Molecular control of acute cystitis and potential new targets of treatment

Ph.D. Thesis
/Short version /

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1 INTRODUCTION

Urinary tract infections (UTIs) are common, dangerous, and interesting. It is estimated that 40–50% of women and 20% of men worldwide will develop symptomatic UTI at least once in their lifetime. As a consequence, UTIs are encountered at all levels of healthcare, whether primary or tertiary. They account for a majority of specialist referrals and hospitalizations, with substantial financial implications and significant consequences to morbidity, mortality, and antibiotic consumption. Fortunately, new insights are now making it possible to explore immune response modifiers as alternatives to antibiotics.

UTIs differ in clinical presentation and severity, depending on the site of infection and molecular basis of disease. In acute pyelonephritis (APN), bacteria reach the renal pelvis and triggers an intense mucosal inflammatory response with progression into the renal parenchyma. Symptoms include high fever, malaise, back pain, or even life-threatening septic shock. In acute cystitis (CY), infection localized to the urinary bladder and cause a rapid and potent innate immune and inflammatory response in the bladder mucosa, and clinical symptoms include urgency, frequency, and supra-pubic pain, without systemic involvement. While the clinical entities of acute cystitis and pyelonephritis usually are quite distinct, the molecular determinants of this difference in clinical presentation and severity are largely unknown. The bacterial interactions with the bladder mucosa have been shown to create inflammatory cascades, which also involve adjacent cells, such as mast cells, macrophages, and the symptom profile indicates that the nervous system is also engaged in the pathogenesis of acute cystitis, but the grade of nerve cell activation by pathogenic bacteria is not well understood.

2 AIMS

Our aim was to examine the molecular and genetic background of acute bladder infection and identify molecular markers and factors specific in cystitis (*in vitro*, *in vivo,* and human).

1. To evaluate IL-1β response in acute cystitis, *in vitro*. (Paper I)

2. To investigate the inflammasome function, the maturation of IL-1β, and the role of inflammasome constituents (ASC, NLRP-3) in experimential bladder infection, *in vivo*. (Paper I)

3. To address if acute cystitis strains activate a neuropeptide- and neuropeptide receptor (SP/NK1R) response in the urinary bladder (*in vivo* and *in vitro*), and investigate the mucosal- and neuronal cell interactions in acute cystitis, *in vivo*. (Paper II)

4. To assess the inhibition of IL-1 receptor, IL-1β processing and NK1R in acute bladder infection, *in vivo*. (Paper I, II)

5. To determine the human relevance of the IL-1β pathway and neuropeptides. (Paper I, II)

3 MATERIAL AND METHODS

3.1 The IL-1β response in acute cystitis, *in vitro*

In experiments addressing how a hyper-inflammatory state is generated in patients with acute cystitis, human bladder epithelial cells (HTB-9) and kidney epithelial cells (A-498) were infected with selected *E. coli* strains to cause acute cystitis (CY) or asymptomatic bacteriuria (ABU). CY and ABU strains were prospectively isolated during a prospective study of childhood UTI in Göteborg, Sweden. The uropathogenic strain *E. coli* CFT073 and the asymptomatic bacteriuria strain *E. coli* 83972 (ABU 8397) were used for reference. Bacteria were cultured on tryptic soy agar (TSA, 16 h, 37˚C), harvested in PBS. For the *in vitro* infection, cells were washed with phosphate-buffered saline (PBS, pH 7.2), and serum-free media were added prior to infection with appropriately diluted bacteria in PBS. To examine if the capacity to elicit an IL-1β response characterizes acute cystitis strains, epithelial cells were exposed to 10^8 colony- forming units (CFU)/ml of bacteria with gentamicin for 4 hours and secreted IL-1β was quantified in epithelial cell supernatants. In remaining experiments, cells were exposed to 10^4 or 10^5 CFU/ml for 1 hour or 4 hours without antibiotics. Immunostaining of epithelial cells was performed, and supernatants or cell lysates were collected for analysis (ELISA, Western Blot) to evaluate induction and processing of IL-1β in acute bladder infection (*in vitro*).

