyelonephritis has an incidence of about 5% in children less than 12 years old [16] and up to 30% of children with APN developed renal scars following UTI, associated with long-term morbidity such as hypertension, complications of pregnancy and renal failure if scarring is extensive.[16,17] The disease burden of acute UTI is therefore significant.

Escherichia coli (*E. coli*) is the predominant pathogen, responsible for 70-90% of cases, followed by other gram-negative bacteria such as *Klebsiella* and *Proteus*.[16,18] Bacteria enter the urinary tract, attack and damage the mucosa of the bladder, ureters, or renal pelvis.[19] The symptoms result largely from the excessive immune response, as the associated inflammatory cell infiltration and nerve cell activation drives both the symptoms and severity of disease. The urinary tract can also harbor substantial numbers of bacteria without causing any symptoms. In cases of asymptomatic bacteriuria (ABU), the bacteria form a balanced, symbiotic relationship with the host that allows them to persist over long periods and may even protect against infection by more aggressive strains.[3,20] The strong association between adherence, virulence and disease severity, indicated that there must be a direct molecular link between the bacteria, their epithelial cell receptors and disease.

The urinary tract relies on innate immunity for its acute anti-bacterial defense. Molecular mechanisms of immune activation and disease have been extensively studied in models of urinary tract infection and validated in clinical studies.[2,12,21] Infections of the urinary bladder cause acute cystitis, characterized by local symptoms such as dysuria, frequency and pain.[15] Kidney infections cause acute pyelonephritis, characterized by extensive inflammation and pain, as well as systemic involvement with fever and general malaise. About 20- 30% of adults with acute pyelonephritis develop urosepsis, which is one of the main causes of death world-wide.[22] Patients with acute pyelonephritis may also develop renal scars, especially during childhood (Figure 1).

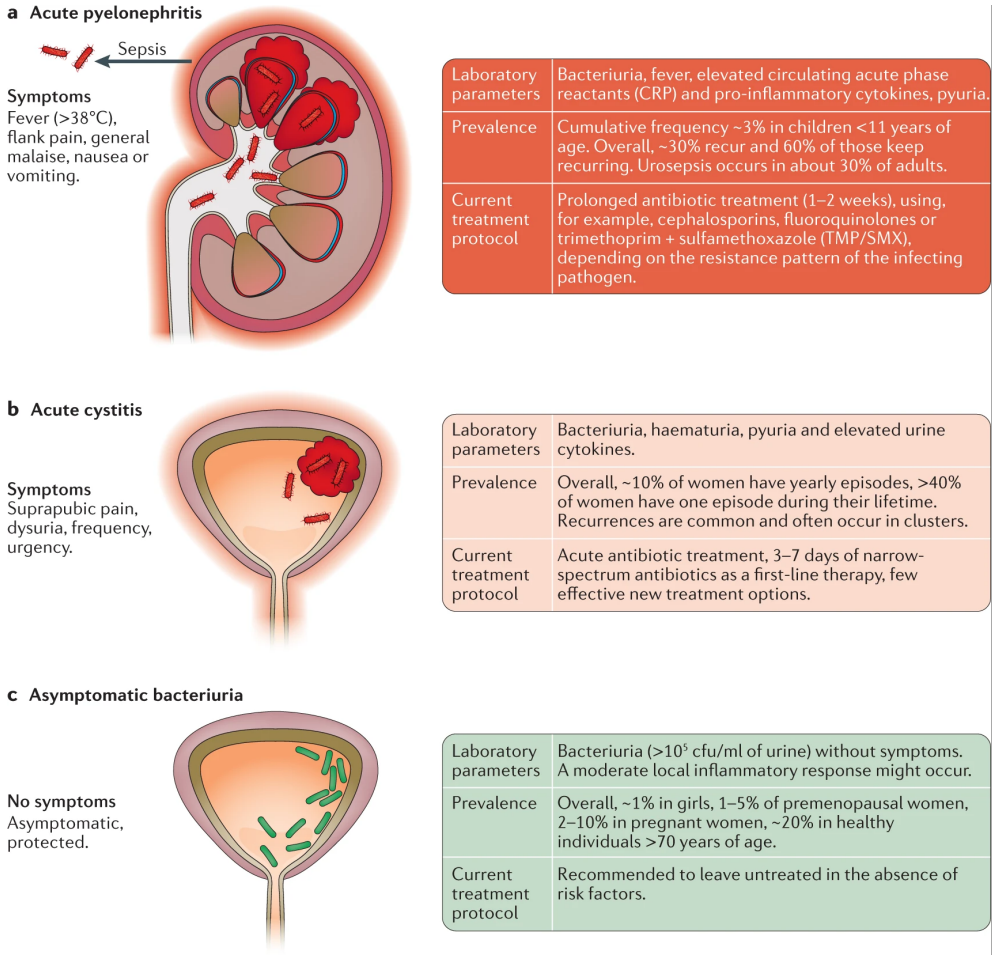


Figure 1. Overview of symptomatic infections and asymptomatic bacteriuria. This image is reproduced from Ambite, I *et al. Nat Rev Urol*. 2021(DOI: 10.1038/s41585-021-00477-x, reference (2).

Immune response to febrile urinary tract infection

The quality of the innate immune response is influenced by the expression of specific host cell receptors and by the signaling pathways that they activate. For example, TLR 4 on mucosal epithelial cells senses the presence of virulent bacteria through co-receptors that bind virulence ligands such as P fimbriae.[23] P-fimbriated uropathogenic *E. coli* strains trigger a TLR4 signaling pathway by inducing phosphorylation of the TLR4 adaptor proteins TRIF, TRAM, MyD88, and TIRAP. The PapG adhesin binds to glycosphingolipid receptors, leading to the release of ceramide, which further activates TLR4 signaling.[2,24] This activation leads to MAP kinase pathway signaling that converges on p38, which in turn regulates the

phosphorylation and activation of IRF3, as well as NF-κB and the activator protein 1 complex, composed of FOS and JUN.[11] In contrast, the activation of IRF7 requires new protein synthesis before it can be phosphorylated.[25] Together, IRF3 and IRF7 influence disease severity by coordinating different components of the antimicrobial defense and inflammatory responses in mice infected with UPEC strains (Figure 2).[2,11,25]

The local innate immune cascade may progress to systemic inflammation in acute pyelonephritis. The communication between the local site of infection and systemic compartments explains symptoms such as fever and the regulation of acute response parameters, such as CRP.[6] Activation of TLRs triggers the release of pro-inflammatory cytokines (IL-6, IL-8, and TNF-α) and chemokines, recruiting neutrophils to the site of infection.[24,26-28] As a result of these interactions, infection activates anti-bacterial effector mechanisms that clear the infection. Neutrophils play a central role by releasing reactive oxygen species and forming neutrophil extracellular traps to contain the infection.[29,30] In addition, infected nociceptive neurons may also participate in a neuroimmune loop, contributing to pain sensation via neuroinflammatory signaling in acute cystitis and acute pyelonephritis.[15] Cytokines also exert paracrine effects on neighboring cells, activating macrophages, eosinophils and a range of different cellular tissue components.[11]

Mucosal signaling activates neutrophil recruitment in urinary tract infection

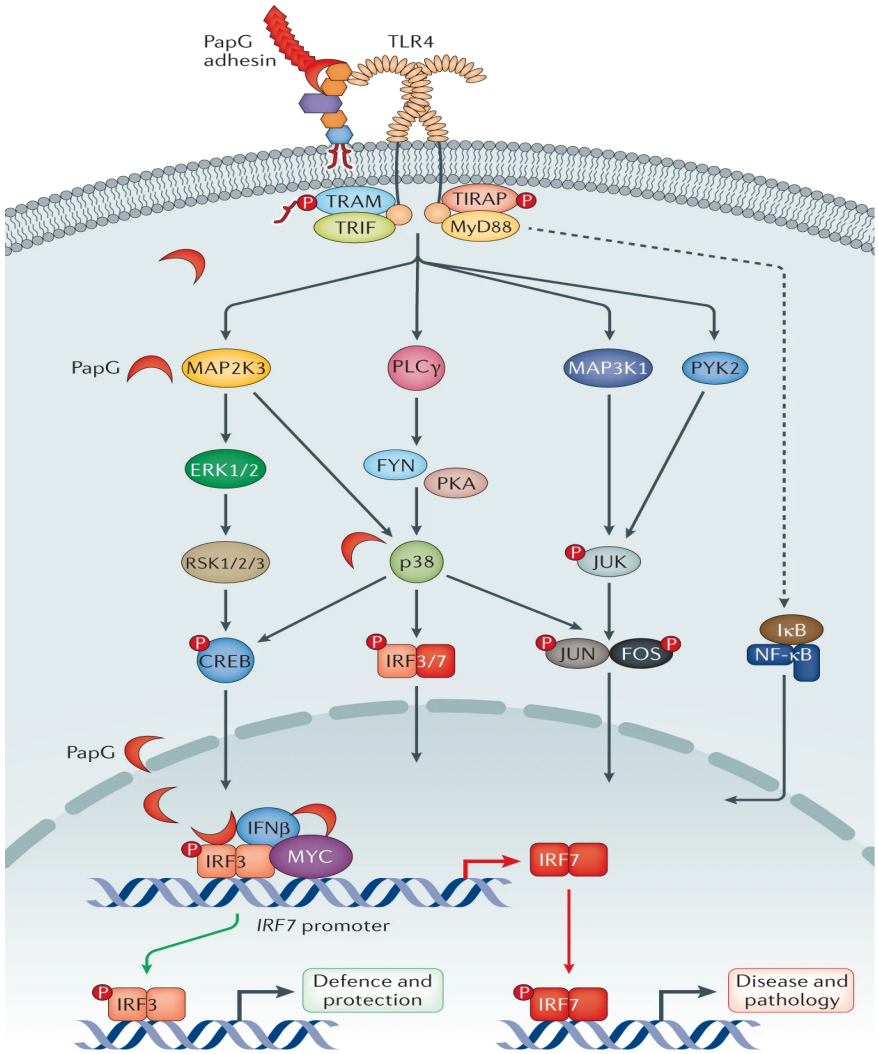
Uroepithelial cells serve as frontline defenders against uropathogenic *E. coli* and rapidly produce pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α.[24,27,28] IL-6 released from the infected mucosa can enter systemic circulation, stimulating hepatic synthesis of acute-phase proteins such as CRP.[31] This mechanism helps explain the elevated CRP levels and fever commonly observed in patients with acute pyelonephritis. Moreover, IL-6, IL-1, and TNF-α secreted by epithelial cells may act to amplify local inflammation, recruit immune cells, and function as early biomarkers detectable in urine.[29,30] Indeed, urinary cytokine measurements are increasingly being explored as non-invasive diagnostic tools for UTI severity and progression.

Chemokines increase the neutrophil recruitment from the blood stream and migration into infected tissues. Infection activates a broad chemokine response to infection, including CXCL family and CC chemokines.[32,33] The main neutrophil chemoattractant is CXCL8 chemokine family, which guides neutrophil migration into the mucosa and the exit across the mucosa into the urine, which is a prerequisite to maintain tissue homeostasis.[34,35] Furthermore, activated neutrophils provide signals for the activation and maturation of macrophages and other mucosal cells,

such as mast cells. [32] TNF-mediated crosstalk between neutrophils and macrophages has been shown to optimize the antibacterial defense in the urinary tract.[36]

Infected epithelial cells also amplify local inflammation by releasing additional cytokines and chemokines that activate resident immune cells, including mast cells, macrophages, and dendritic cells (DCs).[37] Macrophages and DCs play crucial roles in direct antimicrobial defense through phagocytosis and cytokine production, and they also serve as a bridge to adaptive immunity via antigen processing and presentation.[38] Resident mast cells, situated near the basal aspect of the urothelial lining, are among the earliest responders to infection. As inflammation progresses, additional mast cells are recruited into the bladder mucosa, contributing to the evolving immune environment. [37,38]

Studies using mast cell-deficient mice demonstrate that mast cells are required for optimal neutrophil recruitment and effective clearance of uropathogenic *E. coli*, indicating that mast cell–neutrophil crosstalk is vital for early antimicrobial defense.[39,40] The expansion of mast cell populations during bladder infection is supported by IL-3 dependent proliferative signals, providing a mechanistic explanation for the increased mast cell numbers observed during UTI progression. [37] Furthermore, the UPEC adhesin FimH can directly engage CD48 on mast cells, triggering a potent release of TNF-α and thereby enhancing neutrophil activation and accumulation in infected tissues.[40]



Mouse single-gene knockout

Irf3$^{-/-}$	Acute mortality, sepsis, high bacterial counts, large renal abscesses and pathology
Irf7$^{-/-}$	Highly resistant to UTI
Ifnb1$^{-/-}$	Acute mortality, sepsis, high bacterial counts, large renal abscesses and pathology

Human polymorphisms

IRF3 promoter SNPs	Low promoter activity	APN
IRF7 promoter SNP	Low expression	ABU

Figure 2. Overview of TLR4 signalling and transcriptional control of the innate immune response in acute pyelonephritis, bacteriuria. This figure is reproduced from Ambite, I *et al*. *Nat Rev Urol*. 2021(DOI: 10.1038/s41585-021-00477-x, reference (2).

Molecular and genetic basis of susceptibility to urinary tract infection

The severity of febrile UTI is influenced by the host's genetic susceptibility. Studies in both humans and animal models have identified genetic variants associated with increased susceptibility to acute pyelonephritis , including inherited risk observed across generations.[20,41] Evidence from murine UTI models clearly show that innate rather than adaptive immunity is essential for the resistance against acute cystitis and acute pyelonephritis and disease severity.[2] Infections in mice lacking T cell subtypes, B cells or with severe immunodeficiency were not more sensitive to acute infection that immunocompetent counterparts.[42]

Excessive inflammation and disease are essentially products of a failed innate immune response. The importance of innate immunity for host resistance against UTI was first discovered in the early 1980s in the murine UTI model.[42] Continued studies have shown that UTI susceptibility is controlled by the innate immune response through the function of specific genes. Gene deletions that disturb the activation or effector phase of the innate immune response increase the susceptibility to infection by decreasing the efficiency of bacterial clearance.[2] In addition, excessive innate immune activation may cause exaggerated inflammation, resulting in bladder or kidney disease. For example, *mCxcr2*$^{-/-}$ mice with a neutrophil migration and activation deficiency, develop severe APN with urosepsis followed by abscess formation and renal scaring.[43,44] *Irf3*$^{-/-}$ mice develop a similar phenotype with over-activation of the innate immune response, severe, acute disease with urosepsis and renal abscess formation within one week of infection.[11] Susceptibility to acute cystitis is enhanced by genetic defects that affect IL-β processing.[15]

Febrile UTI

Single gene deletions have been used to define mechanisms of susceptibility and disease in the murine acute pyelonephritis model and genes that are critical for immune effector function in the kidneys. The deletion of genes like *Irf3*, *Ifnb1*, and *Cxcr2*, leads to severe infection, including sepsis and renal abscesses.[11,25,45,46] Other proposed susceptibility genes include *Camp*, *Nod2*, and *Il6* and are linked to high bacterial loads and kidney damage.[47,48] Interestingly, overactivation of IRF-7 in *Irf3* deficient mice contributes to kidney inflammation and *Irf7* deficient mice are resistant to APN.[25] Based on these observations, a liposomal siRNA therapy targeting IRF-7 was developed and shown to reduce bacterial burden, inflammation, and abscess formation in susceptible mice, offering a potential therapeutic avenue for genetically predisposed individuals.[25] The efficacy of siRNA therapy was similar to that of cephalosporin treatment.

Clinical studies support the results from the experimental models. *IRF3* promoter polymorphisms have been identified in APN prone patients, and shown to lead to reduced *IRF3* expression.[11] CXCR1 regulates neutrophil migration and activation,

and expression levels are reduced in children with APN, and in family members in a three-generation family study.[49,50] *CXCR1* expression was significantly lower in neutrophils of children with recurrent UTIs, suggesting impaired neutrophil function due to reduced chemokine receptor expression.[45] *CXCR1* polymorphisms were also shown to be associated with lower *CXCR1* mRNA levels in patients with acute pyelonephritis.[11] These findings indicate a potential host-susceptibility factor for recurrent or severe UTI, although further studies are needed to clarify the epidemiology and clinical impact of this deficiency across different UTI presentations.[51]

In contrast, mice lacking *Tlr4* or its *Trif*, or *Tram* adaptors are protected from symptomatic infection and develop asymptomatic bacteriuria due to impaired innate immune responses.[52] In clinical studies, reduced levels of TLR4 protein have been associated with ABU, and identified *TLR4* promoter polymorphisms have been shown to attenuate *TLR4* expression and may be protective against severe urinary tract infections.[53,54]

Transcriptional regulation of immune response to kidney infection

Transcriptional regulation is essential to control the innate immunity and the host defense against bacterial infections.[2,55,56] In acute pyelonephritis caused by uropathogenic *E. coli*,[57] pathogen recognition activates multiple transcriptional regulators including NF-κB, p38, Fos, Jun and the interferon regulatory factors IRF3 and IRF7.[58] Once phosphorylated, IRF3 translocates to the nucleus and drives the expression of IRF7 and type I interferons, including IFN-β which activate interferon-stimulated genes that inhibit bacterial replication and reinforce epithelial barrier functions.[59] Additionally, IFN-γ is produced by NK cells and T lymphocytes and enhances macrophage and epithelial antimicrobial activities by upregulating genes involved in phagocytosis and antigen presentation.[60] These pathways collectively orchestrate a robust innate immune response that restricts bacterial colonization but, if dysregulated, can contribute to excessive inflammation and renal tissue damage.

Epigenetic mechanisms and post-transcriptional regulators further modulate this intricate transcriptional network during kidney infection and recovery. Chromatin remodeling through histone acetylation and methylation dynamically controls the accessibility of promoters for inflammatory cytokines and interferon genes, fine-tuning the magnitude and duration of the immune response.[61] For example, increased acetylation at IFN promoters can amplify IRF3 and IRF7-mediated transcription.[62] MicroRNAs such as miR-146a and miR-155 help restrain excessive TLR and interferon signaling by targeting mRNAs of pathway intermediates and transcription factors.[63] Dysregulation of IRF3 and IRF7 activity whether through mutations, epigenetic silencing, or persistent activation can lead to impaired bacterial clearance or chronic inflammatory injury, contributing to fibrosis and

progression toward chronic kidney disease.[2,25,64] Current research aims to harness these insights for novel therapies, such as enhancing IRF3/IRF7 pathways or modulating IFN-λ3 levels to promote pathogen clearance while minimizing collateral tissue damage.[65,66]

Molecular and genetic basis of acute cystitis

Acute cystitis is characterized by a mucosal inflammatory response in the urinary bladder and caused by bacterial infections.[67] The patients experience pain, dysuria and frequency of urination and a group of susceptible patients develop severe acute cystitis with an excessive innate immune response, severe symptoms and pathology but without fever and general malaise.[68,69]

Molecular determinants of disease severity in acute cystitis is not clear; however, mechanisms of innate immune hyper-activation have been identified using a combination of cellular infection technology, animal models and clinical studies.[15,56] These findings indicate that acute cystitis is an IL-1β driven hyperinflammatory disorder of the bladder, characterized by atypical IL-1β processing mediated by matrix metalloproteinase 7(MMP7) [32] and contributes to pathology, and genetic variation affecting *IL1β*, *ASC*, and *NLRP3* expression may further increase susceptibility to severe disease.[15,56]

Genetic screening revealed a non-canonical pathway of IL-1β hyperactivation that drives severe acute cystitis.[15,56] Although the inflammasome typically mediates pro–IL-1β processing, mice with deletions in *Nlrp3* or *Asc* unexpectedly developed a severe disease phenotype following infection.[56] In $Asc^{-/-}$ mice, the heightened IL-1β response was linked to overexpression of the protease MMP7 and the pain mediators NK1R and substance P.[15,56] Molecular studies demonstrated that MMP7 directly processes pro IL-1β, triggering a downstream cascade of IL-1β dependent genes.[56] Consistent with the central role of IL-1β, $Il1b^{-/-}$ mice were protected from infection and inflammation.[56,70] Supporting human relevance, patients with acute cystitis exhibited significantly higher urinary IL-1β levels than those with asymptomatic bacteriuria.[56]

Clinical cystitis isolates enhanced pro-IL-1β expression and accelerated IL-1β maturation in human bladder epithelial cells *in vitro*. [56] This response is regulated by TLR4 and downstream ERK, p38, and NF-κB signaling.[15,71] IL-1β activation also induces a broader IL-1 dependent cascade, including IL-8, PGE2, substance P, and NK1R.[15,56]

Type 1 fimbriae are important virulence factors in the murine urinary tract, promoting *E. coli* adherence to bladder epithelium.[72,73] The FimH adhesin binds mannosylated host glycoconjugates and may facilitate mucosal cell invasion.[74] Innate immune responses to bladder infection depend on TLR signaling, and Tlr4 deficient mice show protection.[11,73] Type 1 fimbriated strains preferentially activate

MyD88/TIRAP mediated NF-κB pathways.[72,75] While type 1 fimbriae elicit pro-inflammatory effects in mice, human studies using isogenic strains have not detected direct innate immune activation, unlike P fimbriae, which trigger IRF7-dependent responses.[23] Type 1 fimbriae have also been shown to disrupt basic cellular functions, including RNA translation, and may influence neurosensory and solute carrier pathways in the host (Figure 3).[23]

Pain is a hallmark of acute cystitis. Acute cystitis strain directly activate nerve cells both in vitro and within the bladder.[15] Pain signaling involves NK1R and substance P, which are induced in nerve cells and bladder mucosa, and this response is amplified by IL-1β in vivo but blocked by IL-1R antagonists.[15]

These studies suggest that NK1R and SP influence the severity of acute cystitis through a neuroepithelial activation loop that controls pain and mucosal inflammation. IL-1 receptor antagonists and NK1R inhibitors effectively reduced infection and induced pain in mouse models of acute cystitis, markedly diminishing pain-related behaviors alongside inflammation and tissue pathology.[56] Clinical relevance was supported by off-label use of an IL-1RA in patients with bladder pain, which produced rapid pain relief and improved quality of life.[15,56] IL-1RA therapy shows promising clinical potential for treating bladder pain syndrome, but controlled trials are needed to confirm its effectiveness.[3] It may also be useful for addressing IL-1β related symptoms and pathology in acute cystitis, though no clinical data currently exist.

