Host-Pathogen Interactions Mediating Pain of Urinary Tract Infection

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Background. Pelvic pain is a major component of the morbidity associated with urinary tract infection (UTI), yet the molecular mechanisms underlying UTI-induced pain remain unknown. UTI pain mechanisms probably contrast with the clinical condition of asymptomatic bacteriuria (ASB), characterized by significant bacterial loads without lack symptoms.

Methods. A murine UTI model was used to compare pelvic pain behavior elicited by infection with uropathogenic Escherichia coli strain NU14 and ASB strain 83972.

Results. NU14-infected mice exhibited pelvic pain, whereas mice infected with 83972 did not exhibit pain, similar to patients infected with 83972. NU14-induced pain was not dependent on mast cells, not correlated with bacterial colonization or urinary neutrophils. UTI pain was not influenced by expression of type 1 pili, the bacterial adhesive appendages that induce urothelial apoptosis. However, purified NU14 lipopolysaccharide (LPS) induced Toll-like receptor 4 (TLR4)–dependent pain, whereas 83972 LPS induced no pain. Indeed, 83972 LPS attenuated the pain of NU14 infection, suggesting therapeutic potential.

Conclusions. These data suggest a novel mechanism of infection-associated pain that is dependent on TLR4 yet independent of inflammation. Clinically, these findings also provide the rationale for probiotic therapies that would minimize the symptoms of infection without reliance on empirical therapies that contribute to antimicrobial resistance.

Urinary tract infection (UTI) is the second most common infectious disease that sends both men and women to seek treatment. In the United States, UTI accounts for >7 million office visits and >1 million emergency room visits, necessitating 100,000 hospitalizations annually [1]. Most community-acquired UTIs are due to infection by uropathogenic Escherichia coli (UPEC) that elicit an inflammatory response in the bladder during acute bacterial cystitis. Patients with UTI frequently have symptoms that include dysuria, voiding frequency or urgency, and pelvic pain [2]. In contrast, ~5% of patients with UTI do not exhibit any of these symptoms and receive a diagnosis of asymptomatic bacteriuria (ASB) [3]. Although most patients with UTI experience pelvic pain, the precise mechanism underlying UTI-induced pelvic pain remains unknown.

Infection of the urinary tract by UPEC is associated with a robust innate immune response characterized by urothelial production of inflammatory chemokines and cytokines. Local production of inflammatory chemokines results in the rapid recruitment of neutrophils into the bladder lumen, which in turn mediate bacterial clearance [4, 5]. The activation of the innate immune response in the urinary tract is dependent on pattern recognition of UPEC pathogen-associated molecular patterns by so-called pattern recognition receptors. UPEC pathogen-associated molecular patterns include lipopolysaccharide (LPS), flagella, type 1 pili, and pap pili [6, 7], which can act through family members of pattern recognition receptors, including the Toll-like receptors (TLRs) and nucleotide binding and oligomerization domainlike receptors [8].

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The cystitis isolate NU14 is considered archetypal for UPEC and has been used to study many aspects of UTI pathogenesis [9]. Several virulence factors have been characterized in UPEC, such as type 1 pili and LPS; the best-characterized UPEC virulence factor is the type 1 pilus, adhesive organelle [10]. Type 1 pili bind host urothelial cells by virtue of the FimH adhesin protein at the pilus tip, and FimH has lectin activity specific for mannosylated glycoproteins [11]. In seminal studies by Mulvey and colleagues, instillation of the UPEC isolate NU14 induced rapid urothelial apoptosis that was abrogated after instillation of the isogenic ΔfimH mutant NU14–1, indicating a requirement for type 1 pili in the urothelial apoptotic response to UPEC infection [12, 13]. LPS is also a common virulence factor and is a component of the gram-negative cell wall that binds to TLR4, initiating the innate response against UPEC. In culture, TLR4 signaling is required for nuclear factor κB activation and mediates LPS induction of urothelial interleukin (IL)–8 secretion by NU14 [14, 15]. Despite this understanding of specific virulence factors in UTI pathogenesis, the precise mechanisms of UTI-associated pain are unexplored.