3.2 The inflammasome function, the maturation of IL-1β, and the role of the inflammasome constituents (ASC, NLRP-3), *in vivo*

To further address if the pathogenesis of acute cystitis involves *Il1b* and genes in the inflammasome pathway, the response to infection in C57BL/6 mice with intact inflammasome function was compared to mice lacking IL-1β (*Il1b*$^{-/-}$), NLRP-3 (*Nlrp3*$^{-/-}$), and ASC (*Asc*$^{-/-}$). Mice under Isofluorane anesthesia were intravesically infected (10^8 CFU in 0.1 ml) through a soft polyethylene catheter. *E. coli* strains that triggered high IL-1β responses in human bladder epithelial cells, *in vitro* (CFT073, CY-17, or CY-92) were used for infection. Bacterial strains were cultured in Luria-Bertani broth overnight. Urine samples were collected and quantitatively cultured. Neutrophils in uncentrifuged urine were counted using a hemocytometer. IL-1β levels in urine were evaluated by ELISA or Western Blot. Animals were sacrificed under anesthesia; kidneys and bladders were aseptically removed, fixed with 4% paraformaldehyde or frozen for sectioning and RNA extraction. Bladder infection was evaluated by gross pathology and histopathology score, where 0 is unchanged compared to the uninfected controls and 10 is the most edematous, most hyperemic, and destroyed tissue architecture. In addition, urine samples for culture and immune response assessments were obtained after 6, 24 hours, 3 and 7 days. Viable counts in homogenized tissues were determined on TSA (37°C, overnight).

To further characterize the molecular basis of bladder pathology, RNA purified from infected bladders was subjected to genome-wide transcriptomic analysis (*Asc*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice with the highest pathology score after seven days, and C57BL/6 WT and *Il1b*$^{-/-}$ mice with low pathology scores, and from uninfected bladders.) Genes modified by infection were defined in comparison with uninfected tissues from mice of each genetic background (Fold Change (FC) > 1.41, and $P < 0.05$).

3.3 The neuropeptide- and neuropeptide receptor (SP/NK1R) activation in urinary bladder infection, *in vitro* and *in vivo*

To address if acute cystitis strains activate a neuropeptide- and neuropeptide receptor response in the urinary bladder mucosa, nerve cells (SH-SY5Y) and bladder epithelial cells (HTB-9) were infected with selected uropathogenic *E. coli* isolate to cause acute cystitis (CY-17). The well-characterized uropathogens CFT073 and the model ABU strain, *E. coli* 83972, were used

for reference. The infection was performed as before (see 3.1). The well-studied neurokinin-1 receptor (NK1R) and its ligand Substance P (SP) expression were selected for analysis. Immunostaining of epithelial cells was performed, and supernatants or cell lysates were collected for analysis (ELISA, Western Blot, qRT-PCR) to evaluate the Neuro-epithelial response to *E. coli* infection, *in vitro.*

To examine the *in vivo* relevance of the neuro-epithelial activation in bladder infection, C57BL/6WT mice were intravesical infected with CY-17 (see 3.2). Bladder infection was evaluated at sacrifice at 24 hours or 7 days after infection. The severity of acute cystitis was quantified as the gross pathology score. Neuropeptide expression was evaluated by immunohistochemistry after staining with specific antibodies of frozen tissue sections, and by qRT-PCR of whole bladder RNA extracts, using primers specific for *Tacr1* and *Ppt-A*. In addition, urine samples for culture and immune response assessments were obtained after 6, 24 hours, 3 and 7 days. Symptoms were documented by video recording of the mice before and at defined times post-infection.

To address if resident nerve and epithelial cells or neutrophils and macrophages are the main source of NK1R and SP expression, tissue sections from infected C57BL/6WT- and *Nlrp3*$^{-/-}$ mice were stained for NK1R- and SP and counter-stained with neutrophil- or macrophage-specific antibodies.

3.4 The inhibition of IL-1 receptor, IL-1β processing and NK1R in acute bladder infection, *in vivo*

To address the effects of IL-1RA or NK1R inhibition *in vivo*, *Asc*$^{-/-}$ or *Nlrp3*$^{-/-}$ mice were treated with the IL-1 receptor antagonist Anakinra (IL-1RA) or the non-peptide NK1R antagonist SR140333. Anakinra (IL-1RA) were injected intraperitoneally (i.p.), 30 minutes before and daily after infection with *E. coli* CFT073 (1mg in 100 µl of PBS i.p. per mouse per day) for 7 days. SR140333 or vehicle were given intraperitoneally, one hour before infection or 30 minutes after infection with CY-17, mice were sacrificed after 24 h. The severity of acute cystitis was quantified as the gross pathology score and tissue pathology score after sacrificed.