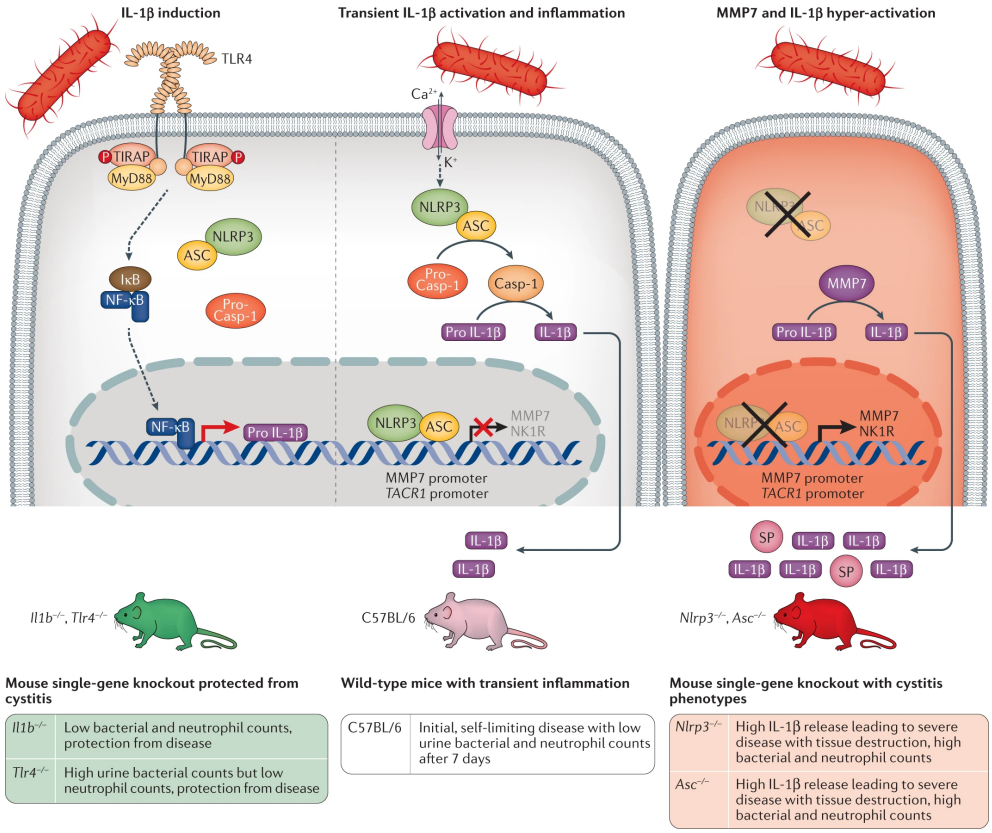


Figure 3. Host determinants of disease severity in acute cystitis; mechanism of IL-1β and SP hyperactivation, This image is reproduced from Ambite, I *et al*. *Nat Rev Urol*. 2021(DOI: 10.1038/s41585-021-00477-x ,reference (2).

Bladder cancer

Background

Bladder cancer is a common malignancy, with higher incidence rates in men than women, and is most frequently diagnosed in individuals over 65 years of age.[76] It is the most prevalent malignancy of the urinary tract, with urothelial carcinoma accounting for approximately 90% of cases. Risk factors include tobacco smoking (the most significant contributor), occupational exposure to carcinogens (e.g., aromatic amines), chronic urinary tract infections, and genetic predisposition.[77] Geographical variation exists, with the highest incidence in Europe, North America, and parts of Asia, while schistosomiasis-related squamous cell carcinoma is

common in endemic regions such as Egypt and sub-Saharan Africa.[77,78] Five-year survival rates depend on disease stage, ranging from over 90% for non-muscle-invasive bladder cancer (NMIBC) to less than 15% for metastatic disease.[78]

Immune response in bladder cancer

Bladder tumors utilize a spectrum of immune evasion mechanisms that collectively attenuate both adaptive and innate anti-tumor immunity. A hallmark feature is the downregulation or complete loss of MHC class I expression, resulting in impaired antigen presentation and diminished recognition by cytotoxic $CD8^+$T lymphocytes.[79] Concurrently, tumor cells frequently upregulate inhibitory immune checkpoint molecules, most prominently PD-L1, but also other co-inhibitory ligands thereby engaging PD-1 and related pathways to induce T-cell exhaustion and functional anergy.[80] In addition, the secretion of immunosuppressive cytokines such as IL-10, TGF-β, and VEGF contributes to a tolerogenic tumor microenvironment by suppressing dendritic cell maturation and effector T-cell activity.[81] Tumor and stromal elements may further evade immune recognition through the expression of non-classical HLA molecules (e.g., HLA-E, HLA-G) and other inhibitory ligands that impede natural killer (NK) cell mediated cytotoxicity.[82] Collectively, these mechanisms facilitate immune escape and promote tumor persistence and progression.

Furthermore, the bladder tumor microenvironment is characterized by a complex network of immunoregulatory myeloid and lymphoid populations that collectively sustain immune suppression and tumor progression. Tumor associated macrophages often skewed toward an M2-like phenotype, secrete IL-10 and TGF-β, promoting angiogenesis and suppressing cytotoxic responses.[81] Myeloid-derived suppressor cells inhibit T-cell proliferation via arginase-1 activity, production of reactive oxygen species (ROS), and nitric oxide (NO), while regulatory T cells release IL-10 and TGF-β to suppress effector T-cell and dendritic cell function.[82] The collective effect of these immunosuppressive populations contributes to T-cell exhaustion, defective antigen presentation, and resistance to immune-based therapies.[81]

Aa high density of activated $CD8^+$ T-cell infiltration and a Th1-skewed cytokine milieu marked by increased production of IFN-γ, IL-2, and TNF-α are associated with improved prognosis and enhanced responsiveness to checkpoint blockade.[81] Functional NK cells and dendritic cell are also key effectors in anti-tumor immunity, particularly in tumors with reduced MHC class I expression; however, both cell types are frequently diminished or functionally impaired in advanced bladder cancer.[79,82]

Bacillus Calmette-Guerin therapy for bladder cancer

Bacillus Calmette-Guérin (BCG) therapy was introduced as an intravesical treatment for bladder cancer in the 1970s.[83] The idea stemmed from the immunostimulatory properties of BCG, a live attenuated strain of *Mycobacterium bovis*, traditionally used as a tuberculosis vaccine. Morales et al (1976) pioneered its use in NMIBC to prevent recurrence and progression after transurethral resection of bladder tumors (TURBT).[84] The mechanism of action was hypothesized to involve immune activation, with BCG inducing a localized immune response in the bladder, leading to tumor destruction.[85]

BCG remains the gold standard treatment for high-risk NMIBC, including carcinoma in situ (CIS), T1 tumors, and high-grade Ta lesions.[86] Its efficacy in reducing recurrence and progression has been consistently demonstrated in numerous clinical trials. Current protocols involve induction therapy followed by maintenance regimens to prolong efficacy.[87] BCG intravesical therapy involves six weekly instillations after tumor resection, followed by maintenance doses every 3–6 months for 1–3 years. BCG induces a complex immune response involving both innate and adaptive immune systems. Key steps include attachment of BCG to the urothelium and internalization by urothelial cells and antigen-presenting cells, follow by activation of immune responses, including recruitment of macrophages, neutrophils, and lymphocytes, cytokine release (e.g., IL-2, TNF-α, IFN-γ).[77,88]

Human Alpha-lactalbumin Made LEthal to Tumor cells (HAMLET)

HAMLET is a protein-lipid complex with potent anticancer properties. It is composed of human alpha-lactalbumin, a protein found in milk, and oleic acid, a fatty acid.[89] This complex selectively targets and kills cancer cells while sparing healthy cells. The tumoricidal activity of HAMLET was discovered unexpectedly during studies investigating the antimicrobial properties of human milk, specifically its influence on bacterial adherence.[90,91] The active component responsible for inducing cell death in transformed and lymphoid cells was subsequently identified as α-lactalbumin, isolated from the casein fraction of human milk via low-pH precipitation.[90]

The cytotoxic effect of HAMLET requires the presence of both partially unfolded α-lactalbumin and oleic acid.[92,93] Reconstitution of biologically active HAMLET like complexes has been achieved using α-lactalbumins derived from various species, as well as recombinant α-lactalbumin expressed in *E. coli*.[92]

Therapeutic effects and clinical studies of HAMLET

HAMLET exhibits broad tumoricidal activity across diverse malignant phenotypes, with efficacy demonstrated in vitro, in multiple rodent cancer models, and in early

clinical studies. Their antitumor properties have been independently validated in murine systems and extended into human therapeutic contexts.

In a rat intracranial xenograft model of human glioblastoma, the therapeutic activity of HAMLET was evaluated following stereotactic implantation of glioblastoma cells. [94] Continuous local infusion of HAMLET significantly delayed intracranial tumor progression and prolonged survival without affecting adjacent non-neoplastic brain tissue, underscoring the high degree of tumor selectivity.[94]

In the *Apc*$^{Min/+}$ mice colon cancer model, peroral HAMLET or BAMLET(the active complex made with bovine α-lactalbumin), administration reduced tumor progression and mortality[95], accumulating specifically in tumor tissue and reduced Wnt/β-catenin signaling and proliferation markers. Remarkably, supplementing the drinking water with HAMLET from the time of weaning, significantly prevented tumor development in *Apc*$^{Min/+}$ mice, which carry mutations relevant to hereditary and sporadic human colorectal tumors.

In the MB49 bladder cancer model, repeated intravesical instillations of HAMLET reduced tumor progression and tumor burden, and significantly delayed disease progression, compared to placebo.[96] Fluorescence imaging showed selective retention in tumor-bearing bladders, and repeated instillations markedly reduced tumor mass and TUNEL staining confirmed abundant apoptosis in tumor tissue, with no detectable effects in non-malignant bladder epithelium.[96]

In a randomized, double-blind, placebo-controlled study of patients with skin papillomas resistant to conventional therapy[97], topical HAMLET application, led to a 75% reduction in lesion volume compared to placebo, increased to 87% reduction with repeated treatement.[97] Repeated treatments resulted in rapid lesion clearance, indicating sustained therapeutic potential and excellent tolerability.[97]

In a clinical pilot study involving patients with superficial bladder cancer, patients received intravesical instillations of HAMLET prior to transurethral tumor resection. Treatment led to rapid shedding of tumor cells into the urine, and evidence of apoptosis in tumor biopsies.[98] In addition, cystoscopic evaluations demonstrated noticeable tumor shrinkage in several patients. Importantly, treatment was well tolerated, with no apparent damage to surrounding healthy tissue.[98]

Clinical studies of alpha1-oleate in bladder cancer patients

Alpha1-oleate, a second-generation derivative of HAMLET, is under clinical investigation for its potential as an anticancer agent. Alpha1-oleate is a synthetic peptide-based investigational new drug formed by the N-terminal α-helical-1 domain of human α-lactalbumin (residues 1–39) complexed with oleic acid. This molecular complex replicates the tumoricidal properties of HAMLET, exhibiting

selective cytotoxicity toward malignant cells while sparing healthy tissue, and holds promise as a novel anti-cancer agent.[91,94,99]

A single-center, randomized, double-blind, placebo-controlled Phase I/II trial was conducted in patients with muscle invasive bladder cancer. Participants received intravesical instillations of alpha1-oleate (1.7 mM) or placebo (PBS) on six occasions (days 1, 3, 5, 8, 15, and 22) prior to tumor removal by transurethral resection. [100] No drug-related toxicity was observed in patients treated with alpha1-oleate compared to placebo. The treatment demonstrated significant therapeutic effects, supporting its safety and potential efficacy for NMIBC management.[100]

Primary study endpoints were successfully achieved, including demonstration of safety, induction of tumor cell shedding, and a significant reduction in tumor size relative to the placebo group. Rapid tumor response was evidenced by the shedding of tumor cells and cell clusters into the urine following intravesical instillation of alpha1-oleate, whereas no such shedding was observed in the placebo cohort.

Secondary endpoints were reached, with apoptosis confirmed via positive TUNEL staining. Treated patients exhibited a robust and rapid apoptotic response in both shed tumor cells and tumor biopsies, in contrast to healthy tissue or placebo-treated samples.[100,101] Alpha1-oleate was detectable in urine-derived tumor cells and in tumor tissue obtained at transurethral resection of bladder tumor. Gene expression profiling of tumor biopsies revealed marked downregulation of cancer-associated genes and pathways, including those specifically implicated in bladder cancer pathogenesis.

Immunofluorescence analysis revealed that alpha1-oleate preferentially accumulated in tumor cells, with markedly higher uptake in shed cells and tumor tissue from patients treated with 8.5 mM compared to 1.7 mM or placebo.[102] This uptake correlated with a dose-dependent induction of apoptosis, as confirmed by TUNEL staining in both shed urine cells and tumor biopsies, with minimal effects on adjacent healthy tissue (Figure 4).[102] RNA sequencing further demonstrated that 8.5 mM alpha1-oleate strongly upregulated apoptosis-related genes while broadly suppressing cancer-associated pathways, including those involved in tumor progression, invasion, and the tumor microenvironment, as well as bladder cancer specific gene signatures, highlighting a comprehensive disruption of tumor cellular programs.[102]

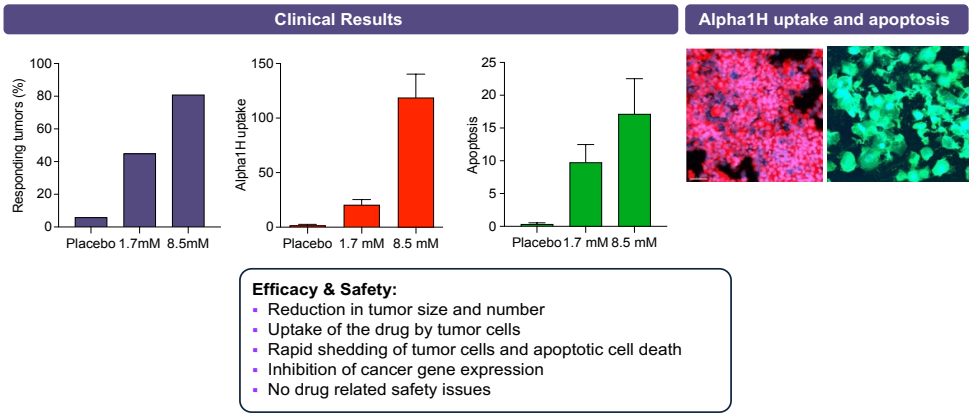


Figure 4 . Overview of clinical study outcomes.

Aims

Paper I AIM

To examine the immune response in infants with their first febrile UTI episode, using proteomic screening technology to analyze the immune response in the urinary tract and gene expression analysis to investigate the systemic response.

Paper II AIM

To analyze molecular determinants of susceptibility and disease severity in infants with their first episode of febrile UTI, using streamlined clinical protocols, DNA exome sequencing, comprehensive global gene expression profiling and proteomic analysis.

Paper III AIM

To investigate, if the IRF3 and IRF7 transcription factors regulate the MYC response to infection and the difference in disease susceptibility between *Irf3*$^{-/-}$ and *Irf7*$^{-/-}$ mice.

Paper IV AIM

To examine the local host response to intravesical alpha1-oleate treatment in patients with non-muscle invasive bladder cancer using urine proteomics and tissue transcriptomic analyses.

Methods

Clinical study protocols

Febrile UTI in infants, Papers I & II

Two prospective, analytical cohort studies were undertaken to investigate molecular determinants of susceptibility and pathogenic mechanisms in infants experiencing their first febrile urinary tract infection. Written informed consent was obtained from the parents or guardians prior to enrolment. Febrile UTI was confirmed by positive urine culture and standard laboratory testing. Patients with discordant urine culture results were excluded. Acute pyelonephritis was defined by a positive dimercaptosuccinic acid (DMSA) scan performed within 7 days of enrolment. Ultrasound imaging was conducted for all patients, and vesicoureteral reflux (VUR) was assessed using micturating cystourethrography (MCU). Renal scarring was determined by a follow-up DMSA scan conducted 6 months after the initial infection.

Cohort I comprised children older than one month, who were admitted to KK Women's and Children's Hospital, Singapore (n = 111). Ethical approval for Cohort I was obtained from SingHealth Centralized Institutional Review Board. Diagnosis and management followed a standardized clinical pathway. Mandatory baseline investigations included urinalysis, full blood count, CRP, and blood cultures. Inclusion criteria consisted of: (i) a positive urine culture defined as single-organism growth $>10^4$ CFU/ml from catheterized specimens or $>10^5$ CFU/ml from midstream clean-catch specimens; (ii) fever >38.5°C; (iii) pyuria; and (iv) CRP positivity. Of the urine samples, 88.7% were obtained via catheterization and 11.3% via midstream clean-catch from older children, with two samples collected for confirmation. Exclusion criteria included recurrent UTI, known renal or urological abnormalities, or newly identified structural abnormalities on admission ultrasound. Among 121 eligible children, eight declined participation and DNA could not be obtained from two, yielding a final cohort of 111 patients. Four children with significant comorbid pathology (renal abscess, pelviureteric junction obstruction with hydronephrosis, nephrocalcinosis, duplex kidneys) were excluded. Seven cases of recurrent UTI occurred: three at ≥6 months and four within 2 months of the initial episode. For the latter, the second DMSA scan was performed 4 months after the

recurrent infection. Blood samples were obtained within 24 hours of diagnosis for exome genotyping and whole-genome transcriptomic profiling (n = 111), while urine samples were collected for proteomic analysis. Additional RNA and urine samples were collected at 6-month follow-up.

Cohort II included children admitted with febrile UTI to 29 pediatric centers in Sweden (n = 52). The study was approved by "Regionala etikprövningsnämnden Lund" according to protocol DNR 2015/884. Complementary ethical applications were approved (DNR 2016/799, 2017/164 and 2017/315). Diagnostic and therapeutic management followed a standardized protocol. Routine investigations included urinalysis, full blood count, and CRP; blood cultures were obtained in severely ill children prior to initiation of intravenous antibiotics. Inclusion criteria were pyuria, fever >38.0°C, and a positive urine culture. A total of 86 infants (<1 year of age) with clinically suspected first febrile UTI were invited, of whom two were excluded due to significant pathology (hydronephrosis, meatal stenosis, or balanich hypospadias). Within 24 hours of diagnosis, blood samples were collected for exome genotyping (n = 39) and whole-genome transcriptomic profiling (n = 27). Additional urine and RNA samples were collected at 6-month follow-up. Detailed analysis of the immune response in infants diagnosed with a first episode of febrile UTI was conducted at three of the centers according to Swedish guidelines. Urine samples and RNA samples were collected within 24 hours of diagnosis and at the scheduled follow up visit after six months. Infants with one or more focal uptake defects (DMSA+) were diagnosed as acute pyelonephritis, the others as febrile UTI without renal involvement (DMSA-). In patients with a 1st positive DMSA scan, a follow-up DMSA scan was performed after at least 6 months to detect renal scarring.