Pain originating from a visceral organ is typically referred to as a corresponding “dermatome” on the skin that shares common spinal cord innervation with the given visceral organ [16]. We have used this phenomenon elsewhere to show that bladder-induced pelvic pain is mast cell dependent and can be positively or negatively modulated by visceral organ cross-talk in a murine neurogenic cystitis model [17, 18]. Therefore, we hypothesized that similar mechanisms mediate pelvic pain in UTI. To elucidate the mechanisms of pelvic pain associated with UTI, we used a murine model of UTI. We found that a UPEC strain induced a pelvic pain response that was not evoked by infection with an ASB strain. This result is similar to that in patients infected with this ASB strain [19, 20]. Pain responses were independent of bacterial pili but could be recapitulated with bladder instillation of purified LPS. Thus, LPS induces pelvic pain, suggesting novel therapeutic approaches to UTI.

**METHODS**

**Animals.** Adult female mice (C57BL/6, C3H-HeOuJ, or C3H-HeJ; 10–14 weeks old) were purchased from Jackson Laboratory. Mast cell–deficient KitW 7sh/KitW 7sh mice (B6.Cg-KitW 7sh/Hifeleahom; 10–14 weeks old) were maintained in facilities, as described elsewhere [17]. All experiments were performed using protocols approved by the Northwestern University Animal Care and Use Committee.

**Origins of UPEC and ASB strains.** NU14 is a clinical isolate of *E. coli* originally obtained from the urine of a patient with cystitis (Table 1) and is considered archetypal for UPEC [13]. NU14–1 is the corresponding fimH mutant that does not express type 1 pili [21]. ASB strain 83972 was isolated from a young Swedish girl who was infected for at least 3 years without symptoms [19, 20] and is one of the most extensively characterized ASB strains [22, 23].

**Infection.** Female mice were anesthetized with isoflurane and instilled via transurethral catheter with a volume of 10 μL containing 1 × 10⁸ colony-forming units of either NU14 or 83972 bacteria. After infection, bladders were harvested, homogenized, and plated on eosin methylene blue agar for colonization, as described elsewhere [24]. Urine was prepared according to manufacturer’s recommendations and assayed for neutrophil myeloperoxidase (MPO) by enzyme-linked immunosorbent assay (Hycult Biosciences). Mice were tested before bacterial infection (baseline) and for up to 14 days after infection. Referred hyperalgesia and tactile allodynia were tested, as described elsewhere [17, 18], using von Frey filaments applied to the abdomen [17, 18, 25] and the plantar region of the hind paw [17, 18, 25].

**Purification of LPS.** LPS was isolated from *E. coli* strains NU14 and 83972 grown overnight on Luria-Bertani agar plates. Cultures were collected by swabbing, suspended in phosphate-buffered saline, and collected by centrifugation. LPS was then isolated using the LPS Extraction Kit (iNTRON Biotechnology), according to the manufacturer’s recommended protocol. Preparations were then further purified to remove any contaminants that could activate additional members of the TLR pathway [26]; this process included ethanol precipitation, digestion with DNase I and RNase, and digestion with proteinase K, followed by a final ethanol precipitation. The concentration of LPS in each sample was determined with the Purpald assay, by mea-

**Table 1. Escherichia coli Strains Used in This Study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Characteristics</th>
<th>MSHA response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NU14</td>
<td>B2 cystitis isolate</td>
<td>Positive</td>
<td>[21]</td>
</tr>
<tr>
<td>NU14–1</td>
<td>NU14 with insertional disruption of fimH</td>
<td>Negative</td>
<td>[21]</td>
</tr>
<tr>
<td>83972</td>
<td>Asymptomatic bacteriuria isolate</td>
<td>Negative</td>
<td>[19]</td>
</tr>
<tr>
<td>83972:pREG153</td>
<td>83972 with vector plasmid pREG153</td>
<td>Negative</td>
<td>[22]</td>
</tr>
<tr>
<td>83972:pGB4</td>
<td>83972 with fim plasmid pGB4</td>
<td>Positive</td>
<td>[22]</td>
</tr>
</tbody>
</table>

**NOTE.** MSHA, mannose-sensitive hemagglutination.
suring 2-keto-3-deoxyoctonate (KDO) levels and comparing them to a KDO standard curve [27].