3.5 The human relevance of IL-1β and neuropeptides

Urine samples were collected from ambulatory patients with a diagnosis of acute cystitis and compared to samples from patients with long-term ABU. Urine IL-1β, MMP-7, and SP concentrations were quantified by ELISA.

Patients with acute cystitis were enrolled at two primary care clinics in Lund, Sweden. Midstream urine specimens were obtained at the time of diagnosis.

The control ABU samples were collected from patients with long-term ABU, who participated in a prospective placebo-controlled study.

4 RESULTS

4.1 The IL-1β response in acute cystitis, *in vitro*

4.1.1 IL-1β response to acute cystitis strains in epithelial cells

A rapid IL-1β response was detected following infection with acute cystitis (CY) strains CY-17, CY-92, and CY-132 (10^8 CFU/ml, 4 hours with gentamicin) ($P < 0.001$, compared to uninfected cells, two-tailed unpaired t-test). *E. coli* CFT073 also triggered IL-1β secretion, but the response to the ABU 83972 strain was low. The kidney epithelial cells (A-498) did not secrete IL-1β.

4.1.2 Induction and processing of IL-1β

The IL-1β response to infection was further characterized by Western blots, using antibodies recognizing pro-IL-1β and mature IL-1β. An increase in pro-IL-1β and mature IL-1β was detected in supernatants suggesting that the acute cystitis strains activate *de novo* IL-1β synthesis and processing. A rapid increase in IL-1β staining intensity was also observed by confocal microscopy. The Western blot analysis of whole-cell extracts confirmed the increased cellular IL-1β levels.

4.1.3 Epidemiologic association of IL-1β with acute cystitis

Most of the acute cystitis strains (85%) triggered an IL-1β response > 5 pg/ml (range 5 to >1000 pg/ml). To further address if the IL-1β response is cystitis associated, the HTB-9 cells were infected with a collection of ABU strains from the same geographic area and background population as the CY strains (n=62). Only 15 % of the ABU strains triggered a high IL-1β

response > 10pg/ml. The mean IL-1β response to infection was 121,8 pg/ml for the acute cystitis strains compared to 32,4 pg/ml for the ABU strains ($P < 0.001$).

4.2 The inflammasome function, the maturation of IL-1β, and the role of the inflammasome constituents (ASC, NLRP-3), *in vivo*

4.2.1 *In vivo* control of acute cystitis by *Il1b* and inflammasome genes

Major, genotype-specific differences in bladder pathology were detected 7 days after infection. Bladders from *Nlrp3*$^{-/-}$ and *Asc*$^{-/-}$ mice were severely inflamed. Similar but less extensive tissue destruction was observed in bladders from *Nlrp3*$^{-/-}$ mice. The mean gross bladder pathology score of infected *Asc*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice was 7.9 and 7.2, respectively. Bacterial numbers in bladders and bacterial counts were elevated in *Nlrp3*$^{-/-}$ and *Asc*$^{-/-}$ mice. Neutrophil counts in urine increased dramatically in *Asc*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice compared to WT mice. In C57BL/6 wild-type (WT) mice, the bladder epithelium was clearly delineated, with little round cell infiltration, distinct sub-epithelial morphology (mean pathology score 1.5). The low level of macroscopic morphology was confirmed by histology, with no evidence of tissue damage. By immunohistochemistry, bacterial staining was weak and very few neutrophils were detected. In contrast, *Il1b*$^{-/-}$ mice showed no macroscopic change of bladder morphology and tissue pathology. Remarkably, bacteria and neutrophils were not detected in tissue sections, consistent with the low bacterial- and neutrophil numbers in the urine of these mice.