Bladder cancer treatment with alpha1-oleate, Paper IV

This single-center study (HP002-001) included patients, who were diagnosed with NMIBC and scheduled for transurethral surgery. The study was approved by the State Institute for Drug Control (SUKL) in the Czech Republic; number 273799/17-I and the Ethics Committee of the Motol University Hospital; number EK-786/17 (ClinicalTrials.gov Identifier: NCT03560479). Patients gave their written informed consent. Demographic data, morbidity and health parameters as well as tumor characteristics were recorded by the study physicians in the electronic Case Report Form (eCRF) and closely monitored by an external monitor.

The first, part of the single center (EudraCTNo:2016-004269-14, ClinicalTrials.gov NCT03560479) was placebo-controlled, enrolling 20 patients in the active arm and 20 patients in the placebo arm. Subjects were randomized 1/1 and received intra-vesical instillations (30 mL) of either alpha1–oleate (1.7 mM) or PBS on six occasions during a 22-day period (days 1, 3, 5, 8, 15, and 22). The inclusion criteria were:

A) Patient with non-muscle invasive papillary bladder cancer based on cystoscopy appearance, on the waiting list for transurethral resection of bladder tumor.

B) Male and female subjects, 18 years or older.

C) Patients should be able to keep the content of the bladder for at least one hour.

D) Signed and dated informed consent form.

E) Negative pregnancy test in women of childbearing potential.

F) Men and women of childbearing potential should use appropriate methods of contraception during the study. Men should also refrain from donating sperm.

The second, dose-finding part of the study, used the same eligibility criteria, schedule of visits, study treatment administration, primary and secondary end points and assessments as the first part of the study. Six patients received intra-vesical instillations of alpha1-oleate at a five times higher dose (8.5 mM) and three patients a 10-times higher dose (17 mM) than patients in the first part of the study, receiving 1.7 mM (n = 20) or placebo (n = 20). The six patients receiving 8.5 mM of alpha1-oleate completed the treatment and samples from these patients were included in this study (Figure 5).

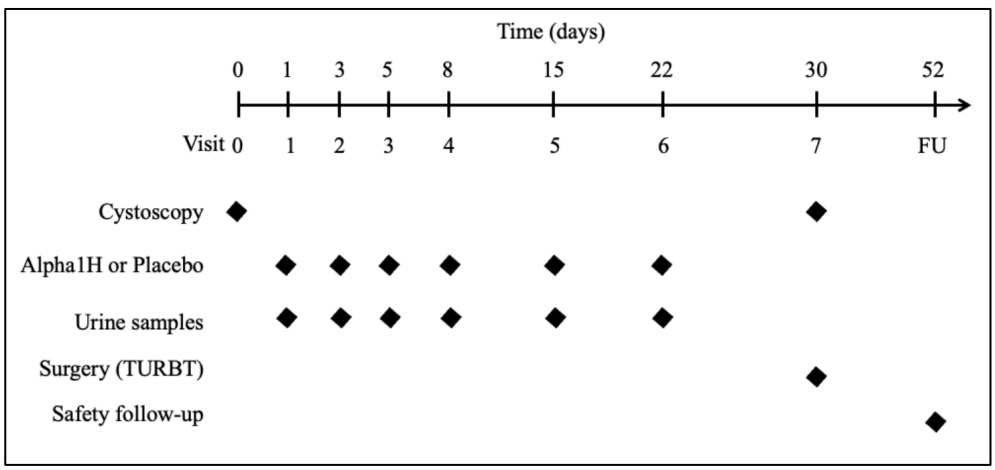


Figure 5. Schematic overview of study protocol in bladder cancer patients treated with alpha1-oleate

Proteomic analysis of urine samples by Luminex and ELISA assays

The Luminex micro-bead assay employs conjugated microsphere particles to immobilize specific antigens on their surface. Using dual fluorescence signals, the instrument determines the analyte attached to the bead and calculates its corresponding concentration based on the detection dye's intensity. As a result, the Luminex system enables the simultaneous detection and quantification of multiple biomarkers.

After thawing on ice, the urine samples were gently mixed and used undiluted. Reverse pipetting was used for high accuracy in all liquid handling steps. High and low controls, all standards and buffer controls were analyzed in duplicate. The assays were performed according to the manufacturer's instructions. Standard, low and high control were reconstituted with diluent RD6-65 sit for 15 min follow by 5 min of gentle agitation. Standard 1 was diluted 3-fold, using diluent RD6-65. Briefly, fifty microliters of different standard dilutions (7-highest to lowest), controls (low and high), or sample were added to each well, and the 50 µL diluted Microparticle Cocktail (0.5 mL Microparticle Cocktail + 5 mL Microparticle diluent) was added with subsequent foil plate sealing and a 2-hour incubation at room temperature on a microplate shaker. Washing involved applying a magnetic device, filling wells with Wash Buffer (100 µL) and repeating this process three times. 50 µL of diluted Biotin-Antibody Cocktail (0.5 mL Biotin-Antibody Cocktail + 5 mL Biotin-Antibody diluent 2) was added, followed by a 1-hour incubation, and a repeat of the wash steps. Subsequently, 50 µL of diluted Streptavidin-PE (220 µL Streptavidin-PE – concentrated + 5.35 mL wash buffer) was added, and after a 30-minute incubation, a final round of washing was performed. Microparticles were resuspended with Wash Buffer (100 µL per well) and incubated for 2 minutes on the shaker. Reading was carried out within 90 minutes using a Luminex or Bio-Rad analyzer, emphasizing immediate microparticle resuspension before reading by shaking the plate for 2 minutes at 800 ± 50 rpm. All assays were validated by the manufacturer for sensitivity, intra-assay precision, and assay linearity. Assays were tested for less than 0.5% cross-reactivity and interference.

In the febrile UTI study, urine samples from Cohort I were thawed, spun down, and supernatants were analyzed using Luminex multiplexed, bead- based kits: Human Cytokine Panel 1 and 2, Human Soluble Cytokine Receptor Panel, Kidney Injury Panel 3 and 4 (Merck Millipore), for a total of 84 measured proteins. Kits were processed as per manufacturer’s protocols and read on the Flexmap 3D system (Luminex). Urine samples from Cohort II were analyzed with the Luminex-based assay for 24 cytokines and chemokines using the Human Immunotherapy Luminex Performance Assay 24-plex Fixed Panel (R&D Systems, Cat #: HCYTMAG-60K-PX41, Lot #: 3090739).

The concentration of IL-17D was quantified using the Human IL-17D ELISA kit (Cat #: EKX-B2Y41N-96, Nordic BioSite), following manufacturer's instructions. The concentration of hCFH was quantified using the Human Complement Factor H ELISA kit (ThermoFisher Scientific, Cat #: EH122RB-96), following manufacturer's instructions. Undiluted urine samples were used and the concentrations of IL-17D and hCFH were determined using standard curves.

RNA transcriptomics analysis

Whole-genome transcriptomic analysis of tissue biopsies from patients with bladder cancer

RNA was extracted from tumor tissue biopsies stabilized in RNAlater, using the AllPrep DNA/RNA/miRNA Universal Kit. Disruption was performed with the TissueLyser system and CK28 Precellys tubes followed by homogenization with QIAshredder columns. The quantity and quality of RNA samples were evaluated using NanoDrop. RNA samples were prepared by Illumina TruSeq Stranded mRNA Library Prep Kit, and libraries were multiplexed and sequenced using NextSeq 500/550 High Output Kits (v2.5 2x75 Cycles) with an average of 22 million reads per sample. Raw sequencing data was demultiplexed using bcl2fastq (version 2.18) and RSEM (1.3) was used for abundance estimation using the human genome release 37/Ensemble 75. Samples were thoroughly quality checked and visualized using dimensionality reduction (i.e Principal component Analysis, PCA), MA-plots as well as RNA-seq intrinsic biases (such as GC bias, transcriptome complexity and alignment quality).

Differential gene expression analysis was performed using R (version 3.4) and the packages Limma and DESeq2. Fold changes were calculated by comparing tumors in the treated patients to the placebo group. Differentially expressed genes were functionally characterized using the Ingenuity Pathway Analysis version 57662101 (IPA, Qiagen) software.

Whole-genome transcriptomics analysis of Blood RNA from patients with febrile UTI

RNA was stabilized and purified from peripheral blood using Tempus blood RNA tubes and purification kit (Applied Biosystems), collected at the time of diagnosis and 6-mo post-infection. RNA was subjected to expression microarray analysis: 100 ng of total RNA was amplified using the Affymetrix WT PLUS Reagent Kit and hy- bridized using the GeneTitan system onto GeneChip Human Gene 2.1 ST Arrays with probe sets measuring the expression of 72,688 transcripts, including a

large number of non-coding and hypo- thetical transcripts. RNA samples collected during follow-up visits post-infection from the same study cohort were used as controls.

Transcriptomic data were normalized using the Robust Multi- Chip Analysis algorithm implemented in the Transcriptome Analysis Console software (TAC v.4.0.1.36, Applied Biosystems; Thermo Fisher Scientific). The TAC software calls the limma differential expression portion of the Bioconductor package to provide fold change. Fold change was calculated by comparing each group to RNA obtained 6 months after enrolment from patients with febrile UTI without renal involvement (acute DMSA−). Relative expression was analyzed by ANOVA using the empirical Bayes (eBayes) method, and Benjamini- Hochberg step-Up FDR-controlling procedure at alpha 0.05 to correct for multiple comparisons (Benjamini & Hochberg, 1995). Genes with a P-value < 0.05, an FDR adjusted P-value < 0.05 and an absolute fold change > 1.5 were considered differentially expressed. Heatmaps were constructed using the Gitools 2.1.1 software. Differentially expressed genes and regulated pathways were analyzed using Ingenuity Pathway Analysis software (QIAGEN), using right- tailed Fisher's Exact test followed by Benjamini-Hochberg correction for multiple testing.

Whole-genome transcriptomics analysis of mouse kidneys

Total RNA was extracted from mouse kidney tissue using the RNeasy Mini Kit (Qiagen) and amplified with the GeneChip 3′IVT Express Kit (Affymetrix). Amplified RNA was hybridized onto either the Clariom S Mouse HT or Mouse Genome 430 PM arrays (Affymetrix) for 16 hours at 45 °C. Following hybridization, samples were washed and stained using the Affymetrix Fluidics Station 450 in accordance with the manufacturer's protocol (Hybridization, Washing, and Staining Kit, Affymetrix). Microarrays were scanned using the GeneTitan or GeneAtlas system (Affymetrix).

Raw data were normalized using the Robust Multi-array Average (RMA) method implemented in the Transcriptome Analysis Console software (version 4.0.1.36, Applied Biosystems). Differentially expressed genes were identified and ranked based on relative expression using a two-way ANOVA model with the empirical Bayes method, applying a threshold of absolute fold change ≥ 2.0 and a significance level of $P < 0.05$. Heatmaps were generated using GraphPad Prism (version 10), and functional enrichment analysis of differentially expressed genes was performed using Ingenuity Pathway Analysis (IPA, Qiagen).

Experimental urinary tract infection

Study protocol, Paper III

Ethical approval for animal experiments was obtained from the Malmö/Lund Animal Experimental Ethics Committee at the Lund District Court, Sweden (#M119-16 and 6551-2021). Animal care and experimental protocols followed institutional, national, and European Union guidelines and were governed by the European Parliament and Council Directive (2016/63, EU), the Swedish Animal Welfare Act (Djurskyddslagen 1988:534), the Swedish Welfare Ordinance (Djurskydssförordningen 1988:539) and Institutional Animal Care and Use Committee (IACUC) Guidelines. Results were reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

For infection, mice were anesthetized via intraperitoneal injection of a ketamine (1.48 mg in 100 µL of 0.9% NaCl; Intervet) and xylazine (0.22 mg in 100 µL of 0.9% NaCl; Vetmedic) cocktail. They were then intravesically inoculated with *E. coli* CFT073 (10^8 CFU in 0.05 mL; 2 × 10^9 CFU/mL, 50 µL) using a soft polyethylene catheter. Mice were euthanized by administering an overdose of isoflurane anesthesia (Dechra, Cat# 200-129).

Bacteria were grown on TSA plates (37 °C, 16 h), harvested in PBS (pH 7.2), and diluted as required. Urine was collected before infection and at 24 h, 3 d, 5 d, and 7 d for bacterial titration (TSA plating) and neutrophil counts (hemocytometer). Mice were euthanized at 24 h or 7 d by isoflurane overdose, and kidneys were collected for RNA extraction or OCT embedding.

Treatment with innate immune inhibitors

Liposomal Irf7 siRNA

Irf3$^{-/-}$ mice received siRNA treatment at a dose of 5 mg/kg. A total volume of 300 µL of Silencer Select Pre-designed Irf7 siRNA (Life Technologies, 4404010 #s79411) was administered 200 µL via tail vein injection and 100 µL via intravesical instillation using the Invivofectamine reagent (Life Technologies, 1377501). To achieve preventive knockdown of Irf7 siRNA was administered three days before and on the day of *E. coli* CFT073 infection. For therapeutic purposes, Irf7 siRNA was administered 24 hours and three days post-infection. Ambion In Vivo Negative Control siRNA (Life Technologies, 4457287) was used as the negative control.

The rLon MYC inhibitor

The Lon protease was recombinantly expressed in *E. coli* BL21 and purified using a Strep-Tactin affinity column. The purified protein was eluted, dialyzed into Tris/NaCl/glycerol buffer, and either used immediately or stored at –80°C. Recombinant bacterial Lon protease (rLon, 100 µL, 250 µg mL-1) was administered intraperitoneally (i.p.) to $Irf3^{-/-}$ mice once a day for eight days starting one day before intravesical infection with *E. coli* CFT073. Placebo-treated mice received 100 µL of PBS i.p. with the same schedule as rLon-treated mice. The mice were sacrificed after seven days by using an overdose of isoflurane anesthesia.

Statistics

Clinical study- febrile UTI

P-values < 0.05 were considered significant. For proteomics, as all data was not following a Gaussian distribution (D'Agostino & Pearson normality test) non-parametric analysis tools were used. Pair-wise analysis of urine proteomic data was performed using Wilcoxon signed-rank test, comparing protein concentrations at the time of acute UTI to follow-up. For group-wise analysis, Mann-Whitney U-test was used to compare protein concentrations in urine samples. For fold change calculations of proteomics data, $\log_{10}$ concentration values were compared between acute and follow-up samples, followed by statistical analysis using Student t-test. Gene expression were tested using using right-tailed Fisher's Exact Test followed by Benjamini-Hochberg correction for multiple testing. Statistical significance was determined using Microsoft Excel or GraphPad Prism (v.10.0.2).

Clinical study-bladder cancer

As all data was not following a Gaussian distribution (D'Agostino & Pearson normality test) non-parametric analysis tools were used. The Mann-Whitney U-test, which is commonly used in studies of responses to BCG in urine[103,104] was selected to compare the pre-V1 samples to the post-inoculation samples in different groups and to compare the treatment groups to the placebo group. The data was further analyzed using the One Way Anova, to compare the distribution of the parameters between the three patient groups. Statistical significance was determined using GraphPad (Prism v.10.0.2) and significance was assigned at * $P< 0.05$, ** $P< 0.01$ and *** $P< 0.001$. For transcriptomic analysis, relative expression levels were analyzed and genes with an absolute fold change > 1.5 and $P < 0.05$ were considered as differentially expressed. Logistic regression analysis was performed using the glm2 function of the statistics package implemented in R 4.3.1. Odds ratios were calculated from the logistic regression coefficients.

Results

Paper I. Analysis of the Innate Immune Response to Febrile UTI in Infants: Evidence of an Acute Cytokine Storm

Background

The innate immune system plays a dual role as defense against disease or cause of pathology. Rapid innate immune responses maintain the first line of defense against infections and when efficient, innate immunity stops pathogen attack and restores health. Understanding the molecular basis of innate immune dysregulation is therefore essential, not least to discover new approaches for targeting and correcting these processes therapeutically. Acute pyelonephritis is a potentially life-threatening infection of the kidneys, associated with urosepsis and mortality in adults. Febrile urinary tract infection is also one of the most common bacterial infections in infancy and childhood and a significant concern, both diagnostically and therapeutically.

Aims

To examine the immune response in infants with their first febrile UTI episode, using proteomic screening technology to analyze the immune response in the urinary tract and gene expression analysis to investigate the systemic response.

Results

Proteomic analysis of the host response to febrile UTI

Urine samples collected at enrolment and at six-month follow-up (29 patients, 58 samples) were analyzed using a Luminex panel. The concentration of each cytokine was quantified relative to a standard curve (pg/mL of urine). Acute samples were compared to samples obtained at the six month follow up visit.

Significant differences were detected for most of the cytokines analyzed (22/24 cytokines). Group-wise analysis detected markedly increased urine concentrations

of the IL-1 family of cytokines (IL-1β, IL-1RA, IL-1α and IL-33). Chemokines and pro-inflammatory cytokines were affected (IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, GM-CSF, IL-6, and TNF-α) as well as IFN-γ. The top regulated cytokines were further identified by calculating the fold change (FC) compared to the follow up samples, with values for IL-1β (FC = 99), IL-8 (FC = 55), IP-10 (FC = 30) and IL-6 (FC = 13) identified as the most strongly activated cytokines during acute febrile infection in this patient group. In addition, mediators of adaptive immunity were activated (IL-17, CD40L, IL-2, Granzyme B, IL-10, IL-15, PD-L1, IL-13, and IL-12). In contrast, no significant difference was observed for IFN-α2 or IL-4.

Confirmation of the acute response by pair-wise analysis

The potent cytokine response to acute infection was confirmed by pair-wise analysis, of the acute (n=55) versus the follow-up samples (n=29). The majority of cytokines (20/24 cytokines) showed significantly elevated levels at enrolment. A significant difference in cytokine concentration was detected for the majority of cytokines including IL-1β, IL-1RA, IL-1α, IL-33,IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, GM-CSF,IL-6, TNF-α, IFN-γ, IL-17, CD40L, Granzyme B, IL-2, IL-10, IL-15, and PD-L1. In contrast, there was no significant difference in the concentration of IFN-α2, IL-12, IL-13 or IL-4 between acute and follow up samples. The results confirm the potent and broad innate immune response to febrile urinary tract infection, identified by group-wise analysis.

Cytokine storm response

The proteomic profile in the acute urine samples was functionally characterized by Ingenuity Pathway Analysis (IPA). Functional analysis identified a systemic cytokine storm in the febrile UTI patient group. The majority of regulated genes were activated ($n = 46/55$, FC 1.5, $P < 0.05$). In addition to cytokine storm signaling, activated canonical pathways identified by IPA, also included neutrophil degranulation, as well as the neuroinflammation, IL-8 and IFN-ɣ signaling pathways. In contrast, the expression of genes involved in adaptive immunity was mainly inhibited. The CTLA4 T cell inhibitor was activated and NF-κB, IL-2 and was inhibited. Key regulators of the systemic response were identified by IPA as TNF, IFNG, IL1B, IL6, IL1B, CSF2, IL17A, IL4, IL33, and IFNA2 (Figure 6).