**LPS instillation.** NU14 or 83972 LPS (2.0µg/25 µl) was instilled via transurethral catheter into the bladder while isoflurane anesthesia was maintained. All mice were tested for referred hyperalgesia with von Frey filaments, before and 1, 4, 24, 48, 72, and 96 h after LPS instillation.

**Lidocaine treatment.** Lidocaine drug therapy was administered as a 2% lidocaine solution in water that was instilled into the colon via a Hamilton syringe catheter, as described elsewhere [18].

**Macrophage responses to LPS.** Splenic macrophages were isolated from 8–10-week-old mice using CD11b MACS in conjunction with MACS LS magnetic columns (Miltenyi); the obtained purity was ~85%. Purified splenic macrophages were cultured under standard conditions in the presence of 100 ng/mL 83972 or NU14 LPS for 4 or 8 h. After incubation, supernatants were collected for quantifying secreted IL-6 by enzyme-linked immunosorbent assay (R&D Systems), and LPS-stimulated cells were immediately analyzed by flow cytometry. Flow cytometry was performed by staining for the following antibodies: allophycocyanin–anti-CD80 or biotin anti-CD86 and streptavidin-conjugated phycoerythrin-indotricarbocyanine (eBioscience), phycoerythrin-CD11b, and anti–CD16-CD32; hamster IgG and rat IgG2a were used as isotype controls (BD Pharmingen). Stained cells were analyzed on a FACS Canto flow cytometer (Becton Dickinson) with FACS Diva acquisition and FlowJo analysis software, version 8.7.3 (Tree Star).

**Statistical analyses.** Results were expressed as means ± standard errors of the mean. Colonization, inflammation, and behavioral data were analyzed with the Student t test or a Kruskal-Wallis test, followed by the Dunn post test, or with analysis of variance, followed by Dunnett’s post test; Prism software, version 5 (GraphPad), was used, as appropriate. Differences were considered statistically significant at P<.05.

## RESULTS

**NU14 induction of pain specific to the pelvic area.** Female C57BL/6J (B6) mice were instilled with 1 × 10⁶ colony-forming units of *E. coli* into the bladder via transurethral catheter (Table 1). To assess tactile sensitivity, mice were stimulated with von Frey filaments. Mechanical stimulation of the pelvic area of saline-instilled mice resulted in a response frequency associated with the applied force, and this response profile did not change during the 14-day course of the experiment (Figure 1A and 1E). Similar to findings in saline-instilled mice, the response profile of 83972-infected mice did not change significantly during the 14-day course of the experiment (Figure 1B and 1E). In contrast, although NU14-infected mice exhibited the same baseline response as saline- and 83972-infected mice, the response to pelvic stimulation was significantly greater by day 1

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**Figure 1.** NU14 inducing pelvic pain in female mice. Referred visceral hyperalgesia was measured as responses to mechanical stimulation of the pelvic region by using von Frey filaments of 5 calibrated forces. Data are reported as mean percentages of positive response ± standard errors of the mean before instillation of bacteria (baseline) and at postinfection day (PID) 1, 2, 3, 4, 5, 6, 7, 10, and 14. A, Responses to pelvic stimulation of saline-instilled female C57BL/6J mice (B6) (n = 15). B, Responses to pelvic stimulation of female B6 mice infected with 83972 (n = 10). C, Responses to pelvic stimulation of female B6 mice infected with NU14 (n = 15). D, Responses to pelvic stimulation of female KitW−/KitW− mice infected with NU14 (n = 9). Analysis of variance indicated a significant increase in response frequency from baseline for all filaments tested in NU14-treated mice on days 1–10 (P<.05), with no significant differences in baseline between saline- and NU14-treated mice. NU14-treated mice exhibited a significant increase in response frequency from baseline in the largest 3 filaments only at day 14 (P<.05). E, Percentage responses for each day were calculated as total responses to all fibers relative to baseline responses. Symbol key in A applies to panels A–D.
after infection, peaked at day 2, and slowly declined but remained significantly elevated until day 10 ($P < .05$) (Figure 1C and 1E). There were no NU14-induced changes in tactile sensitivity of the plantar region of the hind paw or detectable weight changes (data not shown). These data indicate that NU14 induces no changes in gross physiology and pain specific to the pelvic region.