Infection was accompanied by intense mucosal IL-1β staining in bladder tissue sections in WT, *Asc*$^{-/-}$, and *Nlrp3*$^{-/-}$ mice after 24 hours. In parallel with the epithelial staining, IL-1β was detected by ELISA in the urine of infected Asc$^{-/-}$ and *Nlrp3*$^{-/-}$ mice, with lower levels in WT mice. By Western blot analysis, bands of approximately 36 and 18 kDa were detected.

The results suggest that IL-1β, ASC, and NLRP-3 control the pathogenesis of acute cystitis. Remarkably, loss of NLRP-3 and ASC caused exaggerated pathology while the loss of IL-1β was protective, suggesting that IL-1β activation is required to initiate the host response and that a functional inflammasome response is needed to avoid acute disease and pathology.

4.2.2 Gene expression in infected bladders

About 2200 genes were altered exclusively in mice with the highest bladder pathology. Genes with an FC > 100 included metalloproteinase *Mmp7*, the neutrophil and monocyte chemoattractants *Cxcl6* and *Cxcl3*. By top-scoring canonical pathway analysis, genes regulated

in *Asc*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice were shown to control granulocyte and leucocyte diapedesis and signaling, acute phase responses including IL-6 and IL-1β signaling, IL-1R expression and NF-κB signaling, and dendritic cell maturation. These pathways were not significantly regulated in *Il1b*$^{-/-}$ and C57BL/6WT mice, suggesting a direct disease association.

To address the role of IL-1β and the inflammasome for bladder pathology, genes encoding inflammasome complex constituents, inflammasome activators, or downstream effectors were selected for analysis (Qiagen's list of 84 key inflammasome genes). A marked difference was observed between mice with severe acute cystitis (*Asc*$^{-/-}$ *and Nlrp3*$^{-/-}$*)* and resistant mice (C57BL/6WT or *Il1b*$^{-/-}$). Pathology was associated with a drastic increase in overall gene expression in this family, and *Cxcl1*, *Cxcl3*, *Il1b*, and *Il33* expression were most strongly regulated (FC 5-200). Importantly, inflammasome gene expression was virtually absent in *Il1b*$^{-/-}$ mice, further emphasizing that IL-1β is required to drive the response to a bladder infection.

4.2.3 Caspase-1 independent processing of IL-1β

To address if the IL-1β fragments were generated by inflammasome degradation, human bladder epithelial cells were infected in presence of the Caspase-1 inhibitor Z-VAD. Partial inhibition of IL-1β processing was observed (about a 15-40% reduction, compared to cells without Z-VAD), suggesting that the majority of IL-1β processing in response to the acute cystitis strains is caspase-independent.

4.2.4 Mechanism of atypical IL-1β processing

To identify caspase-independent mechanisms of IL-1β processing, genes regulated specifically in Asc$^{-/-}$ and *Nlrp3*$^{-/-}$mice were examined. *Mmp7* was identified as the most strongly regulated gene, and *Mmp7* expression showed a clear association with the overall bladder tissue pathology score and was not regulated in the *Il1b*$^{-/-}$ or C57BL/6WT mice. Furthermore, strong epithelial MMP-7 staining was detected, by immunohistochemistry, in bladder tissue sections from Asc$^{-/-}$ and *Nlrp3*$^{-/-}$ mice. To address if MMP-7 degrades IL-1β, GST-tagged recombinant pro-IL-1β was incubated with the purified enzyme and proteolytic fragments were identified by Western blots using IL-1β specific antibodies. Kinetic analysis showed a time-dependent cleavage of IL-1β with a gradual reduction in full-length protein from 10 to 60 minutes. To address if the cleaved IL-1β fragments were biologically active, human bladder epithelial cells were stimulated with the reaction mixture. The cleaved products activated a dose-dependent

PGE2 response. The results identify a new, MMP-7-dependent mechanism of pro-IL-1β processing in *Asc*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice.

4.3 The neuropeptide- and neuropeptide receptor (SP/NK1R) activation in urinary bladder infection, *in vitro* and in *vivo*

4.3.1 Neuro-epithelial response to *E. coli* infection, *in vitro*

CY-17 infection stimulated cellular NK1R and SP responses. An increase in NK1R and SP staining was observed after infection of differentiated nerve cells with the cystitis isolate (quantified by confocal imaging and western blot analysis). Bladder epithelial cells showed a similar response for infection with CY-17 (10^4 CFU/ml, 4 hours). The APN strain (CFT073) actively induced SP expression in both cells. In contrast, The ABU 83972 strain did not induce an SP or NK1R response.