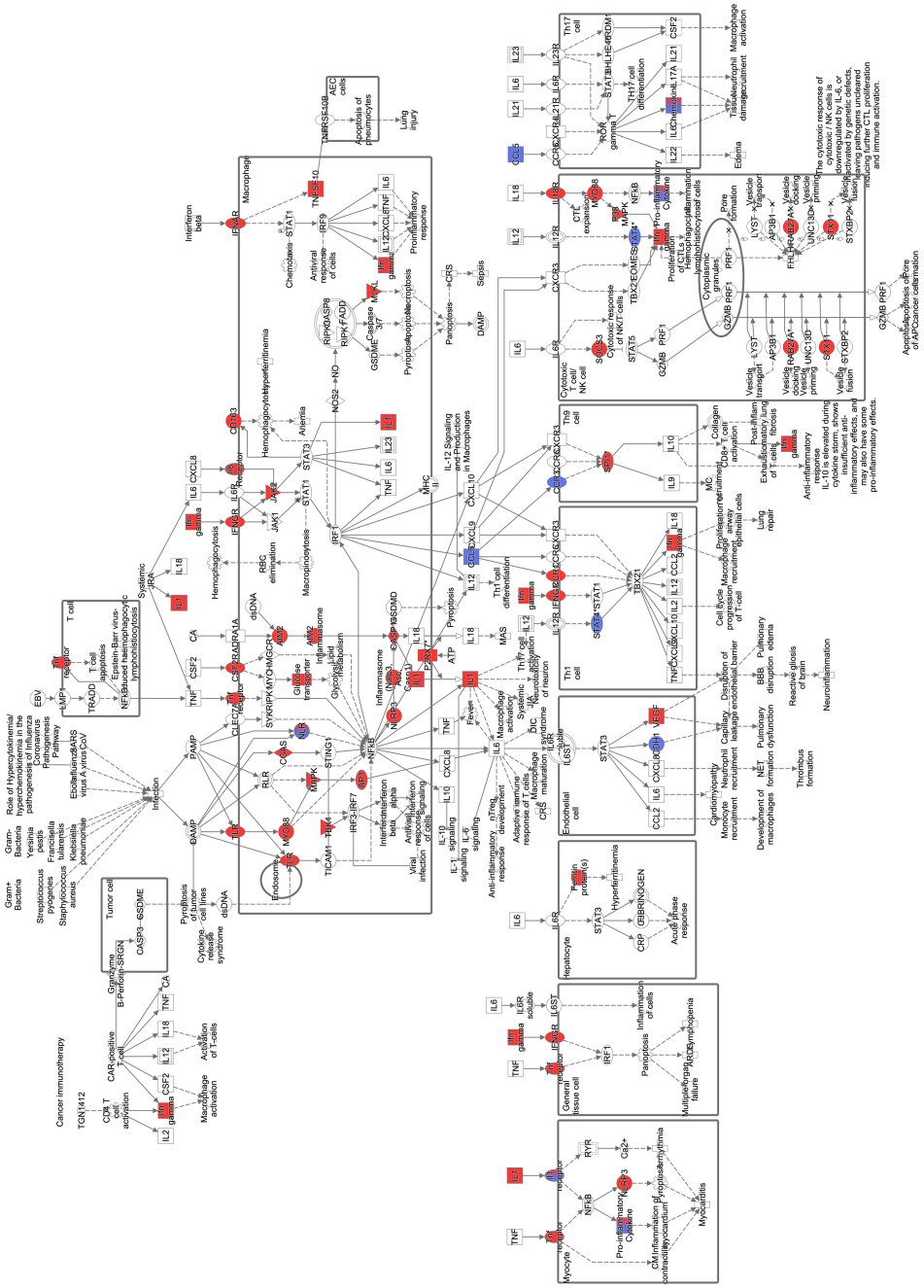


Figure 6: Cytokine storm signaling pathway genes in peripheral blood RNA, quantified by gene expression profiling of acute versus follow-up samples. Significant activation of innate immunity. Gene expression, peripheral blood RNA, acute vs follow-up sample.

Cytokine storm in febrile UTI compared to COVID-19

The term cytokine storm has been coined to define excessive, mainly innate immune responses to infection, where multiple response pathways are activated in parallel. Elevated levels of urinary cytokines and chemokines such as IL-1β, IL-8, IL-6, IL-17A, IFN-γ, MCP-1, GM-CSF, CD40L, Granzyme B, IL-2, TNF-α, IL-1α, MIP-1β, IL-15, IL-10, MIP-1α, IL-12, PD-L1, IL-33 and IL-13, detected in this study, have also been reported in patients with severe or critical SARS-CoV-2 infection compared to patients without COVID-19 or with mild or moderate infections. Additionally, patients with severe or critical COVID-19 showed elevated urinary levels of IL-7, IL-16, IL-18, G-CSF, LIF, CCL-11, CXCL-10, FGFb, M-CSF, VEGF and CTAcK, which were not measured in this study (Figure 7).

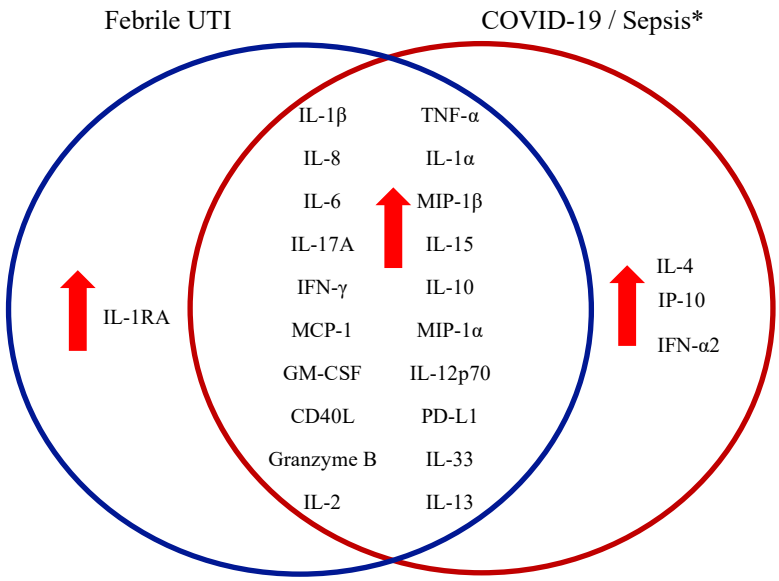


Figure 7: Cytokine storm proteins regulated in patients with febrile UTI compared to COVID-19 or bacterial sepsis*. Venn diagram visualizing the overlap in cytokine storm profiles between febrile UTI patients in this study and COVID-19 or bacterial sepsis, reported in the literature.

Local proteomic response and renal involvement

The cytokine levels in the DMSA+ patients were consistently higher than in the DMSA- group, and IL-1β, IL-8, MCP-1 and IL-6 were identified as the most strongly regulated. A direct comparison further identified IL-1α, IL-6, IL-10, IL-1β, GM-CSF, MIP-1β, IL-8 and MCP-1 as more strongly activated in the 1st DMSA+ than the 1st DMSA- patient group. The results suggest that individual cytokines were more strongly activated in the 1st DMSA+ than in the 1st DMSA- group, but

the results do not identify single cytokines as specific for febrile UTI with acute renal involvement (Figure 8).

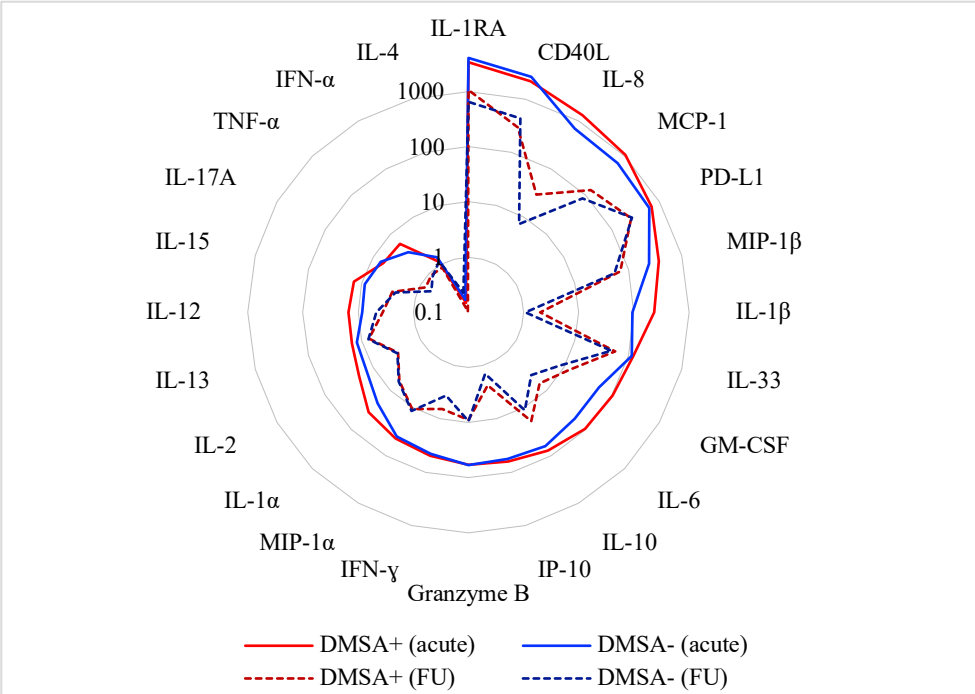


Figure 8: Comparison of the immune response in febrile UTI patients with or without renal involvement. Radar chart comparing mean cytokine levels in patients with or without renal involvement (Red line: DMSA+, Blue line: DMSA-, Red open line: DMSA+ follow up, Blue open line: DMSA- follow up).

Conclusions

Excessive local and systemic innate immune activation, resembling that induced during SARS-CoV-2 infection, characterizes acute pyelonephritis; a common and severe bacterial infection in childhood and a significant cause of urosepsis and mortality in adults. The results define a transient cytokine storm response as characteristic of acute pyelonephritis, rather than individual protein biomarkers. Targeting the excessive innate immune therapeutically is of interest, to inhibit symptoms and pathology.

Paper II. Molecular analysis of acute pyelonephritis - excessive innate and attenuated adaptive immunity

Background

The susceptibility to infection is fundamentally determined by the effectiveness of an individual's antimicrobial host defense and, on a broader scale, by population-level factors such as herd immunity. Rare, primary immunodeficiencies can significantly increase an individual's risk of acquiring specific viral, bacterial, or parasitic infections,[105] while insufficient herd immunity contributes to the spread and persistence of emerging pathogens.[106] Susceptibility to common bacterial infections, including pneumonia, diarrhea, and urinary tract infections, varies considerably within the general population.[107] Although differences in host immune control have been documented, the precise molecular mechanisms and gene targets underlying these variations remain largely undefined.

Aims

To analyze molecular determinants of susceptibility and disease severity in infants with their first episode of febrile urinary tract infection, using streamlined clinical protocols, DNA exome sequencing, comprehensive global gene expression profiling and proteomic analysis.

Results

Study outcome

Two prospective analytical cohort studies were carried out in Singapore and Sweden to investigate molecular susceptibility factors and disease mechanisms in febrile urinary tract infections among infants and young children from diverse genetic backgrounds. Enrolment was limited to each patient's first febrile UTI episode to capture the primary infection response, with diagnosis confirmed by positive urine cultures, fever above 38.5°C, pyuria, and elevated CRP levels. The gender distribution matched previous uncomplicated febrile UTI studies, with most infections caused by *E. coli*, whose genome sequencing revealed a highly conserved virulent genotype including P fimbriae, fim gene cluster, and hemolysin. No correlation was found between bacterial genotype and imaging outcomes.

Acute pyelonephritis was diagnosed in a significant number of patients based on early DMSA scans, with renal scarring observed in follow-up scans, some showing functional impairment and bilateral scars. Vesicoureteral reflux was detected in 17.7% of patients assessed, with renal scarring more frequent in those with high-

grade reflux, though scarring also occurred in patients without reflux. Recurrent UTIs were noted in seven cases, occurring both within two months and after six months of the initial episode, with second DMSA scans performed accordingly.

Disease severity defined by gene expression analysis differences between febrile UTI and APN

In both study cohorts, gene expression was strongly activated during the acute phase of infection. PCA analysis showed that the acute response was clearly separated from follow-up, confirming a distinct disease-related response profile. Comparison between the acute pyelonephritis group (first DMSA+) and the febrile UTI group (first DMSA−) revealed significant overlaps in top canonical pathways, including neutrophil degranulation, pathogen-induced cytokine storm, and neuro-inflammation.

Further analyses showed that gene expression changes were significantly more pronounced in the APN group than in the febrile UTI group, with stronger activation of innate immune pathways such as neutrophil recruitment, TLR and interleukin signaling, and inflammasome pathways.

In contrast, adaptive immunity was inhibited in the APN group including NF-κB signaling, TCR signaling, IL-2 expression, and pathways critical for B, T, and NK-cell development, suggesting an imbalance favoring innate hyperactivation alongside adaptive immune suppression.

Evidence of a local cytokine storm in the urinary tract

In Cohort I, the urinary tract's local response to infection was further investigated by proteomic analysis of 84 cytokines, cytokine receptors, and markers of kidney injury and urine samples collected during the acute infection were compared to follow-up samples. A significantly increase in urine levels was detected for 43 proteins during acute disease, indicating a strong local immune reaction to the infection.

Differentially expressed genes were functionally characterized using the Ingenuity Pathway Analysis software (IPA). This local cytokine response was characterized as a cytokine storm, consistent with the systemic gene expression findings using IPA. Highly upregulated chemokines such as CXCL-8, which promotes neutrophil recruitment and activation, along with central innate immunity mediators like IL-6, IL-1, and downstream effectors including IL-1β, MCP-1, and IP-10 (which is induced by IFN-γ).

These results demonstrate that an excessive innate immune response, typical of a cytokine storm, also occurs locally within the urinary tract during infection. This profile, consistent across cohorts, indicates that febrile UTI, especially with renal involvement, is characterized by an exaggerated innate immune response resembling a cytokine storm and an attenuated adaptive immune function.

Neutrophil accumulation and CD177 staining in infected kidneys

Notably, CD177, a neutrophil surface lipoprotein, and MCEMP1, linked to mast cell maturation, were among the most differentially expressed genes in both cohorts, highlighting robust innate immune activation.

A murine model of acute pyelonephritis was used to examine whether CD177 expression and neutrophil degranulation are altered in infected kidneys. Female *Irf3*$^{-/-}$ mice, which are highly susceptible to APN, were infected intra-vesically with the uropathogenic *E. coli* strain CFT073 and euthanized at 24 hours or seven days post-infection. Neutrophil counts in urine rose quickly and stayed elevated through day seven, with significant neutrophil accumulation observed along the renal pelvis, where bacterial clusters were also detected, leading to micro-abscess formation.

Gene expression analysis showed that the neutrophil degranulation pathway was the most upregulated in infected kidneys seven days after infection, and Cd177 expression was markedly increased. Immunohistochemistry confirmed substantial CD177 protein accumulation in kidney tissues at both 24 hours and seven days post-infection compared to uninfected controls.

Renal toxicity defined by gene expression analysis

APN is a major cause of kidney damage and scarring, and analysis of acute patient samples revealed 215 renal damage-related genes highly activated during infection, mainly linked to neutrophil recruitment, lymphocyte activation, and NK-cell toxicity more so in APN than in febrile UTI without renal involvement. However, analysis of renal toxicity genes did not uncover a distinct pattern among patients who later developed scarring, indicating that these genes, while active during acute infection, are not directly linked to scarring risk. There was also no sign of a persistent systemic hyper-inflammatory response in the scarring group, with only one gene showing differential expression. Locally, urinary cytokine levels at follow-up showed minimal change, although osteopontin levels were slightly higher at baseline in patients who eventually developed scarring compared to those who did not.

DNA-based association studies for APN and renal scarring

To investigate genetic variants linked to APN and renal scarring, patient DNA was analyzed through exome genotyping. Allele frequencies were calculated, by comparing patients with and without renal involvement (first DMSA+ vs. first DMSA−), odds risk ratios (ORRs) resulting in the identification of 714 genes in Cohort I and 824 genes in Cohort II that were significantly associated with acute renal involvement. Patients with positive DMSA scans were accurately distinguished by Eucledian ranking and tSNE clustering in both cohorts with over 98% accuracy.

Compared to a normal population data base database 67 polymorphic genes were consistently linked to APN in both cohorts. These genes were predicted to influence CREB signaling, solute transport, neutrophil migration, kidney injury, and the BACH2 transcription factor, which connects innate immunity with B-cell and regulatory T-cell functions. The gene set associated with acute pyelonephritis susceptibility did not overlap with renal scarring associated genes.

Overall, these findings provide the first clear evidence of distinct genetic patterns linked to both acute renal involvement and subsequent scarring in patients with febrile UTI, highlighting specific host genetic factors that may influence susceptibility to infection-related kidney damage.

Conclusions

This study explored the molecular mechanisms underlying disease severity in acute pyelonephritis, a common and potentially severe bacterial infection. Two cohorts of infants with febrile urinary tract infections were analyzed, with renal involvement determined by DMSA scans and disease mechanisms assessed through gene expression profiling and proteomic analysis at diagnosis and six months later. Neutrophil degranulation and renal injury genes were highly upregulated in the APN group (first DMSA+), while adaptive immune suppression further indicated an imbalance in immune response. DNA exome sequencing showed distinct genetic profiles for APN and febrile UTI and identified genes linked to scarring, though activation of renal toxicity genes during infection did not predict scarring. Overall, the study defines APN as a hyper-inflammatory condition marked by excessive innate immunity and weakened adaptive responses, aligning with known genetic susceptibilities involving neutrophil dysfunction and innate immune defects. The results revealed pronounced innate immune hyper-activation, both systemically and within the urinary tract, characteristic of a cytokine storm.

Paper III. Targeting a transcriptional node formed by IRF3, MYC and IRF7 to treat bacterial infections

Background

The mucosal innate immune system is the first line of defense against infection, relying on transcriptional regulators like NF-κB, CREB, AP-1, and STAT to maintain balance in immune response and prevent excessive inflammation.[108,109] IRF-3 and IRF-7 are key transcription factors in this response to infection, originally known for their antiviral roles but now also recognized as crucial in bacterial infections like acute pyelonephritis.[110] TLR4 signaling activates IRF-3 and upregulates IRF-7, and deleting these genes results in opposing outcomes: *Irf3*$^{-/-}$ mice show severe infection, hyper immune activation with renal damage, while Irf7$^{-/-}$ mice are protected.[25]

The MYC transcription factor, a central regulator of gene expression and immune cell function, has also been linked to kidney infection severity. Recent studies show MYC expression is influenced by IRF3 and IRF7, suggesting they form a transcriptional network that controls susceptibility to infection.[111,112] Targeting MYC by Lon protease or IRF7 by Irf7 siRNA reduced disease severity, pointing to a potential non-antibiotic treatment strategy for bacterial infections by modulating innate immune transcriptional responses.[113]

Aims

To investigate, if the IRF3 and IRF7 transcription factors regulate the MYC response to infection and the difference in disease susceptibility between *Irf3*$^{-/-}$ and *Irf7*$^{-/-}$ mice.

Results

Myc expression in infected kidneys and regulation by Irf3 or Irf7

To determine whether MYC activation during infection is regulated by IRF3 and IRF7, mice were infected with *E. coli* CFT073 and scarified at 24 hours and seven days. *Irf3*$^{-/-}$ mice developed severe pyelonephritis with high urine bacterial counts, neutrophil infiltration, and strong MYC expression. In contrast, *Irf7*$^{-/-}$ mice were protected, and wild-type mice showed only a mild, transient response. Transcriptomic analysis revealed a strong MYC dependent gene network and activation of cytokine storm and innate immune pathways in *Irf3*$^{-/-}$ mice. These responses were absent or weak in Irf7$^{-/-}$ and wild-type mice. The findings show that

IRF3 promotes a pathogenic MYC driven immune response, while IRF7 counteracts it, identifying them as key regulators of kidney infection severity.

Renal toxicity genes regulated by MYC and IRF7 in infected mice

Acute pyelonephritis, characterized by acute renal inflammation, can lead to long term kidney damage. In this study, gene expression analysis revealed that infected *Irf3*$^{-/-}$ mice exhibited strong activation of renal damage associated genes seven days post-infection, with nearly half (162 of 353) defined as MYC related (Figure 9). Additionally, a majority of the IRF related genes activated in these mice overlapped with the MYC network. In contrast, infected *Irf7*$^{-/-}$ mice showed minimal gene activation, and C57BL/6 wild type mice displayed only a weak response, highlighting the pronounced renal toxicity response in the absence of IRF3 regulation.

Network analysis confirmed that the renal toxicity response involved MYC dependent gene pathways were central to the observed in *Irf3*$^{-/-}$ mice, particularly those associated with kidney damage, injury, and cell death. A smaller subset of IRF7 regulated genes also overlapped with MYC related pathways, suggesting a complex interaction. These MYC driven networks were not significantly activated in either *Irf7*$^{-/-}$ or C57BL/6 mice. Overall, the findings underscore the dominant role of MYC in mediating renal damage during infection in the absence of IRF3, with a minor but notable contribution from IRF7 regulated genes.