**Mast cells and NU14-induced pelvic pain.** In a recent study, we showed that a mouse model of interstitial cystitis developed bladder-associated pelvic pain that is dependent on mast cells [17], and other studies have shown that mast cells are important in defense from bacterial infection (reviewed in [28]). To test whether mast cells play a role in NU14-induced pain, we infected mast cell–deficient (KitW-sh/KitW-sh) mice with NU14. Similar to NU14-infected B6 mice, NU14-infected KitW-sh/KitW-sh mice exhibited responses to pelvic stimulation that were significantly greater by day 1 after infection, peaked at day 2, and remained significantly elevated until day 10 ($P < .05$) (Figure 1D and 1E). In contrast to the wild-type NU14-infected mice, NU14-infected KitW-sh/KitW-sh mice exhibited a prolonged increase in pelvic sensitivity until day 7 (Figure 1E), suggesting that mast cells may help resolve the pelvic pain.

**Organ cross-talk in UTI-associated pelvic pain.** We have shown elsewhere that instillation of 2% lidocaine directly into the colon attenuates bladder-induced pelvic pain in mice, demonstrating organ cross-talk in pelvic pain relief [18]. We used a similar strategy to determine whether NU14-induced pelvic pain was modulated by organ cross-talk. Lidocaine instilled into the colon significantly reduced the response to mechanical stimulation with von Frey filaments, by $\sim 66\%$ in wild-type infected mice (Figure 2B) and $\sim 56\%$ in mast cell–deficient mice (Figure 2C), whereas 83972 animals exhibited no loss of pelvic sensitivity after saline instillation (Figure 2A). These data suggest that neurogenic cystitis and UTI-associated pelvic pain are similarly modulated by organ cross-talk.

**NU14-induced pelvic pain not correlated with bladder colonization.** Infection of the urinary tract by UPEC is the most frequent cause of UTI, so we hypothesized that bacterial colonization directly correlates with UTI-associated pelvic pain. Mice were infected with either 83972 or NU14, and bacterial colonization was measured 24 h and 14 days after infection. Both 83972 and NU14 colonized the bladder, but NU14 colonization was significantly greater than 83972 colonization at 24 h and 14 days after infection ($P < .01$) (Figure 3A and 3C). However, pelvic pain was not correlated with bladder colonization at 24 h ($r = −0.20$) (Figure 3B) or 14 days ($r = −0.17$) (Figure 3D) after infection.

**Type 1 pilus status and UTI-associated pelvic pain.** A major difference between NU14 and 83972 strains is that NU14 bacteria express type 1 pili on their surface, whereas 83972 bacteria are not piliated (Table 1). Therefore, we hypothesized that type 1 pilus mediate NU14-induced pelvic pain. To test this hypothesis, we varied pilus expression for NU14 and 83972 bacteria (Table 1), instilled these bacteria into the bladder of B6 mice, and then assessed pain responses. Before instillation, a hemagglutination assay was performed on each bacterial strain to confirm the presence or absence of type 1 pili (data not shown). We found that endowing 83972 with functional type 1 pilus expression did not induce significant pelvic pain (Figure 3A–C and 4F). In contrast, both NU14 ($fimH^+$) and NU14–1 ($fimH^-$) induced significant pelvic pain in infected mice (Figure 4D–F) ($P < .05$). These data demonstrate that type 1 pilus status does not influence UTI-associated pelvic pain, suggesting that pain is initiated by other bacterial factors.