4.3.2 Neuro-epithelial response to a bladder infection, *in vivo*

NK1R and SP staining intensity were increased after infection with CY-17 ($P = 0.004$ and $P = 0.02$ compared to uninfected controls). NK1R was clearly visible in infected bladders, with a distinct staining pattern of the mucosal nerve plexus in the lamina propria. The increase in NK1R and SP expression was confirmed by qRT-PCR of total bladder RNA. Urine SP levels were elevated after 24 hours and 7 days in infected mice ($P < 0.05$). Symptoms were documented by video recording, and a significant change in behavior was detected. The results suggest that acute cystitis in C57BL/6WT mice is accompanied by a mucosal neuropeptide response and symptoms from the site of infection.

4.3.3 Contributions of neutrophils and macrophages

Despite the massive neutrophil influx in the severely inflamed bladder, only minor evidence of colocalization was observed with NK1R and SP. Suggesting that the resident nerve and epithelial cells are the main sources of NK1R and SP in the inflamed bladder mucosa.

4.4 The inhibition of IL-1 receptor, IL-1β processing and NK1R in acute bladder infection, *in vivo*

4.4.1 Efficacy of the IL-1β receptor antagonist and MMP-7 inhibitor

The IL-1β receptor antagonist Anakinra (IL-1RA) treatment abrogated the macroscopic pathology, virtually removing bladder enlargement, edema, and hyperemia, resulting in a significantly lower pathology score ($P < 0.001$) compared to untreated *Asc*$^{-/-}$ mice. Consistent with this reduction in inflammation, urine neutrophil numbers were low. To further address the contribution of MMP-7, *Asc*$^{-/-}$ mice were also treated with an MMP inhibitor (Batimastat). Treatment reduced the enlargement, edema, and hyperemia that characterized untreated bladders in *Asc*$^{-/-}$ mice, and a marked reduction in pathology was observed ($P=0.002$). The dramatic aggregation of neutrophils and bacteria in the mucosa of untreated *Asc*$^{-/-}$ mice was prevented in the treated mice.

4.4.2 Effects of NK1R inhibition on mucosal inflammation

The SR140333 treatment abrogated the macroscopic pathology, resulting in a significantly lower pathology ($P < 0.05$ for pre-and post-infection treatment), and tissue pathology score ($P = 0.005$ and $P = 0.03$ for pre-and post-infection treatment). The urine neutrophil recruitment also was decreased ($P = 0.02$ and $P = 0.002$ for pre-and post-infection treatment). SR140333 treatment inhibited NK1R staining in infected bladders and the reduction in NK1R expression was confirmed by qRT-PCR. The results identify NK1R as a potential therapeutic target in acute cystitis.

4.5 The human relevance of IL-1β and neuropeptides

Significantly higher mean IL-1β concentrations were found in CY patients than ABU group (264.5 pg/ml and 1.5 pg/ml, respectively, $P < 0.001$). All the patients with acute cystitis had positive MMP-7 levels above the detection limit of 0.15 ng/ml, resulting in mean concentrations of 15.4 ng/ml. In contrast, the mean MMP-7 concentration was significantly ($P<0.001$) lower in the ABU group (4,3 ng/ml). Patients with acute cystitis had significantly higher urine SP (161,1 pg/ml), than patients with asymptomatic bacteriuria (69,7 pg/ml) ($P < 0.001$). The results show that patients with acute cystitis have more elevated concentrations of IL- 1β, MMP-7, and

SP and prove the importance of these molecules, identifying as potential new biomarkers of acute cystitis.