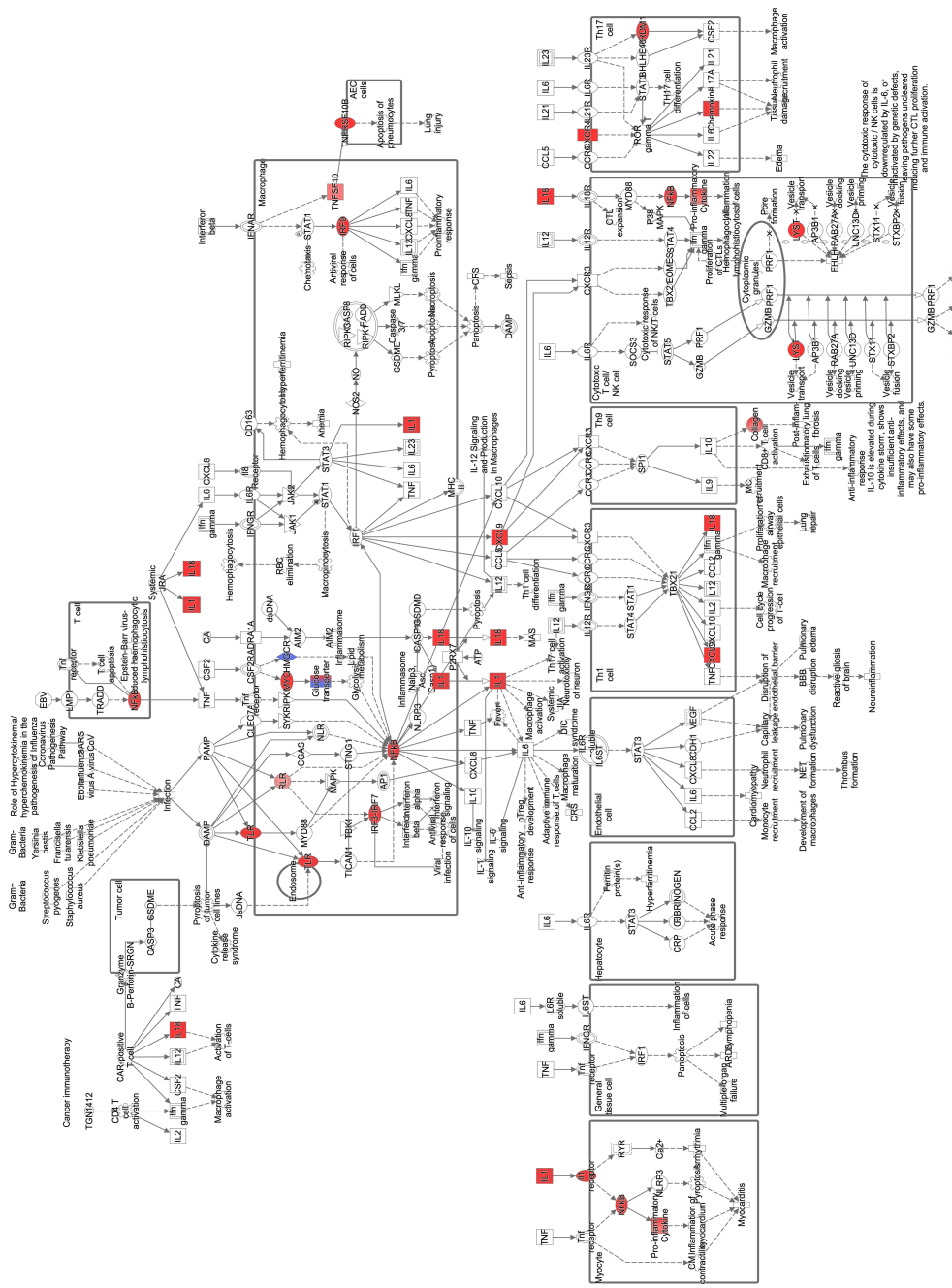


Figure 9: Cytokine storm signaling pathway, MYC-dependent genes (CFT073 vs. uninfected, FC ≥ 2, P < 0.05). Pathway analysis identifying the majority of regulated genes in the cytokine storm network as MYC-related (35 ↑ 2 ↓**).**

Effects of Irf7 siRNA treatment on the MYC response to infection

To investigate if IRF7 regulates MYC expression in infected kidneys, *Irf3*$^{-/-}$ mice were treated with IRF7 siRNA. siRNA was administered intraperitoneally three days before and on the day of infection with *E. coli* CFT073. This targeted inhibition of Irf7 significantly reduced the MYC response to infection, as shown by a significant decrease in MYC dependent gene expression in the treated group compared to untreated *Irf3*$^{-/-}$ mice.

Network and functional analyses confirmed that Irf7 knockdown suppressed MYC driven innate immune responses. In particular, key immune pathways such as the acute phase response, neutrophil degranulation, IL-6 and IL-8 signaling, cytokine storm, and TLR signaling were all inhibited in the siRNA treated group. The cytokine storm pathway, highly activated in untreated *Irf3*$^{-/-}$ mice, was suppressed by Irf7 silencing, indicating that IRF7 plays a critical role in sustaining the MYC dependent immune response to kidney infection.

Effects of MYC inhibition by rLon protease treatment on IRF7 expression

To explore if MYC regulates the IRF7 response, mice were treated with recombinant Lon protease, a recently identified MYC inhibitor. *Irf3*$^{-/-}$, *Irf7*$^{-/-}$ and C57BL/6 mice received daily intraperitoneal injections of rLon for eight days, beginning one day before infection with *E.coli* CFT073. rLon treatment significantly reduced the IRF7 response to infection in *Irf3*$^{-/-}$ mice, decreasing the number of regulated IRF7 dependent genes from 128 to only 1. In contrast, this effect was not observed in *Irf7*$^{-/-}$ mice, and only marginal effects were seen in C57BL/6 mice. Functional pathway analyses identified MYC dependent innate immune responses such as cytokine storm, neutrophil degranulation, IL-6 and IL-8 signaling, and TLR pathways as the most strongly affected by rLon. These findings highlight the critical role of MYC in mediating both innate immune and IRF7 responses in kidney infection, supporting a central regulatory role for the MYC, IRF3, and IRF7 transcriptional network in driving disease pathology. Network and functional analyses confirmed that MYC dependent gene networks and innate immune pathways were strongly inhibited in rLon-treated *Irf3*$^{-/-}$ mice.

In addition, rLon treatment markedly suppressed the MYC response, most notably in *Irf3*$^{-/-}$ mice, where the number of MYC regulated genes was reduced from 1087 to 5. Similar, though less pronounced effects, were observed in C57BL/6 and *Irf7*$^{-/-}$ mice.

MYC and interferon signaling in children with febrile UTI

To assess the relevance of these findings for human disease, peripheral blood RNA from infants with febrile urinary tract infection was subjected to genome wide transcriptomic analysis. MYC, IRF3, and IRF7 dependent genes were activated during acute infection, with enrichment in innate immune pathways such as

neutrophil degranulation, acute phase response, and IL-1, IL-6, IL-8, and interferon signaling mirroring responses seen in mice.

Using the Boruta feature selection algorithm, a subset of 33 interferon related genes was identified as predictors of renal involvement, including IFNAR1, SOCS3, and PTPN6. Additionally, 63 MYC related genes, including IRF4, were found to predict acute infection, suggesting a potential link between MYC and interferon signaling in the pathogenesis of human UTI.

Conclusions

A transcriptional node comprising MYC, IRF3, and IRF7 is shown to control the innate immune response during febrile UTI. Treatment of *Irf3*$^{-/-}$ mice with Irf7 siRNA inhibited MYC expression and treatment with the MYC inhibitor rLon inhibited IRF7 expression. Both treatments efficiently prevented disease and pathology and accelerated bacterial clearance. IRF3 and IRF7 were shown to differentially regulate MYC expression and drive the expression of MYC dependent genes linked to innate immunity and renal pathology. Clinical data support these findings, linking MYC and interferon signaling to acute pyelonephritis and implicating MYC dependent interferon genes in disease susceptibility. These results identify MYC and IRF7 as promising targets for novel, non-antibiotic therapies aimed at restoring innate immune balance and treating both antibiotic sensitive and resistant uropathogenic *E. coli* infections.

Paper IV. Similar immune responses to alpha1-oleate and Bacillus Calmette-Guérin (BCG) treatment in patients with bladder cancer

Background

Bladder cancer is a prevalent malignancy, ranking fourth in the United States and fifth in Europe, with a global incidence of approximately 500,000 new cases annually. BCG immunotherapy is the preferred treatment for high-risk NMIBC and an option for intermediate-risk NMIBC. The innate and adaptive immune systems are activated in patients receiving BCG immunotherapy and both have been associated with protection against bladder cancer.

The HAMLET complex, formed by partially unfolded alpha-lactalbumin and oleic acid, exhibits strong tumoricidal activity, effectively killing various tumor cells and showing therapeutic efficacy in preclinical models of colon, glioblastoma, and bladder cancers. The N-terminal alpha-helical peptide reproduces these effects, when bound to oleic acid. Alpha1-oleate has been used successfully to treat bladder cancer. Pathological processes and different therapies change the molecular composition of urine, and a variety of markers have been analyzed in patients with bladder cancer. Biomarkers would be useful for the diagnosis of bladder cancer but are not readily available. Urine proteins would potentially provide useful information at the time of diagnosis and to follow the effect of treatment with local or systemic compounds.

Aims

To examine the host response to intravesical alpha1-oleate treatment in patients with non-muscle invasive bladder cancer using urine proteomic and tissue RNA transcriptomic analyses.

Results

Initial proteomic analysis of urine samples

An initial screen included a bladder cancer panel with 16 proteins (MCP-1, MIP-1α, MIP-1β, IP-10, IFN-α, IL-1α, IL-1RA, IL-8, IL-7, IL-31, IL-15, TNF-β, Eotaxin, SDF-1α, Rantes and GRO). A significant increase in urine concentrations of IL-1RA, IL-1α, IL-8, Rantes and MIP-1α was detected in patients treated with 1.7 mM of alpha1-oleate, compared to placebo. In contrast, the concentrations of Eotaxin and IL-7 were reduced.

Extended proteomic analysis of urine samples from treated patients

The proteomic analysis was expanded to include 24 proteins to provide a more detailed characterization of the response to alpha1-oleate treatment. The extended screen confirmed a rapid innate immune response, including the IL-1 cytokine family (IL-1RA, IL-1α, IL-1β, IL-33), along with the proinflammatory cytokines TNF-α and the chemokine MIP-1α. Additionally, alpha1-oleate treatment activated an interferon response. Proteins such as MCP-1, IP-10, MIP-1β, and IL-6, which were elevated in urine before treatment, showed a decrease post-treatment. CD40, PD-L1, Granzyme B and IL-17D were rapidly activated and concentrations in urine increased over time. IL-2 and IL-12 levels increased during treatment, except for a decline at the final visit. In contrast, IL-4, IL-13, IL-15 and IL-17A did not change in response to treatment, compared to the placebo group.

Dose-dependence of the cytokine response

The concentrations of IL-1RA, IL-1α, IL-1β, IL-33, MIP-1α, TNF-α, IFN-α2, IFN-γ, CD40, PD-L1, Granzyme B, IL-12 and IL-17D were significantly higher in the 8.5 mM treatment group than in the 1.7 mM treatment group. In contrast, MCP-1, MIP-1β and IP-10 concentrations were significantly lower in the 8.5 mM treatment group than in the 1.7 mM group. Increased IL-12, TNF-α, IL-2 and PD-L1 responses were only observed in the 8.5 mM treatment group and a dose-dependent increase in urine IL-17D concentrations was detected, compared to the placebo group. IL-17A concentrations were low in the groups treated with 1.7 mM or 8.5 mM of alpha1-oleate.

Response to alpha1-oleate treatment defined by RNA sequencing of tumor tissue

Expression profiling detected significant changes in tumors from patients treated with 1.7 mM or 8.5 mM of alpha1-oleate compared to the placebo group, with a predominance of downregulated genes in both groups, more significantly higher in the 8.5 mM treatment group consistent with the increased anti-tumor effect. In addition to the overall downregulation of genes, the immune-related genes were also downregulated in both treatment groups. According to the IPA analysis, innate immune response biofunctions were strongly inhibited in both 1.7 mM and 8.5 mM groups, suggesting an acute anti-inflammatory response of alpha1-oleate treatment. The cytokine storm genes, which include genes related to phagocytes, monocytes, granulocytes, and neutrophils were strongly downregulated in both treatment groups with a dose dependent decrease in the 8.5 mM treated group. Furthermore, genes related to adaptive immune response functions were shown to decrease with longer treatment.

Cytokine response and treatment outcome

The results showed a significant association of alpha1-oleate treatment with increased cell shedding (odds ratio > 1 and P-value < 0.0001), and the likelihood of IL-1RA activation, suggesting IL-1RA as a predictor of cell shedding and a response marker of alpha1-oleate treatment. Interestingly, an increase in IFN-α2 protein concentrations predicted outcome defined by the remaining cytokines in the alpha1-oleate treated group with an odd ratio > 15, suggesting IFN-α2 as a potential response marker of the immune response to alpha1-oleate treatment.

Comparison of the urine proteomes in alpha1-oleate treated and BCG treated patients

Several proteins reported to increase during BCG treatment were activated in patients treated with alpha1-oleate as well. These included IL-1RA, IL-1α, IL-1β, IL-8, IFN-α2, IFN-γ and Granzyme B, which were activated in alpha1-oleate treated groups. This response was more rapid than that reported in BCG treated patients. Additional proteins that have been reported to increase during BCG treatment were activated in patients receiving 8.5 mM of alpha1-oleate, including IL-2, IL-12, TNF-α and IL-17D. In contrast IL-6 and GM-CSF, which are activated by BCG treatment, were inhibited in alpha1-oleate treated patients in alpha1-oleate treated groups. Increased levels of IL-33, MIP-1α and CD40 and decreased levels of IP-10 were observed in the alpha1-oleate treated group but have not been reported in BCG treated patients, suggesting additional effects of alpha1-oleate. IL-4 and IL-15 responses, which occur in BCG treated patients, were not observed in the alpha1-oleate treatment groups (Figure 10).

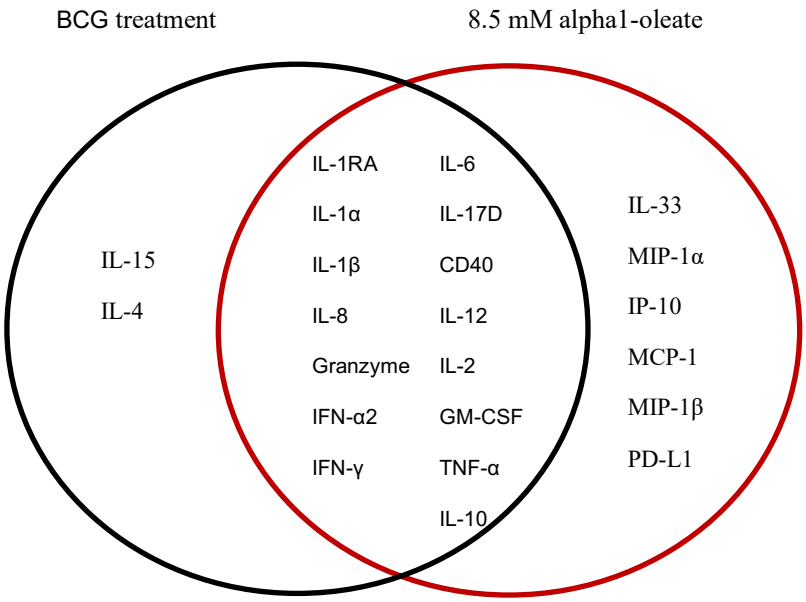


Figure 10: Comparison of cytokine responses detected in alpha1-oleate treated patients and responses to BCG treatment reported in the literature.

Conclusion

Proteomic analysis of urine from treated patients revealed a rapid immune response, with cytokine profiles resembling those seen in BCG treated patients and associated with favorable treatment outcomes. Importantly, no significant toxicity was observed with alpha1-oleate. In future studies, it might be interesting to combine BCG with alpha1-oleate treatment, to optimize anti-tumor effects.

Discussion

This thesis aimed to investigate the host immune response to urinary tract infection and alpha1-oleate treatment of bladder cancer, using proteomic and transcriptomic analyses of samples from controlled clinical studies. The local urinary tract immune response and systemic gene expression patterns were investigated in infants experiencing their first febrile urinary tract infection. Additionally, this study explored the transcriptional control of the disease response in acute pyelonephritis in a murine model, showing potent regulation by the MYC, IRF3 and IRF7 transcription factors and identifying specific inhibitors of IRF7 or MYC as potent therapeutics. Finally, the local host immune response is evaluated in patients with non-muscle invasive bladder cancer, who receive intravesical alpha1-oleate treatment (1.7 or 8.5 mM) and compared to local immune responses reported during BCG therapy.

Proteomic analysis of urine samples

In this study, we employed the Luminex proteomic assay, a bead-based multiplex technology that allows simultaneous quantification of multiple proteins in small-volume samples. This method was chosen for its high throughput, sensitivity, and efficiency. The Luminex micro-bead assay employs conjugated microsphere particles to immobilize specific antigens on their surface. Using dual fluorescence signals, the instrument determines the analyte attached to the bead and calculates its corresponding concentration based on the detection dye's intensity. This technology is particularly valuable when analyzing complex biological fluids such as serum urine and plasma. A key strength of the Luminex system is its capacity to detect numerous analytes in a single run, significantly reducing sample usage and processing time compared to traditional ELISAs.

Potential weaknesses include variability in bead coupling efficiency, cross-reactivity among antibodies, and a limited dynamic range for certain targets. Additionally, the requirement for rigorous standardization and validation of each analyte panel can be resource intensive. To overcome this, we troubleshooted it by using the Luminex kit for different targets with minimal cross reactivity between antibodies and using ELISA test for validation and for specific protein targets with high cross reactivity. Additionally, the instrument was validated and calibrated on

the same day the experiment was conducted to ensure consistency and accuracy. Despite these challenges, the Luminex assay provided us robust and reproducible data that were integral to the proteomic profiling in this study.

Cytokine storm in acute pyelonephritis

In earlier studies using ELISA-based cytokine assays, innate immune responses were detected in urine of patients with UTI, describing the mucosal immune response during urinary tract infection by *E. coli*.[5,34,114] In vitro, urinary tract epithelial cell lines produce IL-1α, IL-6, and IL-8 when stimulated with *E. coli*, with P-fimbriae enhancing this cytokine response.[31,43,115] These findings were confirmed in vivo, patients deliberately inoculated with *E. coli* showed increased urinary but not serum IL-8 levels, which strongly correlated with neutrophil counts.[43] Overall, the early studies demonstrate that *E. coli* elicits a local IL-6 and IL-8 mediated inflammatory response in the urinary tract, implicating epithelial cells as a key source of IL-8 and contributors to pyuria. [31,43,115]

To understand the local immune response to infection in more detail, using a broad panel of cytokines, chemokines and adaptive immune regulators. Urine proteomic analysis identified a strong local cytokine storm in the infant's during febrile UTI in the two study cohorts. Gene expression profiling of peripheral blood detected a systemic cytokine storm, particularly pronounced in infants with renal involvement. A cytokine storm is an exaggerated and dysregulated immune response characterized by the excessive and uncontrolled release of pro-inflammatory cytokines.[116] This hyperinflammatory state is often triggered by infections, such as severe viral infections (e.g., influenza, SARS-CoV-2) or by autoimmune diseases, or certain cancer therapies, including chimeric antigen receptor (CAR) T-cell therapy.[117-119] The excessive cytokine production causes systemic inflammation, capillary leakage, multiorgan damage, and potentially fatal outcomes, such as acute respiratory distress syndrome or multi-organ failure. Key cytokines involved include IL-6, TNF-α, IL-1 and IFN-γ, among others.[119-121]

Comparative analysis revealed a significant overlap between the febrile UTI group, studied here, and published analyses of patients with SARS-CoV-2 infection or bacterial sepsis identifying excessive cytokine activation and an out-of-control innate immune responses as a cause of disease severity in infants with febrile UTI.