**NU14-induced pelvic pain not correlated with bladder inflammation.** Because the inflammatory response to UPEC

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**Figure 2.** Urinary tract infection (UTI)–associated pelvic pain is attenuated by organ cross-talk. Modulation of referred visceral hyperalgesia by colonic lidocaine was measured as responses to mechanical stimulation of the pelvic region with von Frey filaments of 5 intensities. Responsiveness was characterized before infection (baseline), on postinfection day (PID) 1, and 45 min after colonic administration of 2% lidocaine on day 1. Instilling 50 $\mu$L of lidocaine into the colon of B6 mice ($n = 15$; B) or mast cell–deficient KitW-sh mice ($n = 5$; C) reduced pelvic pain responses ($P < .05$), whereas lidocaine had no significant effect on mice infected with 83972 ($n = 10$; A). Data are reported as mean percentages of positive responses $\pm$ standard errors of the mean.
is dominated by neutrophil influx, we quantified MPO as a marker of inflammation in the murine UTI model, in a study reported elsewhere [29]. Here we quantified MPO to determine whether pelvic pain correlated directly with inflammation. Mice were infected with either 83972 or NU14, and MPO levels in the urine were measured 6 or 24 h after infection. MPO was not significantly different between 83972- and NU14-infected mice at 6 or 24 h after infection (Figure 5A and 5C, respectively). Pelvic pain was not correlated with MPO levels at 6 h \((r = –0.20; \, B)\) or 24 h \((r = 0.39)\) after infection. Furthermore, in primary cultures of macrophages, both NU14 and 83972 LPS induced similar significant increases in CD80+ cells at 4 h (Figure 5E and 5G), with recovery by 8 h (Figure 5F), whereas IL-6 supernatant levels were significantly elevated only at 8 h (Figure 5H). In contrast, CD86+ cell numbers were unchanged at either 4 or 8 h after NU14 or 83972 LPS application, compared with medium (data not shown).

**Induction of pelvic pain by NU14 LPS.** Other studies have shown that when LPS was injected intraperitoneally or into the footpad of rats it produced hyperalgesia [30, 31], and LPS has been shown to be important in bacteria-induced bladder inflammation [32, 33]. Therefore, we tested the hypothesis that LPS mediates NU14-induced pelvic pain. LPS was purified from 83972 or NU14 and instilled directly into the bladder of female B6 mice. Interestingly, the response profile of 83972 LPS-instilled mice did not change during the 4-day course of the experiment (Figure 6B). In contrast, the response to pelvic stimulation was significantly greater 1 h after NU14 LPS instillation, peaking at 4 h after instillation and declining to baseline levels by 96 h after instillation \((P < 0.05)\) (Figure 6A and 6B). Furthermore, levels of urinary MPO 6 h after instillation did not differ significantly between 83972 and NU14 LPS–instilled mice (Figure 6C). These data suggest that NU14 LPS is capable of inducing pelvic pain independent of neutrophil-induced inflammation.

LPS acts through TLR4, and previous studies have shown that TLR4 is expressed on nociceptive neurons [34]. Therefore, we tested the hypothesis that NU14 LPS induces pelvic pain through TLR4, using TLR4-deficient C3H-HeJ and the isogenic wild-type C3H-HeOuJ mouse strain. C3H-HeJ mice exhibited significantly reduced pelvic pain compared with the C3H-HeOuJ mice 4 h after instillation of NU14 LPS \((P < 0.05)\) (Figure 6D). These data suggest that NU14 LPS is acting through TLR4 to initiate NU14-induced pelvic pain.

Because 83972 LPS did not cause pelvic pain, we speculated

**Figure 3.** NU14-induced pelvic pain is not correlated with bacterial colonization of the bladder. Wild-type mice were infected with either 83972 or NU14, and bacterial colonization was measured 24 h \((n = 10\) for 83972, \(n = 20\) for NU14) or 14 days \((n = 10\) for 83972, \(n = 15\) for NU14) after infection. A and C, Both 83972 and NU14 colonized the bladder, but NU14 colonization was significantly greater than 83972 colonization at 24 h \((A)\) and 14 days \((C)\) after infection; cfu, colony-forming units; PID, postinfection day; \(* * P < .01\). Mean colonization levels are indicated by solid lines; bladder homogenates without detectable bacterial colonization appear on the x-axis. B and D, Pelvic pain was not correlated with bladder colonization at 24 h \((r = 0.20; \, B)\) or 14 days \((r = 0.17; \, D)\) after infection.
LPS-Induced UTI Pelvic Pain