5 DISCUSSION

The results prove, that the cystitis is a hyperinflammatory disorder of the urinary bladder, driven by IL-1β in hosts with defective inflammasome function. In addition, the matrix metalloproteinase-7 was identified as a molecular player in mucosal inflammation, acting by proteolytically cleaving pro-IL-1β in susceptible hosts. The mucosal immune response in acute cystitis is regulated by direct bacterial effects on nerve cells and epithelial cells through the activation of neuropeptides- (SP) and neuropeptide receptors (NK1R). The importance of IL-1β, MMP-7, and NK1R/SP is further proven by the treatment of susceptible mice with IL-1 RA, MMP, or NK1R inhibitor. Elevated IL-1β levels were also detected in the urine of patients with acute cystitis compared to patients with ABU, and MMP-7, SP showed a similar pattern. These results are the first to provide a molecular context for acute cystitis, to reproduce the disease phenotype of acute cystitis patients in an animal model and to validate the IL-1β, MMP-7 and SP response in clinical studies. Furthermore, these findings suggest that IL-1β and MMP-7 may serve as targets for immunomodulatory therapy, or NK1R may be targeted therapeutically to alleviate symptoms associated with acute infection, complementing the increasingly problematic use of antibiotics in this patient group.

5.1 The IL-1β response in acute cystitis

The uropathogenic cystitis strains triggered a rapid IL-1β response in bladder epithelial cells, but not in kidney epithelial cells. The severity of acute cystitis was influenced by bacterial virulence as the acute cystitis strains activated IL-1β more efficiently than ABU strains. This comparison was especially valid, as the CY and ABU strains were isolated from the same pediatric population and geographic area.

IL-1β responses may also be detrimental, however dysregulation of IL-1β has been observed in autoimmune and auto-inflammatory disorders. This dichotomy was also apparent in the present study, where a controlled IL-1β response accompanied the clearance of infection in WT mice. The association of a dysregulated IL-1β response with disease suggested that acute cystitis is an infection-induced, hyper-inflammatory disorder of the urinary bladder. The protected

phenotype in *Il1b*$^{-/-}$mice and the therapeutic efficacy of the IL-1R inhibitor identified IL-1β as the possible main effector principle in bladder pathology, linking acute cystitis to other hyper-inflammatory, IL-1β-driven disorders.

5.2 The inflammasome function, the maturation of IL-1β, and the role of the inflammasome constituents (ASC, NLRP-3)

The dramatic disease phenotype in *Asc*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice strongly suggested that a functional inflammasome response is required to maintain tissue homeostasis in infected bladders. The presence of large quantities of mature IL-1β in the urine of *Asc*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice demonstrated that pro-IL-1β is processed in these mice. Yet, the effects of caspase inhibition were limited, suggesting that additional mechanisms must be involved.

These findings and the results of transcriptomic analysis (*Mmp7* was identified as the most strongly activated gene) add MMP-7 to the list of metalloproteinases (MMP-2, MMP-3, and MMP-9) that cleave pro-IL-1β or degrade IL-1β in other cell types.

The proteolytic cleavage by MMP7 was identified as a potential mechanism of IL-1β activation, and evidence for IL-1β fragmentation by MMP-7 was obtained by direct cleavage of the purified components *in vitro, h*ighlighted, that the cleaved IL-1β fragments by MMP-7 were also biologically active.

The results emphasize the difference in pathogenesis between acute cystitis and acute pyelonephritis. The mechanism used by acute cystitis strains to generate bladder pathology differ from the well-known acute pyelonephritis response caused by fimbriae-mediated activation of TLR4 signaling. This discrepancy was supported by transcriptomic analysis, where the acute cystitis strains were shown to activate MyD88-dependent *Il1b* and *Tnf* expression, potentially explaining the increase in the expression of IL-1β and IL-1β-dependent genes.

5.3 The neuropeptide- and neuropeptide receptor (SP/NK1R) activation in urinary bladder infection

The mucosal epithelial cells are important modulators of the nervous system and immune system and multidirectional interactions between the systems have been documented in autoimmune disease and inflammation. The results showed that the pathogenesis of acute cystitis involves infected nerve cells. Furthermore, the epithelial cells resemble nerve cells and

they express neuropeptide receptors and secrete neuropeptides in response to infection, suggesting that combined action of these two cell types may contribute significantly to pain at the site of infection. In addition, ligand release by each infected cell type was shown to trigger an amplification loop for coactivation of both cell types. It suggests that the symptoms of acute cystitis might be caused by the combined activation of the epithelial barrier and mucosal nerve cells. Additional cells in the lamina propria might play a role in this loop as well, including eosinophils and mast cells, which play an important role in acute cystitis and are known to produce and release SP in mice models and patients with interstitial cystitis/bladder pain syndrome.