The high innate immune response with high concentration of neutrophil degranulation proteins such as myeloperoxidase, elastase, defensins, and S100 family proteins was showed in previous study of inflammatory disease.[57,122,123] High concentration of cytokines and chemokines including IL-6, IL-8, MCP-1, NGAL, VEGF and IP-10, as well as antimicrobial defenses mediator such as cathelicidin and β-defensins was observed in urinary tract infection.[5,19,114,124,125] Although

weaker, but the adaptive immune cytokines Th1/Th17, antigen-presentation proteins, and urinary immunoglobulins shows the activation of innate response during more severe or longer infections.[124,125] In the current study, the results suggest that a loss of immune control and excessive responses affecting both innate and adaptive immunity characterizes acute pyelonephritis, not the activation of single cytokines.

Transcriptional control of kidney infection

Studies in the acute pyelonephritis model identified a transcriptional network involving MYC and the interferon regulatory factors IRF3 and IRF7 that governs the innate immune response. The interplay among these factors influences gene expression linked to immune activation and tissue damage, especially in renal tissues. The findings highlight how IRF3 and IRF7 inversely regulate MYC, affecting susceptibility to infections and positioning these transcription factors as potential therapeutic targets for bacterial diseases.

Earlier research demonstrated that MYC and IRF7 mutually influence each other's expression in various immune cells. For instance, MYC can regulate IRF7 activity and promote components like p48 in interferon pathways. These interactions appear context dependent: while they show a one-way regulation in dendritic cells and macrophages, in kidney epithelial cells, MYC and IRF7 seem to form a feedback loop. This suggests a distinct, tissue specific mechanism that operates outside traditional immune cell populations, allowing for nuanced control of local immune responses.

In *Irf3*$^{-/-}$ mice, MYC activation was shown to depend on IRF7, indicating a functional link between the two. Both transcription factors are expressed in the renal mucosa and are locally regulated during infection. The co-localization of MYC and IRF7 in the renal pelvic epithelium supports a model where localized, rather than systemic, immune responses dominate. This tissue-specific regulation enhances bacterial clearance and could help overcome challenges posed by transcription factor redundancy in designing targeted immune therapies.

Targeting the hyper-immune response to infection

The conventional treatment of UTIs relies heavily on antibiotics, which provide rapid bacterial clearance by directly targeting the bacteria. Antibiotics often lose their efficacy against UPEC, particularly in the context of recurrent UTI, where *E. coli* strains and other pathogens develop resistance through repeated exposure to broad-spectrum antibiotics.[126,127] Moreover, repeated antibiotic courses may interfere with the host's ability to develop natural immune tolerance or resistance to colonization, as shown that using ABU strains that modulate the immune system rather than trigger inflammation.[128,129] This necessitates the use of higher doses, extended treatment durations, or combination therapies approaches that increase the risk of side effects, antibiotic-associated complications, and the selection of multidrug-resistant organisms.[130,131]

Excessive innate immune activation in infectious diseases suggests the potential for therapeutic intervention to inhibit over reactivity and pathology. The goal is to develop therapies that selectively attenuate out-of-control immune responses while enhancing antimicrobial defense. In this study, treatments targeting the IRF-7 transcription factor by small RNA, and MYC activation using Lon protease, showed strong protective effects in animal models of acute pyelonephritis. Other examples include the NlpD protein that suppresses RNA Polymerase II phosphorylation and pro-inflammatory gene expression, and IL-1 receptor antagonist that blocks IL-1 and inflammasome-driven pathology.[132,133]

-*Therapeutic effects of MYC inhibition.* Recombinant Lon protein (rLon) was tested for its therapeutic potential via MYC inhibition in a murine acute pyelonephritis model. *Irf3*$^{-/-}$ mice treated with rLon daily for eight days (starting before infection) were protected from developing severe kidney disease. rLon treatment nearly eliminated renal Myc gene expression and c-MYC protein levels compared with placebo. Although cefotaxime reduced bacterial counts in vitro, rLon had no direct antibacterial activity.[113] Instead, its protective effect was due to strong inhibition of the MYC-dependent innate immune response during infection. Importantly, rLon had minimal effects on MYC signaling in healthy mice, indicating that MYC inhibition by rLon is disease-context dependent and does not significantly disrupt normal MYC homeostasis.[113,134]

-*Therapeutic effects of NlpD treatment in models of acute cystitis and acute pyelonephritis.* Recombinant NlpD protein has demonstrated strong therapeutic potential in mouse models of urinary tract infection. In an acute cystitis model (*Asc*$^{-/-}$ mice), NlpD treatment reduced kidney inflammation, limited tissue damage, accelerated bacterial clearance, and suppressed Pol II dependent gene expression.[132] In susceptible mice that develop severe cystitis due to excessive IL-1 driven innate immune activation, NlpD reversed transcriptional overactivation, attenuated the IL-1 response, and reduced disease severity. Similarly, treatment with recombinant

NlpD protected *Irf3*$^{-/-}$ mice from acute pyelonephritis by reducing RNA Pol II phosphorylation and inflammation, thereby mitigating disease progression.[132] NlpD acts as transcriptional regulators that protect against excessive innate immune activation during bacterial infection. They enhance bacterial clearance with efficacy comparable to antibiotics while limiting immune-mediated tissue damage. Their therapeutic effect is driven by inhibition of RNA polymerase II phosphorylation, which tempers the host immune response. This mechanism highlights NlpD as a promising non-antibiotic strategy for controlling bacterial infections by balancing effective clearance with reduced inflammatory harm.[132]

-IL-1 inhibition therapy using the IL-1 receptor antagonist anakinra. The therapeutic potential of IL-1 inhibition in cystitis was evaluated using *Asc*$^{-/-}$ mice, which display pronounced bladder disease and heightened IL-1 responses.[56] Treatment with anakinra significantly attenuated the inflammatory response to infection, as indicated by reduced neutrophil counts in urine and tissue, as well as decreased expression of IL-1 dependent genes, resulting in approximately 75–80% less tissue damage compared to untreated mice.[133] Moreover, anakinra enhanced bacterial clearance of antibiotic-sensitive or resistant *E. coli* strains, suggesting that modulating IL-1 signaling restores immune balance and strengthens the host's innate immune defenses. The efficacy of anakinra was further confirmed in C57BL/6 wild-type mice, which have normal inflammasome function, where IL-1RA treatment significantly alleviated the milder, more transient disease phenotype observed in these mice.[56] Therapeutic targeting of the IL-1 pathway can rebalance dysregulated innate immunity, yielding improved outcomes even in antibiotic-resistant infections.[133] In a recent clinical study, IL-1RA treatment was reduce the chronic pain, relief symptom and an improved quality of life in about 50% of treated patients and the urinary SP levels were lowered, and IL-1 dependent gene networks were inhibited, confirming the effects on the host response.[135] A placebo-controlled phase II study has been initiated in this patient group.

Other novel therapies in UTI

- *Lactobacilli.* In women, uropathogenic bacteria responsible for most UTIs originate from the intestinal microbiota and initially colonize the vaginal introitus and periurethral region. These organisms subsequently ascend through the urethra to the bladder and, in some cases, to the kidneys, resulting in cystitis or pyelonephritis. Therefore, factors that influence the composition and stability of the vaginal microbiota are central to the pathogenesis of UTIs and represent important targets for preventive interventions.[136,137] Culture-based studies of vaginal samples from healthy women demonstrate that *Lactobacillus* species dominate the vaginal microbiota, accounting for approximately 90% of organisms present and among these, 80–90% are H_2O_2 producing strains, predominantly *Lactobacillus crispatus*

and *Lactobacillus jensenii*.[138,139] Conversely, the absence of vaginal *lactobacilli* has been associated with several disease states. Women with diminished or absent vaginal lactobacilli, especially H_2O_2 producing species, exhibit a significantly increased susceptibility to urogenital infections, these include bacterial vaginosis, HIV infection, and *Neisseria gonorrhoeae*, as well as vaginal colonization by *E. coli*, the principal causative agent of urinary tract infections in women.[140-142]

These findings suggest that vaginal colonization with H_2O_2 producing *lactobacilli* plays a protective role by inhibiting *E. coli* colonization of the vaginal introitus and reducing the risk of UTI.[143-145] This hypothesis has been tested by developing therapeutic approaches to the treatment of recurrent UTI that include *lactobacilli*. Research on urinary tract infections in women has highlighted the importance of the vaginal microbiota, particularly the presence of *Lactobacillus* species, in determining susceptibility to recurrent UTI.[136,146] Depletion of vaginal lactobacilli is associated with increased UTI risk, suggesting that restoration of these organisms may be protective. In a randomized, double-blind, placebo-controlled phase 2 trial, intravaginal administration of *Lactobacillus crispatus* (via the probiotic product Lactin-V) after acute cystitis reduced the rate of recurrent UTI: 15% of women receiving Lactin-V experienced recurrent UTI versus 27% in the placebo group.[147] High-level, sustained vaginal colonization with *Lactobacillus crispatus* was strongly associated with UTI protection in the Lactin-V group and this data support the concept that re-establishing a healthy lactobacilli-dominated vaginal flora may serve as a promising non-antibiotic strategy to prevent recurrent UTIs in women, though larger trials are needed to confirm efficacy and long-term colonization.[146,147]

- *Adhesion inhibitors.* Furthermore, adherence of uropathogenic *E. coli* to host tissues via fimbriae is a critical step in UTI pathogenesis and can be competitively inhibited by soluble receptor analogues.[148] Oligosaccharides and glycolipid receptor antagonists that block fimbrial adhesins particularly the P fimbrial adhesin PapG and the type 1 fimbrial lectin FimH, have demonstrated protective effects in multiple rodent UTI models, significantly reducing bacterial adhesion, colonization, and bladder bacterial burden.[148,149] High affinity mannosides targeting FimH have been shown to decrease intestinal colonization by fimbriated UPEC strains while simultaneously protecting against UTIs, without disrupting the native intestinal microbiota.[150] Early studies using α-methyl-D-mannose and low molecular weight mannosides further confirmed inhibition of type 1 fimbrial binding, reduced bacterial persistence, and altered host cell shedding.[151,152] Importantly, oral FimH inhibitors were found to be non-inferior to trimethoprim sulfamethoxazole in clearing bladder infection in mouse models.[152,153] Collectively, these findings support bacterial adhesion blockade as a promising therapeutic strategy for UTIs, although clinical translation has yet to be achieved despite the near-universal presence of FimH among cystitis isolates.[154-156]

- *Antimicrobial peptides.* Antimicrobial peptides, such as defensins, cathelicidins, and RNase7, are key effectors of the innate immune system that directly target

bacteria, similar to antibiotics.[157,158] In the kidney, they contribute to defense by disrupting microbial membranes. Lipocalin 2 inhibits bacterial iron acquisition, while pentraxin 3 enhances bacterial clearance by neutrophils.[159] The cathelicidin LL-37 and its mouse homologue CRAMP specifically target uropathogenic *E. coli*.[160] Studies in knockout mice suggest CRAMP plays a protective antimicrobial role, although further research is needed to evaluate AMPs as therapeutic agents.[160,161]

Host immune response in bladder cancer

The immune system plays dual roles in cancer. On the one hand, immunosurveillance helps detect and eliminate malignant cells.[162] On the other hand, chronic inflammation can foster tumor growth and progression by promoting angiogenesis, genomic instability, and immune evasion.[163] A defining hallmark is "avoiding immune destruction," which cancer cells develop different mechanisms to escape recognition and elimination by immune cells including cytotoxic T lymphocytes and natural killer cells.[164] The cancer tumors achieve this mechanism through upregulation of immune checkpoint molecules such as PD-L1 and CTLA-4 which suppress T-cell activity, secretion of immunosuppressive cytokines (TGF-β, and IL-10), and recruitment of regulatory immune cells such as Tregs and myeloid-derived suppressor cells.[165] Additionally, inflammation enabling tumor initiation and progression by promoting genomic instability, angiogenesis, and tissue remodeling.[164] The interplay between tumor-promoting inflammation and immune evasion underscores the dynamic relationship between cancer and the host immune system.[166]

Furthermore, therapies like immune checkpoint inhibitors (anti-PD-1), which have revolutionized cancer treatment, can unleash immune-related adverse events where the immune system attacks healthy tissues, mimicking autoimmune diseases.[167] These examples illustrate that the immune system must be tightly regulated to balance its protective and pathogenic roles. Understanding this balance is essential for designing effective therapies that modulate immune function whether to boost immunity in cancer and infection or suppress it in autoimmunity and transplantation.

Alpha1-oleate therapy in NMIBC

In this study, proteomic screening technology was used to further evaluate the complexity of the local immune response in the urinary tract and in bladder cancer patients treated with alpha1- oleate. Alpha1-oleate treatment induced a strong innate immune response, characterized by elevated IL-1RA levels, which remained high throughout the treatment. IL-1RA, a key regulator of the response, along with IL-

1α, IL-1β, and IL-33, exhibited significant pro-inflammatory effects. Shed tumor cells from treated patients contained IL-1RA, IL-1β, IFN-α2, and IL-17D, highlighting a localized immune reaction. Elevated IL-1RA levels, previously associated with reduced bladder cancer recurrence risk, suggest a potential role in enhancing the therapeutic effects of alpha1-oleate. Additionally, the activation of IL-17D by the treatment indicates a possible link between innate and adaptive immunity. The treatment also activated IFN-α2 and IFN-γ, which possess anti-cancer properties such as inducing apoptosis, inhibiting angiogenesis, and modulating immune responses. IFN-α2 triggered TRAIL expression and caspase-8 activation, while IFN-γ promoted cytotoxic effects and immune checkpoint regulation. Alpha1-oleate reduced IL-10 and IL-6 levels, potentially supporting a TH1 response and mitigating pathological angiogenesis and tumor dissemination. Adaptive immune markers like CD40 were affected, further enhancing cytokine production and immune activation. Together, these findings suggest alpha1-oleate's ability to elicit a robust anti-tumor immune response, offering promise for bladder cancer treatment.

The results suggest that in addition to killing tumor cells, alpha1-oleate activates a strong anti-tumor response that is not present in the placebo group. The innate response, highlighted by IL-1RA and interferons, plays a critical role, while the later adaptive response, involving CD40, PD-L1, IL-17, and Granzyme B, suggests tumor microenvironment reprogramming and apoptosis induction. The eventual cytokine reduction likely reflects the loss of tumor tissue. Alpha1-oleate has shown efficacy in reducing tumor size and cell shedding in patients with non-muscle invasive bladder cancer, who are not eligible for BCG therapy, due to early, less severe disease.

Safety and lack of toxicity of alpha1-oleate the treatment

The investigational drug alpha1-oleate shows promise as a neoadjuvant therapy for non-muscle-invasive bladder cancer. Nonclinical studies report minimal toxicity, with no adverse effects on body or organ weights, pathology, or histology. Preliminary clinical trial data confirm that alpha1-oleate is safe, well-tolerated, and exhibits dose-dependent effects on tumor size and cell shedding. This novel therapy could reduce the extent of surgery, enhance surgical efficacy, and improve tumor response to subsequent treatments. Additionally, it may improve patient outcomes by addressing subclinical lesions, limiting cancer spread, and potentially reducing recurrences, thereby enhancing quality of life for non-muscle-invasive bladder cancer patients.

BCG immunotherapy in NMIBC and similarity to alpha1- oleate therapy

BCG stimulates urothelial and antigen-presenting cells to release cytokines including IL-6, IL-8, TNF, GM-CSF, and chemokines such as IL-1β, IL-15, IL-18, CXCL10, CCL2, and CCL3, recruiting immune cells such as granulocytes and mononuclear cells to the bladder. In vitro and clinical studies show that BCG upregulates cytokines and chemokines in urine within 24 hours of treatment.[168-172] Alpha1-oleate therapy exhibits a similar cytokine response profile to BCG but triggers a faster and more robust response, with significantly higher cytokine levels and earlier activation of key molecules like IL-1RA, IL-1β, Granzyme B, TNF-α, and IL-12. These findings suggest that alpha1-oleate reproduces critical immune aspects of BCG therapy and that combination therapy might be beneficial to maximize the effect on bladder cancer outcomes.

The similarities of the responses to alpha1-oleate treatment to patients with BCG immunotherapy suggest that a combined or sequential treatment approach could be beneficial. Priming and debulking of the tumor using alpha1-oleate and subsequent BCG treatment, in patients with disease progression. The potential benefits of such approaches would require validation in future clinical trials.

Future Perspectives

The data presented here and additional recent data highlight the potential of targeted immunotherapy as a promising alternative to traditional antibiotic treatment for bacterial infections such as acute pyelonephritis and cystitis. Building on findings that modulation of transcription factors and inflammatory mediators can accelerate bacterial clearance while minimizing tissue damage future studies should focus on defining the precise immune signatures associated with each therapeutic approach.

Comprehensive proteomic profiling of cytokines, chemokines, and other immune mediators will be essential to delineate the molecular mechanisms underlying these responses. In particular, the application of proteomic assays offers a powerful approach to analyze systemic and local immune responses induced by different infectious agents, that can subsequently by targeted by immunomodulation.

Proteomic data with molecular and functional immunological analysis will facilitate a deeper understanding of host pathogen interactions and treatment-specific immune modulation. Cytokine storm counter to single biomarkers Such an approach may also reveal biomarkers predictive of therapeutic efficacy or adverse inflammatory responses. Ultimately, these insights could guide the development of precision immunotherapeutic strategies that optimize host defense while minimizing tissue injury, thereby reduce dependence on conventional antibiotics.

Alpha1-oleate as a neoadjuvant therapy for NMIBC include the larger controlled clinical trials to confirm its safety profile and the preliminary dose-dependent effects on tumor burden and cells shedding. This approach may help refine surgical strategies, enhance responsiveness to subsequent treatments, and potentially lower recurrence rates by addressing subclinical lesions. Continued clinical development will be essential to fully define its therapeutic value and its potential to improve long-term outcomes and quality of life for patients with NMIBC.

Acknowledgments

First and foremost, I would like to express my deepest gratitude to my supervisors, **Catharina**, **Gabriela** and **Ines** for their invaluable guidance, support, and encouragement throughout this journey. Reaching this milestone would not have been possible without your mentorship and the generosity you have shown me over the years.

My sincere thanks go especially to my supervisor **Catharina**, mentor, and friend. Your insight, patience, and grace have shaped not only this project but also my growth as a researcher. Being mentored by you has been an immense learning experience. Your empathy and understanding helped me navigate difficult times, and your unwavering support allowed me to experience and embrace every facet of this profession. I could not have imagined a better mentor.

To **Gabriela**, thank you for always being cheerful, approachable, and ever willing to help. More than just supervisors, it is my peers - past and present, who have stood by me through the highs and lows, without whom I would not have been able to come this far. I am grateful for your mentorship and kind presence throughout my PhD journey.

Thank you to my co-supervisor, **Ines**, for always being there when I needed guidance and motivation. Your enthusiasm for science and curiosity about new ideas were inspiring, and our discussions really helped me grow as a scientist. I've learned a lot from your patience and your talent for connecting with people.

To my lab partners, with special thanks to **Farhan**, **Arunima**, **Hien**, and **Murphy**, thank you for your help with the final reading of this thesis and for so much more. I consider myself incredibly fortunate to have worked with such fantastic colleagues. You have become precious friends and my family away from home.

To **Michele T** and **Sid**, your optimism and can-do attitude brightened even the toughest days. Thank you for your friendship and unwavering support. To **Susan** and **Atefeh**, thank you for being kind colleagues and true friends, always ready to help in any way possible. It has been a pleasure working with you. To **Samudra**, thank you for your constant kindness and readiness to help. I have truly enjoyed our time working together.

To all past lab members **Michele C**, **Daniel**, **Antonio**, and **Mario** thank you for making the lab a fun and vibrant place to work. I have valued our late-evening discussions, which ranged from scientific debates to open-ended conversations that expanded my perspectives and encouraged me to think outside the box. A warm thank-you to **Komal** and **Camilla** from Gabriela's lab for your friendship and for always being open to conversation.

To **Jakob**, your patience and willingness to help made navigating administrative and visa matters far easier. Thank you for being so dependable.

To all the students who have crossed my path **Axel**, **Ben**, **Vincent**, **Rita**, **Nicolas**, and **Hanna** thank you for your thoughtful questions and stimulating discussions. You broadened my perspectives, and I sincerely hope I was able to support you in return.

To my family: **Mahshid**, **Bahram**, **Arash**, **Mahvash**, **Nazanin**, and **Bahareh** thank you for your love, patience, and unwavering belief in me. Your support has meant everything. To **Reza** and **Zoherh**, thank you for your heartfelt encouragement and prayers throughout this journey. Knowing that you were always cheering me on brought me strength and comfort.