Figure 4. Type 1 pilus status does not influence urinary tract infection (UTI)–associated pelvic pain. Referred visceral hyperalgesia was measured as responses to mechanical stimulation of the pelvic region by von Frey filaments of 5 intensities. Responsiveness was characterized at baseline and 24 h after bacterial infection. A, Responses to pelvic stimulation of female B6 mice infected with 83972 without the fimB-fimD gene cluster (no type 1 pili; n = 10); PID, postinfection day. B, Responses to pelvic stimulation of female B6 mice infected with 83972:pREG153 without the fimB-fimD gene cluster (no type 1 pili; n = 10). C, Responses to pelvic stimulation of female wild-type mice infected with 83972:pGB4 with the fimB-fimD gene cluster (expresses type 1 pili; n = 10). D, Responses to pelvic stimulation of wild-type mice infected with NU14 with fimH (expresses type 1 pili; n = 20). E, Responses to pelvic stimulation of wild-type mice infected with NU14–1 without fimH (greatly reduced expression of type 1 pili; n = 10). Data are reported as the mean percentages of positive responses ± standard errors of the mean. Analysis of variance indicated a significant increase in responses from baseline at all filaments tested in NU14- and NU14–1–treated mice at 24 h after infection (P < .05), with no significant differences in baseline between any of the 5 groups of infected mice. F, Percentage responses 24 h after infection were calculated as total responses to all fibers relative to baseline responses for 83972 (white bar), 83972:pREG153 (light gray bar), 83972:pGB4 (dark gray bar), and NU14 with or without fimH (black bars).

that it could act as a TLR4 antagonist to block NU14-induced pelvic pain. To test this possibility, B6 mice were instilled with NU14 bacteria and then instilled with saline 24 h later; Saline instillation did not alter the development of significant pelvic pain (P < .05) (Figure 6E). Similarly, mice instilled with both NU14 bacteria and 83972 LPS and then instilled with saline 24 h later also developed significant pelvic pain (data not shown). In contrast to these groups, mice instilled with NU14 bacteria and then instilled with 83972 LPS 24 h later exhibited a 40% reduction in NU14-induced pelvic pain 24 h after instillation of 83972 LPS (P = .051) (Figure 6F). These data suggest that 83972 LPS acts as a TLR4 antagonist to attenuate UTI-induced pelvic pain.