5.4 The inhibition of IL-1 receptor, IL-1β processing and NK1R in acute bladder infection

Recurrent or acute cystitis is a handicap, socially, professionally, and emotionally but despite its prevalence and importance for patients and society, acute cystitis is a poorly understood disease and novel therapeutic approaches are needed in this large patient group. The results showed that the pathology in acute cystitis is prevented by the IL-1β receptor blockade and the inhibition of MMP-7 was also protective. Furthermore, NK1R inhibition might constitute an interesting alternative approach to prevent inflammation and pathology in cystitis. These findings suggest that the use of NK1R antagonist or short-term immunotherapy might be a realistic treatment option in patients with acute cystitis or recurrent UTIs, where antibiotic resistance is creating an urgent need for novel therapeutic alternatives.

5.5 The human relevance of IL-1β and neuropeptides

IL-1β is one of the first cytokines detected at the onset and elevation of urinary IL-1β was observed in patients with bacterial cystitis by several groups. Consistent with these, patients with acute cystitis have more elevated concentrations of IL-1β in urine than patients with ABU. The MMP-7 levels also were elevated proving the importance of Il-1β in acute bladder infection. The importance of SP/NK1R in lower urinary tract symptoms, especially in bladder pain, has previously been documented in chronic pelvic pain, interstitial cystitis, and bladder inflammation. Furthermore, increased *Tac1* gene expression was detected after bladder infection in mice, and elevated urine SP concentrations were observed in patients with

interstitial cystitis and in UTI. Consistent with these studies, elevated SP levels were found in patients with acute cystitis compared to patients with ABU.

The identified molecular determinants may also be helpful to address the unmet need for diagnostic tools in this patient group. The frequency of genetic variants, such as ASC mutations, and their relevance to disease would be an interesting focus of prospective clinical studies.

6 CONCLUSION

1. Acute cystitis is an Interleukin-1 beta-driven, hyper-inflammatory condition of the infected urinary bladder. (Paper I)
2. Disease severity was controlled by the mechanism of IL-1β processing, and mice with intact inflammasome function developed a moderate, self-limiting form of cystitis. The most severe form of acute cystitis was detected in mice lacking the inflammasome constituents ASC or NLRP-3, and IL-1β processing was hyperactive in these mice, due to a new, non-canonical mechanism involving the matrix metalloproteinase-7 (MMP-7). (Paper I)
3. The infection activates Neurokinin-1 receptor (NK1R) and Substance P (SP) expression in nerve cells and bladder epithelial cells *in vitro* and *in vivo* in the urinary bladder mucosa, and a neuro-epithelial activation loop was identified that participates in the control of mucosal inflammation and pain in acute cystitis. (Paper II)
4. The IL-1 receptor antagonist and NK1R inhibitors attenuated acute cystitis in susceptible mice, supporting a role in disease pathogenesis. Furthermore, the MMP inhibitor had a similar therapeutic effect. (Paper I, II)
5. Elevated levels of IL-1β, SP, and MMP-7 were detected in patients with acute cystitis, suggesting a potential role as biomarkers and potential therapeutic targets. (Paper I, II)

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Publications directly related to the Ph.D. thesis

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*These authors contributed equally to this work.

II. Butler DSC, Ambite I, Nagy K, Cafaro C, Ahmed A, Nadeem A, Filenko N, Tran TH, Andersson KE, Wullt B, Puthia M, Svanborg C; Neuroepithelial control of mucosal inflammation in acute cystitis. SCIENTIFIC REPORTS 8 Paper: 11015, 15 p. (2018) **IF: 4.122**

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Full papers

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Regarding our joint results referred to in his / her thesis, were obtained

- as the result of joint contribution by the applicant and myself;
- the applicant's contribution was prominent in obtaining the results referred.
- the applicant has a shared first authorship in this article

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Regarding our joint results referred to in his / her thesis, were obtained

- as the result of joint contribution by the applicant and myself;
- the applicant's contribution was prominent in obtaining the results referred.

Title:	Neuroepithelial control of mucosal inflammation in acute cystitis
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