To my mother, **Madineh** no words can fully express my gratitude for the life you have given me. Your sacrifices, your strength, and the dreams you set aside so that I could pursue mine are gifts I will carry forever. Everything I am, and everything I will become, is rooted in your endless love and unwavering belief in me. I am forever indebted to you.

Finally, and most importantly, to my wife, **Ladan** thank you for your unconditional love, support, and patience. I will never forget the incredible sacrifice you made in moving to Sweden so I could pursue this dream. Your belief in me, even when I doubted myself, kept me going. I truly could not have done this without you. I look forward to standing by your side and supporting your dreams, just as you have supported mine.

References

1 Chaplin, D. D. Overview of the immune response. *J Allergy Clin Immunol* **125**, S3-23 (2010). https://doi.org/10.1016/j.jaci.2009.12.980

2 Ambite, I. *et al.* Molecular determinants of disease severity in urinary tract infection. *Nat Rev Urol* **18**, 468-486 (2021). https://doi.org/10.1038/s41585-021-00477-x

3 Butler, D. *et al.* Immunomodulation therapy offers new molecular strategies to treat UTI. *Nat Rev Urol* **19**, 419-437 (2022). https://doi.org/10.1038/s41585-022-00602-4

4 Svanborg, C., Agace, W., Hedges, S., Lindstedt, R. & Svensson, M. L. Bacterial adherence and mucosal cytokine production. *Ann N Y Acad Sci* **730**, 162-181 (1994). https://doi.org/10.1111/j.1749-6632.1994.tb44247.x

5 Hedges, S. R., Agace, W. W. & Svanborg, C. Epithelial cytokine responses and mucosal cytokine networks. *Trends Microbiol* **3**, 266-270 (1995). https://doi.org/10.1016/s0966-842x(00)88941-6

6 Beutler, B. Innate immunity: an overview. *Mol Immunol* **40**, 845-859 (2004). https://doi.org/10.1016/j.molimm.2003.10.005

7 Kumar, H., Kawai, T. & Akira, S. Pathogen recognition by the innate immune system. *Int Rev Immunol* **30**, 16-34 (2011). https://doi.org/10.3109/08830185.2010.529976

8 Chen, R. *et al.* Pattern recognition receptors: function, regulation and therapeutic potential. *Signal Transduction and Targeted Therapy* **10**, 216 (2025). https://doi.org/10.1038/s41392-025-02264-1

9 Hannan, T. J., Mysorekar, I. U., Hung, C. S., Isaacson-Schmid, M. L. & Hultgren, S. J. Early severe inflammatory responses to uropathogenic E. coli predispose to chronic and recurrent urinary tract infection. *PLoS Pathog* **6**, e1001042 (2010). https://doi.org/10.1371/journal.ppat.1001042

10 Chen, L. *et al.* Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* **9**, 7204-7218 (2018). https://doi.org/10.18632/oncotarget.23208

11 Fischer, H. *et al.* Pathogen specific, IRF3-dependent signaling and innate resistance to human kidney infection. *PLoS Pathog* **6**, e1001109 (2010). https://doi.org/10.1371/journal.ppat.1001109

12 Svanborg, C. *et al.* Uropathogenic Escherichia coli as a model of host-parasite interaction. *Curr Opin Microbiol* **9**, 33-39 (2006). https://doi.org/10.1016/j.mib.2005.12.012

13 Wu, J. & Abraham, S. N. The Roles of T cells in Bladder Pathologies. *Trends Immunol* **42**, 248-260 (2021). https://doi.org/10.1016/j.it.2021.01.003

14 Edén, C. S., Hanson, L. A., Jodal, U., Lindberg, U. & Akerlund, A. S. Variable adherence to normal human urinary-tract epithelial cells of Escherichia coli strains associated with various forms of urinary-tract infection. *Lancet* **1**, 490-492 (1976).
15 Butler, D. S. C. *et al.* Neuroepithelial control of mucosal inflammation in acute cystitis. *Sci Rep* **8**, 11015 (2018). https://doi.org/10.1038/s41598-018-28634-0
16 Roberts, K. B. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* **128**, 595-610 (2011). https://doi.org/10.1542/peds.2011-1330
17 Brandström, P. & Hansson, S. Urinary Tract Infection in Children. *Pediatr Clin North Am* **69**, 1099-1114 (2022). https://doi.org/10.1016/j.pcl.2022.07.003
18 Salo, J., Ikäheimo, R., Tapiainen, T. & Uhari, M. Childhood urinary tract infections as a cause of chronic kidney disease. *Pediatrics* **128**, 840-847 (2011). https://doi.org/10.1542/peds.2010-3520
19 Asadi Karam, M. R., Habibi, M. & Bouzari, S. Urinary tract infection: Pathogenicity, antibiotic resistance and development of effective vaccines against Uropathogenic Escherichia coli. *Mol Immunol* **108**, 56-67 (2019). https://doi.org/10.1016/j.molimm.2019.02.007
20 Ambite, I. *et al.* Susceptibility to Urinary Tract Infection: Benefits and Hazards of the Antibacterial Host Response. *Microbiol Spectr* **4** (2016). https://doi.org/10.1128/microbiolspec.UTI-0019-2014
21 Kurts, C., Panzer, U., Anders, H. J. & Rees, A. J. The immune system and kidney disease: basic concepts and clinical implications. *Nat Rev Immunol* **13**, 738-753 (2013). https://doi.org/10.1038/nri3523
22 Hsiao, C. Y. *et al.* Urolithiasis Is a Risk Factor for Uroseptic Shock and Acute Kidney Injury in Patients With Urinary Tract Infection. *Front Med (Lausanne)* **6**, 288 (2019). https://doi.org/10.3389/fmed.2019.00288
23 Ambite, I. *et al.* Fimbriae reprogram host gene expression - Divergent effects of P and type 1 fimbriae. *PLoS Pathog* **15**, e1007671 (2019). https://doi.org/10.1371/journal.ppat.1007671
24 Wicherska-Pawłowska, K., Wróbel, T. & Rybka, J. Toll-Like Receptors (TLRs), NOD-Like Receptors (NLRs), and RIG-I-Like Receptors (RLRs) in Innate Immunity. TLRs, NLRs, and RLRs Ligands as Immunotherapeutic Agents for Hematopoietic Diseases. *Int J Mol Sci* **22** (2021). https://doi.org/10.3390/ijms222413397
25 Puthia, M. *et al.* IRF7 inhibition prevents destructive innate immunity-A target for nonantibiotic therapy of bacterial infections. *Sci Transl Med* **8**, 336ra359 (2016). https://doi.org/10.1126/scitranslmed.aaf1156
26 Janeway, C. A., Jr. & Medzhitov, R. Innate immune recognition. *Annu Rev Immunol* **20**, 197-216 (2002). https://doi.org/10.1146/annurev.immunol.20.083001.084359
27 Gauthier, A. E., Rotjan, R. D. & Kagan, J. C. Lipopolysaccharide detection by the innate immune system may be an uncommon defence strategy used in nature. *Open Biol* **12**, 220146 (2022). https://doi.org/10.1098/rsob.220146

28 Kawai, T. & Akira, S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nature Immunology* **11**, 373-384 (2010). https://doi.org/10.1038/ni.1863

29 Kaplan, M. J. & Radic, M. Neutrophil extracellular traps: double-edged swords of innate immunity. *J Immunol* **189**, 2689-2695 (2012). https://doi.org/10.4049/jimmunol.1201719

30 Retter, A., Singer, M. & Annane, D. "The NET effect": Neutrophil extracellular traps-a potential key component of the dysregulated host immune response in sepsis. *Crit Care* **29**, 59 (2025). https://doi.org/10.1186/s13054-025-05283-0

31 Hedges, S. & Svanborg, C. The mucosal cytokine response to urinary tract infections. *Int J Antimicrob Agents* **4**, 89-93 (1994). https://doi.org/10.1016/0924-8579(94)90039-6

32 Otto, G., Braconier, J., Andreasson, A. & Svanborg, C. Interleukin-6 and disease severity in patients with bacteremic and nonbacteremic febrile urinary tract infection. *J Infect Dis* **179**, 172-179 (1999). https://doi.org/10.1086/314534

33 Godaly, G., Otto, G., Burdick, M. D., Strieter, R. M. & Svanborg, C. Fimbrial lectins influence the chemokine repertoire in the urinary tract mucosa. *Kidney Int* **71**, 778-786 (2007). https://doi.org/10.1038/sj.ki.5002076

34 Agace, W. W., Hedges, S. R., Ceska, M. & Svanborg, C. Interleukin-8 and the neutrophil response to mucosal gram-negative infection. *J Clin Invest* **92**, 780-785 (1993). https://doi.org/10.1172/jci116650

35 Wullt, B. *et al.* The host response to urinary tract infection. *Infect Dis Clin North Am* **17**, 279-301 (2003). https://doi.org/10.1016/s0891-5520(03)00028-x

36 Zec, K. *et al.* Neutrophil Migration into the Infected Uroepithelium Is Regulated by the Crosstalk between Resident and Helper Macrophages. *Pathogens* **5** (2016). https://doi.org/10.3390/pathogens5010015

37 Engel, D. *et al.* Tumor necrosis factor alpha- and inducible nitric oxide synthase-producing dendritic cells are rapidly recruited to the bladder in urinary tract infection but are dispensable for bacterial clearance. *Infect Immun* **74**, 6100-6107 (2006). https://doi.org/10.1128/iai.00881-06

38 Mora-Bau, G. *et al.* Macrophages Subvert Adaptive Immunity to Urinary Tract Infection. *PLoS Pathog* **11**, e1005044 (2015). https://doi.org/10.1371/journal.ppat.1005044

39 Malaviya, R. & Abraham, S. N. Mast cell modulation of immune responses to bacteria. *Immunol Rev* **179**, 16-24 (2001). https://doi.org/10.1034/j.1600-065x.2001.790102.x

40 Malaviya, R., Ikeda, T., Ross, E. & Abraham, S. N. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. *Nature* **381**, 77-80 (1996). https://doi.org/10.1038/381077a0

41 Scholes, D. *et al.* Family history and risk of recurrent cystitis and pyelonephritis in women. *J Urol* **184**, 564-569 (2010). https://doi.org/10.1016/j.juro.2010.03.139

42 Hagberg, L. *et al.* Difference in susceptibility to gram-negative urinary tract infection between C3H/HeJ and C3H/HeN mice. *Infect Immun* **46**, 839-844 (1984). https://doi.org/10.1128/iai.46.3.839-844.1984

43 Hang, L., Frendéus, B., Godaly, G. & Svanborg, C. Interleukin-8 receptor knockout mice have subepithelial neutrophil entrapment and renal scarring following acute pyelonephritis. *J Infect Dis* **182**, 1738-1748 (2000). https://doi.org/10.1086/317599

44 Svensson, M. *et al.* Natural history of renal scarring in susceptible mIL-8Rh-/- mice. *Kidney Int* **67**, 103-110 (2005). https://doi.org/10.1111/j.1523-1755.2005.00060.x

45 Frendéus, B. *et al.* Interleukin 8 receptor deficiency confers susceptibility to acute experimental pyelonephritis and may have a human counterpart. *J Exp Med* **192**, 881-890 (2000). https://doi.org/10.1084/jem.192.6.881

46 Svensson, M., Irjala, H., Svanborg, C. & Godaly, G. Effects of epithelial and neutrophil CXCR2 on innate immunity and resistance to kidney infection. *Kidney Int* **74**, 81-90 (2008). https://doi.org/10.1038/ki.2008.105

47 Wang, C. *et al.* NOD2 is dispensable for ATG16L1 deficiency-mediated resistance to urinary tract infection. *Autophagy* **10**, 331-338 (2014). https://doi.org/10.4161/auto.27196

48 Ching, C. B. *et al.* Interleukin-6/Stat3 signaling has an essential role in the host antimicrobial response to urinary tract infection. *Kidney Int* **93**, 1320-1329 (2018). https://doi.org/10.1016/j.kint.2017.12.006

49 Lundstedt, A. C. *et al.* Inherited susceptibility to acute pyelonephritis: a family study of urinary tract infection. *J Infect Dis* **195**, 1227-1234 (2007). https://doi.org/10.1086/512620

50 Javor, J. *et al.* Genetic variations of interleukin-8, CXCR1 and CXCR2 genes and risk of acute pyelonephritis in children. *Int J Immunogenet* **39**, 338-345 (2012). https://doi.org/10.1111/j.1744-313X.2012.01096.x

51 Svanborg, C. *et al.* The 'innate' host response protects and damages the infected urinary tract. *Ann Med* **33**, 563-570 (2001). https://doi.org/10.3109/07853890109002101

52 Fischer, H., Yamamoto, M., Akira, S., Beutler, B. & Svanborg, C. Mechanism of pathogen-specific TLR4 activation in the mucosa: fimbriae, recognition receptors and adaptor protein selection. *Eur J Immunol* **36**, 267-277 (2006). https://doi.org/10.1002/eji.200535149

53 Ragnarsdóttir, B. *et al.* TLR- and CXCR1-dependent innate immunity: insights into the genetics of urinary tract infections. *Eur J Clin Invest* **38 Suppl 2**, 12-20 (2008). https://doi.org/10.1111/j.1365-2362.2008.02004.x

54 Ragnarsdóttir, B. *et al.* Toll-like receptor 4 promoter polymorphisms: common TLR4 variants may protect against severe urinary tract infection. *PLoS One* **5**, e10734 (2010). https://doi.org/10.1371/journal.pone.0010734

55 Ahmadi, S. *et al.* Analysis of the Innate Immune Response to Febrile UTI in Infants: Evidence of an Acute Cytokine Storm. *Pediatr Infect Dis J* **44**, 1188-1200 (2025). https://doi.org/10.1097/inf.0000000000004914

56 Ambite, I. *et al.* Molecular Basis of Acute Cystitis Reveals Susceptibility Genes and Immunotherapeutic Targets. *PLoS Pathog* **12**, e1005848 (2016). https://doi.org/10.1371/journal.ppat.1005848

57 Ambite, I. *et al.* Molecular analysis of acute pyelonephritis-excessive innate and attenuated adaptive immunity. *Life Sci Alliance* **8** (2025). https://doi.org/10.26508/lsa.202402926

58 Schilling, J. D., Mulvey, M. A., Vincent, C. D., Lorenz, R. G. & Hultgren, S. J. Bacterial invasion augments epithelial cytokine responses to Escherichia coli through a lipopolysaccharide-dependent mechanism. *J Immunol* **166**, 1148-1155 (2001). https://doi.org/10.4049/jimmunol.166.2.1148

59 Kotenko, S. V., Rivera, A., Parker, D. & Durbin, J. E. Type III IFNs: Beyond antiviral protection. *Semin Immunol* **43**, 101303 (2019). https://doi.org/10.1016/j.smim.2019.101303

60 Mysorekar, I. U. & Hultgren, S. J. Mechanisms of uropathogenic Escherichia coli persistence and eradication from the urinary tract. *Proc Natl Acad Sci U S A* **103**, 14170-14175 (2006). https://doi.org/10.1073/pnas.0602136103

61 Tang, J. & Zhuang, S. Epigenetics in acute kidney injury. *Curr Opin Nephrol Hypertens* **24**, 351-358 (2015). https://doi.org/10.1097/mnh.0000000000000140

62 Au, W. C. & Pitha, P. M. Recruitment of multiple interferon regulatory factors and histone acetyltransferase to the transcriptionally active interferon a promoters. *J Biol Chem* **276**, 41629-41637 (2001). https://doi.org/10.1074/jbc.M105121200

63 Nahid, M. A., Satoh, M. & Chan, E. K. MicroRNA in TLR signaling and endotoxin tolerance. *Cell Mol Immunol* **8**, 388-403 (2011). https://doi.org/10.1038/cmi.2011.26

64 Liu, Y., Peng, B., Wu, S. & Xu, N. Epigenetic Regulation of Regulatory T Cells in Kidney Disease and Transplantation. *Curr Gene Ther* **17**, 461-468 (2017). https://doi.org/10.2174/1566523218666180214093813

65 Honda, K. & Taniguchi, T. IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. *Nat Rev Immunol* **6**, 644-658 (2006). https://doi.org/10.1038/nri1900

66 Honda, K. *et al.* IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* **434**, 772-777 (2005). https://doi.org/10.1038/nature03464

67 Foxman, B. The epidemiology of urinary tract infection. *Nat Rev Urol* **7**, 653-660 (2010). https://doi.org/10.1038/nrurol.2010.190

68 Fihn, S. D. Clinical practice. Acute uncomplicated urinary tract infection in women. *N Engl J Med* **349**, 259-266 (2003). https://doi.org/10.1056/NEJMcp030027

69 Stamm, W. E. & Hooton, T. M. Management of urinary tract infections in adults. *N Engl J Med* **329**, 1328-1334 (1993). https://doi.org/10.1056/nejm199310283291808

70 Yadav, M. *et al.* Inhibition of TIR domain signaling by TcpC: MyD88-dependent and independent effects on Escherichia coli virulence. *PLoS Pathog* **6**, e1001120 (2010). https://doi.org/10.1371/journal.ppat.1001120

71 Cogswell, J. P. *et al.* NF-kappa B regulates IL-1 beta transcription through a consensus NF-kappa B binding site and a nonconsensus CRE-like site. *J Immunol* **153**, 712-723 (1994).

72 Wu, X. R., Sun, T. T. & Medina, J. J. In vitro binding of type 1-fimbriated Escherichia coli to uroplakins Ia and Ib: relation to urinary tract infections. *Proc*

Natl Acad Sci U S A **93**, 9630-9635 (1996). https://doi.org/10.1073/pnas.93.18.9630

73 Hagberg, L. *et al.* Contribution of adhesion to bacterial persistence in the mouse urinary tract. *Infect Immun* **40**, 265-272 (1983). https://doi.org/10.1128/iai.40.1.265-272.1983

74 Eto, D. S., Jones, T. A., Sundsbak, J. L. & Mulvey, M. A. Integrin-mediated host cell invasion by type 1-piliated uropathogenic Escherichia coli. *PLoS Pathog* **3**, e100 (2007). https://doi.org/10.1371/journal.ppat.0030100

75 Svanborg-Edén, C. *et al.* Bacterial virulence versus host resistance in the urinary tracts of mice. *Infect Immun* **55**, 1224-1232 (1987). https://doi.org/10.1128/iai.55.5.1224-1232.1987

76 Lobo, N. *et al.* Epidemiology, Screening, and Prevention of Bladder Cancer. *Eur Urol Oncol* **5**, 628-639 (2022). https://doi.org/10.1016/j.euo.2022.10.003

77 Kamat, A. M. *et al.* Bladder cancer. *Lancet* **388**, 2796-2810 (2016). https://doi.org/10.1016/s0140-6736(16)30512-8

78 Lenis, A. T., Lec, P. M., Chamie, K. & Mshs, M. D. Bladder Cancer: A Review. *Jama* **324**, 1980-1991 (2020). https://doi.org/10.1001/jama.2020.17598

79 Gil-Julio, H. *et al.* Tumor Escape Phenotype in Bladder Cancer Is Associated with Loss of HLA Class I Expression, T-Cell Exclusion and Stromal Changes. *Int J Mol Sci* **22** (2021). https://doi.org/10.3390/ijms22147248

80 Wang, X. *et al.* Bladder cancer cells induce immunosuppression of T cells by supporting PD-L1 expression in tumour macrophages partially through interleukin 10. *Cell Biol Int* **41**, 177-186 (2017). https://doi.org/10.1002/cbin.10716

81 Di Spirito, A., Balkhi, S., Vivona, V. & Mortara, L. Key immune cells and their crosstalk in the tumor microenvironment of bladder cancer: insights for innovative therapies. *Explor Target Antitumor Ther* **6**, 1002304 (2025). https://doi.org/10.37349/etat.2025.1002304

82 Salomé, B. *et al.* NKG2A and HLA-E define an alternative immune checkpoint axis in bladder cancer. *Cancer Cell* **40**, 1027-1043.e1029 (2022). https://doi.org/10.1016/j.ccell.2022.08.005

83 Moss, J. T. & Kadmon, D. BCG and the treatment of superficial bladder cancer. *Dicp* **25**, 1355-1367 (1991). https://doi.org/10.1177/106002809102501215

84 Morales, A., Eidinger, D. & Bruce, A. W. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. *J Urol* **116**, 180-183 (1976). https://doi.org/10.1016/s0022-5347(17)58737-6