DISCUSSION

We previously showed that mast cells mediate bladder-associ-
ated pelvic pain in a murine neurogenic cystitis model of in-
terstitial cystitis [17]. As in those studies, we show here that
colic lidocaine attenuated UTI-associated pelvic pain, indi-
cating that UTI pain is similarly modulated by organ cross-talk
[18]. Given these parallels and other studies showing that mast
cells are important for clearing bacterial infection and are ac-
tivated by UPEC FimH (reviewed in [28]), we hypothesized
that mast cells also mediate UTI-associated pelvic pain. How-
ever, our data demonstrate that mast cell–deficient mice in-
fected with NU14 developed the same magnitude of pelvic pain as infected wild-type mice, suggesting that UTI pelvic pain
develops independent of mast cells. The differential role of mast
cells in these pelvic pain models may result from the mechanism
of pain induction. Pelvic pain in the neurogenic cystitis is de-
pendent on mast cell–derived histamine and is transduced by
histamine receptors 1 and 2 [17]. In contrast, pelvic pain in-
duced in murine UTI originates from bacterial LPS that is
transduced by TLR4 (see below). Therefore, the differential role
of mast cells in cystitis models may be due to the nature of
the initiating insult, which neurogenic cystitis is initiated in the
Figure 5. NU14-induced pelvic pain is not correlated with urine neutrophil myeloperoxidase (MPO). Female B6 mice were infected with 83972, 83972:pGB4, NU14, or NU14–1, and neutrophil MPO, a measure of inflammation, was quantified 6 h (n = 28, 10, 23, and 10, respectively) or 24 h (n = 30, 8, 15, and 10, respectively) after infection. A, NU14–1 MPO levels are significantly lower than those for both NU14 and 83972:pGB4 (P < .05) 6 h after infection, with no other statistically significant differences between any groups. C, NU14–1 MPO levels were significantly lower than those for 83972:pGB4, and NU14 MPO levels were significantly higher than those for 83972 (P < .05) 24 h after infection, with no other statistically significant differences between any groups. B and D, Mean MPO levels are indicated by solid lines. Pelvic pain was not correlated with MPO levels at 6 h (r = −0.01; B) or 24 h (r = 0.39; D) after infection. E and F, Histograms of CD80+ cells 4 h (E) and 8 h (F) after lipopolysaccharide (LPS) stimulation. Solid gray line with shading, medium; dashed line, isotype control; dotted black line, 83972; solid black line, NU14. G, Both NU14 and 83972 LPS significantly increased numbers of CD80+ macrophages compared with medium; *P < .05; for comparison with medium at 4 h after LPS stimulation (analysis of variance [ANOVA]). H, Both NU14 and 83972 LPS induced significantly increased levels of interleukin (IL)-6; *P < .05; for comparison with medium at 8 h after LPS stimulation (ANOVA).
Lipopolysaccharide (LPS) induces pelvic pain and represents a therapeutic target for pain relief. Referred visceral hyperalgesia was measured as responses to mechanical stimulation of the pelvic region with von Frey filaments of 5 intensities. Responsiveness was characterized at baseline and 1, 4, 24, 48, 72, and 96 h after 83972 or NU14 LPS instillation.

A. Responses to pelvic stimulation of female B6 mice instilled with NU14 LPS. Data are reported as mean percentages of positive responses ± standard errors of the mean (n = 8 for 83972 and NU14). Analysis of variance indicated a significant increase in response frequency from baseline for all filaments tested in NU14 LPS–treated mice at 1, 4, and 24 h after instillation (P < .05), with no significant differences in baseline between 83972- and NU14-treated mice. B. Percentage responses 1, 4, 24, 72, and 96 h after instillation were calculated as total responses to all fibers relative to baseline responses for 83972 or NU14 LPS–instilled mice. C. Myeloperoxidase (MPO) levels in 83972 and NU14 LPS–instilled mice. D. C3H-HeJ mice (n = 10) instilled with NU14 LPS exhibited a significant reduction in pelvic pain compared with C3H-HeOuJ mice (n = 10) instilled with NU14 LPS (* P < .05). Percentage responses 4 h after instillation were calculated as total responses to all fibers relative to baseline responses for all groups of mice (NU14 followed by saline, n = 8; NU14 followed by 83972 LPS, n = 9).

Our recent studies demonstrated that mast cells played roles in bladder pathophysiology that were separable from bladder-associated pain, and the data presented here suggest similarly separable host responses induced by UPEC infection. NU14 induced pelvic pain behavior in mice that was not observed after infection with the ASB strain 83972, thus recapitulating human behavioral responses to UPEC and ASB strains [19, 20]. FimH induces rapid urothelial apoptosis and formation of urothelial lesions, suggesting that FimH bladder pathology mediates pain. However, we found no relationship between type 1 pilus expression and pain behavior, for either NU14 or 83972 (Figure 4).

Inflammation is often assumed to underlie infection pain, but we also failed to observe a consistent relationship between pain and inflammation. Pain did decay over time as urinary MPO decayed, but both whole bacteria and LPS purified from NU14 and 83972 induced similar inflammatory responses in vitro and in vivo. This observation is also consistent with clinical findings that patients with ASB, who lack pain by definition, nonetheless often have pyuria [2, 3]. Thus, the inflammation of cystitis is not sufficient by itself to mediate the pain of cystitis. Although our data suggest that LPS mediates cystitis pain through TLR4, the effects on pain appear independent of inflammatory actions.

Our data demonstrate that LPS mediates pelvic pain behavior, yet the structural determinants of this effect are unknown. NU14 LPS confers a smooth colony morphology, whereas 83972 exhibits the rough phenotype, together suggesting that O antigen may mediate the pain response. However, ECOR reference strain 71 is an ASB strain with O78 serotype, indicating that O antigen alone is insufficient to endow a strain with a pain-causing phenotype. Alternatively, it is possible that pain is another activity mediated by the lipid A moiety that is separable from inflammatory effects, but the specific LPS structural elements influencing pain are unknown.

Our results show that LPS isolated from NU14 induced pelvic pain through a TLR4-dependent mechanism, providing a novel pathway of pelvic pain induction and relief. C3H-OuJ and C3H-HeJ are co-isogenic mouse strains, making them ideal for studying LPS-induced TLR4 signaling. Over time, the C3H-HeJ strain developed a spontaneous mutation in the TLR4 gene, rendering these mice defective in TLR4 signaling [35]. Although central nervous system, whereas UTI in the peripheral nervous system.
TLR4 involvement is well documented for bacterial defense, it has only recently been implicated in nociception. Similar to the results presented here, 1 previous study demonstrated enhanced thermal sensitivity in mice infected with bacteria [36]. Furthermore, our study is the first, to our knowledge, to demonstrate that LPS is the initiator of UTI-induced pelvic pain. TLR4 signaling appears to be important in patients with ASB and animal models [37–39]. Ragnarsdottir et al [37] report lower neutrophil TLR4 expression levels in patients with ASB that correspond with elevated levels of the TRIF adaptor protein and reduced levels of the TLR4 inhibitor SIGIRR. These data collectively support the role for TLR4 in UTI-induced pelvic pain.

Little is known about how TLR4 contributes to pain generally, much less pelvic-specific pain in UTI. Broadly, however, LPS could trigger UTI pain peripherally or centrally. TLR4 has been implicated in neuropathic pain because activation of microbial TLR4 leads to nuclear factor κB–dependent cyclooxygenase 2 up-regulation that contributes to central sensitization in peripheral injury models [40–43]. Supporting this possibility, intrathecal LPS induces enhanced dorsal horn neuronal firing that correlates with allodynia and hyperalgesia [44]. Either such mechanism would require that LPS from the bladder diffuses into the central nervous system, perhaps an unlikely scenario given that we observed pain responses within 1 h. Alternatively, TLR4 could mediate pain peripherally, either through sensory nerves or via the urothelium itself. Nociceptors have recently been shown to express TLR4, which could then lead to LPS-induced firing due to TLR-induced protein kinase C activation [34, 45–48]. Finally, urothelial TLR4 could mediate pain responses indirectly, by stimulating urothelial production of reactive oxygen species that can then activate peripheral nociceptors [49]. Regardless of the precise mechanism, the involvement of TLR4 identifies a novel therapeutic target for managing UTIs.

Current UTI treatment practice employs empirical use of antimicrobials to eradicate acute infection, although the clinical condition of ASB already indicates that bladder infection is not necessarily deleterious in itself. Moreover, experimental infection of human subjects demonstrated that UTI was resolved naturally, over the course of several days, in the absence of intervention with antimicrobials [50]. Together, these observations suggest that an alternative therapeutic strategy for UTIs is to treat the symptoms that drive patients to visit their physicians: pain and discomfort. We show that LPS purified from 83972 attenuated pelvic pain during NU14 infection (Figure 6E). Whereas 83972 is being explored as a potential probiotic, it has been assumed that its mechanism of action was due to its ability to compete with UPEC for colonization of the urinary tract, thus preventing UTI [23]. Instead, our data suggest that 83972—or LPS derived from an ASB strain—may actually suppress UTI pain by interfering with TLR4-dependent pain induced by UPEC. These observations suggest a novel treatment strategy using a probiotic that would minimize the symptoms of infection without reliance on empirical therapies that contribute to antimicrobial resistance.

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References