85 Han, J., Gu, X., Li, Y. & Wu, Q. Mechanisms of BCG in the treatment of bladder cancer-current understanding and the prospect. *Biomed Pharmacother* **129**, 110393 (2020). https://doi.org/10.1016/j.biopha.2020.110393

86 Babjuk, M. *et al.* European Association of Urology Guidelines on Non-muscle-invasive Bladder Cancer (Ta, T1, and Carcinoma in Situ). *Eur Urol* **81**, 75-94 (2022). https://doi.org/10.1016/j.eururo.2021.08.010

87 Hannouneh, Z. A. *et al.* Novel immunotherapeutic options for BCG-unresponsive high-risk non-muscle-invasive bladder cancer. *Cancer Med* **12**, 21944-21968 (2023). https://doi.org/10.1002/cam4.6768

88 Fuge, O., Vasdev, N., Allchorne, P. & Green, J. S. Immunotherapy for bladder cancer. *Res Rep Urol* **7**, 65-79 (2015). https://doi.org/10.2147/rru.S63447

89 Ho, J. C. S., Nadeem, A. & Svanborg, C. HAMLET - A protein-lipid complex with broad tumoricidal activity. *Biochem Biophys Res Commun* **482**, 454-458 (2017). https://doi.org/10.1016/j.bbrc.2016.10.092

90 Håkansson, A., Zhivotovsky, B., Orrenius, S., Sabharwal, H. & Svanborg, C. Apoptosis induced by a human milk protein. *Proc Natl Acad Sci U S A* **92**, 8064-8068 (1995). https://doi.org/10.1073/pnas.92.17.8064

91 Svanborg, C. *et al.* HAMLET kills tumor cells by an apoptosis-like mechanism--cellular, molecular, and therapeutic aspects. *Adv Cancer Res* **88**, 1-29 (2003). https://doi.org/10.1016/s0065-230x(03)88302-1

92 Svensson, M., Håkansson, A., Mossberg, A. K., Linse, S. & Svanborg, C. Conversion of alpha-lactalbumin to a protein inducing apoptosis. *Proc Natl Acad Sci U S A* **97**, 4221-4226 (2000). https://doi.org/10.1073/pnas.97.8.4221

93 Pettersson-Kastberg, J. *et al.* alpha-Lactalbumin, engineered to be nonnative and inactive, kills tumor cells when in complex with oleic acid: a new biological function resulting from partial unfolding. *J Mol Biol* **394**, 994-1010 (2009). https://doi.org/10.1016/j.jmb.2009.09.026

94 Fischer, W. *et al.* Human alpha-lactalbumin made lethal to tumor cells (HAMLET) kills human glioblastoma cells in brain xenografts by an apoptosis-like mechanism and prolongs survival. *Cancer Res* **64**, 2105-2112 (2004). https://doi.org/10.1158/0008-5472.can-03-2661

95 Puthia, M., Storm, P., Nadeem, A., Hsiung, S. & Svanborg, C. Prevention and treatment of colon cancer by peroral administration of HAMLET (human α-lactalbumin made lethal to tumour cells). *Gut* **63**, 131-142 (2014). https://doi.org/10.1136/gutjnl-2012-303715

96 Mossberg, A. K., Hou, Y., Svensson, M., Holmqvist, B. & Svanborg, C. HAMLET treatment delays bladder cancer development. *J Urol* **183**, 1590-1597 (2010). https://doi.org/10.1016/j.juro.2009.12.008

97 Gustafsson, L., Leijonhufvud, I., Aronsson, A., Mossberg, A. K. & Svanborg, C. Treatment of skin papillomas with topical alpha-lactalbumin-oleic acid. *N Engl J Med* **350**, 2663-2672 (2004). https://doi.org/10.1056/NEJMoa032454

98 Mossberg, A. K. *et al.* Bladder cancers respond to intravesical instillation of HAMLET (human alpha-lactalbumin made lethal to tumor cells). *Int J Cancer* **121**, 1352-1359 (2007). https://doi.org/10.1002/ijc.22810

99 Hien, T. T. *et al.* Bladder cancer therapy without toxicity-A dose-escalation study of alpha1-oleate. *Int J Cancer* **147**, 2479-2492 (2020). https://doi.org/10.1002/ijc.33019

100 Brisuda, A. *et al.* Bladder cancer therapy using a conformationally fluid tumoricidal peptide complex. *Nat Commun* **12**, 3427 (2021). https://doi.org/10.1038/s41467-021-23748-y

101 Ho, J. C. S., Ambite, I., Mok, K. H., Babjuk, M. & Svanborg, C. A scientific journey from discovery to validation of efficacy in cancer patients: HAMLET and alpha1-oleate. *Mol Cell Oncol* **8**, 1974278 (2021). https://doi.org/10.1080/23723556.2021.1974278

102 Haq, F. *et al.* Clinical and molecular response to alpha1-oleate treatment in patients with bladder cancer. *Cancer Med* **13**, e70149 (2024). https://doi.org/10.1002/cam4.70149
103 Frantzi, M. & Vlahou, A. Ten Years of Proteomics in Bladder Cancer: Progress and Future Directions. *Bladder Cancer* **3**, 1-18 (2017). https://doi.org/10.3233/BLC-160073
104 Kamat, A. M. *et al.* Cytokine Panel for Response to Intravesical Therapy (CyPRIT): Nomogram of Changes in Urinary Cytokine Levels Predicts Patient Response to Bacillus Calmette-Guérin. *European Urology* **69**, 197-200 (2016). https://doi.org/10.1016/j.eururo.2015.06.023
105 Fodil, N., Langlais, D. & Gros, P. Primary Immunodeficiencies and Inflammatory Disease: A Growing Genetic Intersection. *Trends Immunol* **37**, 126-140 (2016). https://doi.org/10.1016/j.it.2015.12.006
106 Tangye, S. G. Impact of SARS-CoV-2 infection and COVID-19 on patients with inborn errors of immunity. *J Allergy Clin Immunol* **151**, 818-831 (2023). https://doi.org/10.1016/j.jaci.2022.11.010
107 Telenti, A. & di Iulio, J. Regulatory genome variants in human susceptibility to infection. *Hum Genet* **139**, 759-768 (2020). https://doi.org/10.1007/s00439-019-02091-9
108 Patalano, S. D., Fuxman Bass, P. & Fuxman Bass, J. I. Transcription factors in the development and treatment of immune disorders. *Transcription* **16**, 118-140 (2025). https://doi.org/10.1080/21541264.2023.2294623
109 Takaoka, A., Tamura, T. & Taniguchi, T. Interferon regulatory factor family of transcription factors and regulation of oncogenesis. *Cancer Sci* **99**, 467-478 (2008). https://doi.org/10.1111/j.1349-7006.2007.00720.x
110 Sato, M. *et al.* Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. *Immunity* **13**, 539-548 (2000). https://doi.org/10.1016/s1074-7613(00)00053-4
111 Zeller, K. I. *et al.* Global mapping of c-Myc binding sites and target gene networks in human B cells. *Proc Natl Acad Sci U S A* **103**, 17834-17839 (2006). https://doi.org/10.1073/pnas.0604129103
112 Wierstra, I. & Alves, J. The c-myc promoter: still MysterY and challenge. *Adv Cancer Res* **99**, 113-333 (2008). https://doi.org/10.1016/s0065-230x(07)99004-1
113 Ambite, I. *et al.* Multitarget mechanism of MYC inhibition by the bacterial lon protease in disease. *Sci Rep* **15**, 6778 (2025). https://doi.org/10.1038/s41598-025-88093-2
114 Svanborg, C., Agace, W., Hedges, S., Linder, H. & Svensson, M. Bacterial adherence and epithelial cell cytokine production. *Zentralbl Bakteriol* **278**, 359-364 (1993).
115 Hedlund, M., Svensson, M., Nilsson, A., Duan, R. D. & Svanborg, C. Role of the ceramide-signaling pathway in cytokine responses to P-fimbriated Escherichia coli. *J Exp Med* **183**, 1037-1044 (1996). https://doi.org/10.1084/jem.183.3.1037
116 Fajgenbaum, D. C. & June, C. H. Cytokine Storm. *N Engl J Med* **383**, 2255-2273 (2020). https://doi.org/10.1056/NEJMra2026131

117 Cron, R. Q., Goyal, G. & Chatham, W. W. Cytokine Storm Syndrome. *Annu Rev Med* **74**, 321-337 (2023). https://doi.org/10.1146/annurev-med-042921-112837
118 Jarczak, D. & Nierhaus, A. Cytokine Storm-Definition, Causes, and Implications. *Int J Mol Sci* **23** (2022). https://doi.org/10.3390/ijms231911740
119 Wahid, B., Rani, N. & Idrees, M. Cytokine storm syndrome in SARS-CoV-2: a review. *Z Naturforsch C J Biosci* **77**, 65-69 (2022). https://doi.org/10.1515/znc-2021-0062
120 Gao, Y. M., Xu, G., Wang, B. & Liu, B. C. Cytokine storm syndrome in coronavirus disease 2019: A narrative review. *J Intern Med* **289**, 147-161 (2021). https://doi.org/10.1111/joim.13144
121 Hu, B., Huang, S. & Yin, L. The cytokine storm and COVID-19. *J Med Virol* **93**, 250-256 (2021). https://doi.org/10.1002/jmv.26232
122 Riaz, B. & Sohn, S. Neutrophils in Inflammatory Diseases: Unraveling the Impact of Their Derived Molecules and Heterogeneity. *Cells* **12** (2023). https://doi.org/10.3390/cells12222621
123 Jones, H. R., Robb, C. T., Perretti, M. & Rossi, A. G. The role of neutrophils in inflammation resolution. *Semin Immunol* **28**, 137-145 (2016). https://doi.org/10.1016/j.smim.2016.03.007
124 Lacerda Mariano, L. & Ingersoll, M. A. The immune response to infection in the bladder. *Nat Rev Urol* **17**, 439-458 (2020). https://doi.org/10.1038/s41585-020-0350-8
125 Morozov, D. *et al.* Urinary cytokines as markers of latent inflammation in children with chronic pyelonephritis and anorectal malformations. *J Pediatr Urol* **12**, 153.e151-156 (2016). https://doi.org/10.1016/j.jpurol.2016.01.013
126 Prestinaci, F., Pezzotti, P. & Pantosti, A. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health* **109**, 309-318 (2015). https://doi.org/10.1179/2047773215y.0000000030
127 Tacconelli, E. *et al.* Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* **18**, 318-327 (2018). https://doi.org/10.1016/s1473-3099(17)30753-3
128 Zdziarski, J., Svanborg, C., Wullt, B., Hacker, J. & Dobrindt, U. Molecular basis of commensalism in the urinary tract: low virulence or virulence attenuation? *Infect Immun* **76**, 695-703 (2008). https://doi.org/10.1128/iai.01215-07
129 Lutay, N. *et al.* Bacterial control of host gene expression through RNA polymerase II. *J Clin Invest* **123**, 2366-2379 (2013). https://doi.org/10.1172/JCI66451
130 Rybak, J. M., Barber, K. E. & Rybak, M. J. Current and prospective treatments for multidrug-resistant gram-positive infections. *Expert Opin Pharmacother* **14**, 1919-1932 (2013). https://doi.org/10.1517/14656566.2013.820276
131 Kapoor, K. *et al.* Intravenous colistin for multidrug-resistant gram-negative infections in critically ill pediatric patients. *Pediatr Crit Care Med* **14**, e268-272 (2013). https://doi.org/10.1097/PCC.0b013e31828a740f
132 Ambite, I. *et al.* Active bacterial modification of the host environment through RNA polymerase II inhibition. *J Clin Invest* **131** (2021). https://doi.org/10.1172/JCI140333

133 Ambite, I. *et al.* Therapeutic Effects of IL-1RA against Acute Bacterial Infections, including Antibiotic-Resistant Strains. *Pathogens* **13** (2023). https://doi.org/10.3390/pathogens13010042

134 Butler, D. S. C. *et al.* A bacterial protease depletes c-MYC and increases survival in mouse models of bladder and colon cancer. *Nat Biotechnol* **39**, 754-764 (2021). https://doi.org/10.1038/s41587-020-00805-3

135 Wullt, B. *et al.* Immunomodulation-A Molecular Solution to Treating Patients with Severe Bladder Pain Syndrome? *Eur Urol Open Sci* **31**, 49-58 (2021). https://doi.org/10.1016/j.euros.2021.07.003

136 Beerepoot, M. & Geerlings, S. Non-Antibiotic Prophylaxis for Urinary Tract Infections. *Pathogens* **5** (2016). https://doi.org/10.3390/pathogens5020036

137 Czaja, C. A. *et al.* Prospective cohort study of microbial and inflammatory events immediately preceding Escherichia coli recurrent urinary tract infection in women. *J Infect Dis* **200**, 528-536 (2009). https://doi.org/10.1086/600385

138 Antonio, M. A., Hawes, S. E. & Hillier, S. L. The identification of vaginal Lactobacillus species and the demographic and microbiologic characteristics of women colonized by these species. *J Infect Dis* **180**, 1950-1956 (1999). https://doi.org/10.1086/315109

139 Eschenbach, D. A. *et al.* Prevalence of hydrogen peroxide-producing Lactobacillus species in normal women and women with bacterial vaginosis. *J Clin Microbiol* **27**, 251-256 (1989). https://doi.org/10.1128/jcm.27.2.251-256.1989

140 Hawes, S. E. *et al.* Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. *J Infect Dis* **174**, 1058-1063 (1996). https://doi.org/10.1093/infdis/174.5.1058

141 Martin, H. L. *et al.* Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis* **180**, 1863-1868 (1999). https://doi.org/10.1086/315127

142 Zheng, H. Y., Alcorn, T. M. & Cohen, M. S. Effects of H2O2-producing lactobacilli on Neisseria gonorrhoeae growth and catalase activity. *J Infect Dis* **170**, 1209-1215 (1994). https://doi.org/10.1093/infdis/170.5.1209

143 Gupta, K. *et al.* Inverse association of H2O2-producing lactobacilli and vaginal Escherichia coli colonization in women with recurrent urinary tract infections. *J Infect Dis* **178**, 446-450 (1998). https://doi.org/10.1086/515635

144 Hooton, T. M., Roberts, P. L. & Stamm, W. E. Effects of recent sexual activity and use of a diaphragm on the vaginal microflora. *Clin Infect Dis* **19**, 274-278 (1994). https://doi.org/10.1093/clinids/19.2.274

145 Pfau, A. & Sacks, T. The bacterial flora of the vaginal vestibule, urethra and vagina in premenopausal women with recurrent urinary tract infections. *J Urol* **126**, 630-634 (1981). https://doi.org/10.1016/s0022-5347(17)54661-3

146 Stapleton, A. E. The Vaginal Microbiota and Urinary Tract Infection. *Microbiol Spectr* **4** (2016). https://doi.org/10.1128/microbiolspec.UTI-0025-2016

147 Stapleton, A. E. *et al.* Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. *Clin Infect Dis* **52**, 1212-1217 (2011). https://doi.org/10.1093/cid/cir183

148 Aronson, M. *et al.* Prevention of colonization of the urinary tract of mice with Escherichia coli by blocking of bacterial adherence with methyl alpha-D-mannopyranoside. *J Infect Dis* **139**, 329-332 (1979). https://doi.org/10.1093/infdis/139.3.329

149 Michaels, E. K., Chmiel, J. S., Plotkin, B. J. & Schaeffer, A. J. Effect of D-mannose and D-glucose on Escherichia coli bacteriuria in rats. *Urol Res* **11**, 97-102 (1983). https://doi.org/10.1007/bf00256954

150 Edén, C. S. *et al.* Inhibition of experimental ascending urinary tract infection by an epithelial cell-surface receptor analogue. *Nature* **298**, 560-562 (1982). https://doi.org/10.1038/298560a0

151 Lindberg, F., Lund, B., Johansson, L. & Normark, S. Localization of the receptor-binding protein adhesin at the tip of the bacterial pilus. *Nature* **328**, 84-87 (1987). https://doi.org/10.1038/328084a0

152 Spaulding, C. N. *et al.* Selective depletion of uropathogenic E. coli from the gut by a FimH antagonist. *Nature* **546**, 528-532 (2017). https://doi.org/10.1038/nature22972

153 Ofek, I., Mirelman, D. & Sharon, N. Adherence of Escherichia coli to human mucosal cells mediated by mannose receptors. *Nature* **265**, 623-625 (1977). https://doi.org/10.1038/265623a0

154 Chen, S. L. *et al.* Positive selection identifies an in vivo role for FimH during urinary tract infection in addition to mannose binding. *Proc Natl Acad Sci U S A* **106**, 22439-22444 (2009). https://doi.org/10.1073/pnas.0902179106

155 Cusumano, C. K. *et al.* Treatment and prevention of urinary tract infection with orally active FimH inhibitors. *Sci Transl Med* **3**, 109ra115 (2011). https://doi.org/10.1126/scitranslmed.3003021

156 Micoli, F., Bagnoli, F., Rappuoli, R. & Serruto, D. The role of vaccines in combatting antimicrobial resistance. *Nat Rev Microbiol* **19**, 287-302 (2021). https://doi.org/10.1038/s41579-020-00506-3

157 Nielsen, K. L. *et al.* Role of urinary cathelicidin LL-37 and human β-defensin 1 in uncomplicated Escherichia coli urinary tract infections. *Infect Immun* **82**, 1572-1578 (2014). https://doi.org/10.1128/iai.01393-13

158 Spencer, J. D. *et al.* Ribonuclease 7 is a potent antimicrobial peptide within the human urinary tract. *Kidney Int* **80**, 174-180 (2011). https://doi.org/10.1038/ki.2011.109

159 Goetz, D. H. *et al.* The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cell* **10**, 1033-1043 (2002). https://doi.org/10.1016/s1097-2765(02)00708-6

160 Chromek, M. *et al.* The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. *Nat Med* **12**, 636-641 (2006). https://doi.org/10.1038/nm1407

161 Danka, E. S. & Hunstad, D. A. Cathelicidin augments epithelial receptivity and pathogenesis in experimental Escherichia coli cystitis. *J Infect Dis* **211**, 1164-1173 (2015). https://doi.org/10.1093/infdis/jiu577

162 Postow, M. A., Sidlow, R. & Hellmann, M. D. Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. *N Engl J Med* **378**, 158-168 (2018). https://doi.org/10.1056/NEJMra1703481
163 Singh, N. *et al.* Inflammation and cancer. *Ann Afr Med* **18**, 121-126 (2019). https://doi.org/10.4103/aam.aam_56_18
164 Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646-674 (2011). https://doi.org/10.1016/j.cell.2011.02.013
165 Schreiber, R. D., Old, L. J. & Smyth, M. J. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* **331**, 1565-1570 (2011). https://doi.org/10.1126/science.1203486
166 Hanahan, D. Hallmarks of Cancer: New Dimensions. *Cancer Discov* **12**, 31-46 (2022). https://doi.org/10.1158/2159-8290.Cd-21-1059
167 Chen, D. S. & Mellman, I. Elements of cancer immunity and the cancer-immune set point. *Nature* **541**, 321-330 (2017). https://doi.org/10.1038/nature21349
168 de Boer, E. C. *et al.* Role of interleukin-8 in onset of the immune response in intravesical BCG therapy for superficial bladder cancer. *Urol Res* **25**, 31-34 (1997). https://doi.org/10.1007/bf00941903
169 Bisiaux, A. *et al.* Molecular analyte profiling of the early events and tissue conditioning following intravesical bacillus calmette-guerin therapy in patients with superficial bladder cancer. *J Urol* **181**, 1571-1580 (2009). https://doi.org/10.1016/j.juro.2008.11.124
170 Luo, Y., Chen, X. & O'Donnell, M. A. Role of Th1 and Th2 cytokines in BCG-induced IFN-gamma production: cytokine promotion and simulation of BCG effect. *Cytokine* **21**, 17-26 (2003). https://doi.org/10.1016/s1043-4666(02)00490-8
171 De Boer, E. C. *et al.* Induction of urinary interleukin-1 (IL-1), IL-2, IL-6, and tumour necrosis factor during intravesical immunotherapy with bacillus Calmette-Guérin in superficial bladder cancer. *Cancer Immunol Immunother* **34**, 306-312 (1992). https://doi.org/10.1007/bf01741551
172 Böhle, A. & Brandau, S. Immune mechanisms in bacillus Calmette-Guerin immunotherapy for superficial bladder cancer. *J Urol* **170**, 964-969 (2003). https://doi.org/10.1097/01.ju.0000073852.24341.4a