LPS-sensory peptide communication in experimental cystitis


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Saban, M. R., R. Saban, T. G. Hammond, M. Haak-Frendscho, H. Steinberg, M. W. Tengowski, and D. E. Bjorling.  LPS-sensory peptide communication in experimental cystitis.  Am J Physiol Renal Physiol  282:F202–F210, 2002. First published August 21, 2001; 10.1152/ajprenal.00163.2001.—Stimulation of sensory nerves can lead to release of peptides such as substance P (SP) and consequently to neurogenic inflammation. We studied the role of bacterial lipopolysaccharide (LPS) in regulating SP-induced inflammation. Experimental cystitis was induced in female mice by intravesical instillation of SP, LPS, or fluorescein-labeled LPS. Uptake of fluorescein-labeled LPS was determined by confocal analysis, and bladder inflammation was determined by morphological analysis. SP was infused into the bladders of some mice 24 h after exposure to LPS. In vitro studies determined the capacity of LPS and SP to induce histamine and cytokine release by the bladder. LPS was taken up by urothelial cells and distributed systemically. Twenty-four hours after instillation of LPS or SP, bladder inflammation was characterized by edema and leukocytic infiltration of the bladder wall. LPS pretreatment enhanced neutrophil infiltration induced by SP, increased in vitro release of histamine, tumor necrosis factor-α, and interferon-γ, and significantly reduced transforming growth factor-β release. These findings suggest that LPS amplifies neurogenic inflammation, thereby playing a role in the pathogenesis of neurogenic cystitis.

PAIN IS OFTEN THE BODY’S FIRST response to injury or infection (15). During infection, bacterial products such as lipopolysaccharide (LPS) can influence visceral sensitivity (48–49). Indeed, intraperitoneal LPS produces somatic hyperalgesia through a mechanism involving mast cell degranulation, release of interleukin (IL)-1β and tumor necrosis factor (TNF)-α, and activation of vagal afferents (11).

Transmission of nociceptive impulses to the central nervous system is mediated by release of sensory neuropeptides, particularly substance P (SP), by peripheral sensory afferent C fiber neurons (46). In addition, SP is spontaneously released within the bladder wall (40), and its action is terminated by neutral endopeptidase (NEP or EC3.4.24.11) (40). Deletion of the gene for NEP by homologous recombination resulted in an increase in basal plasma extravasation in the bladder, an effect that was reversed by treatment with recombinant NEP (31). Experimentally, several lines of evidence support a central role for SP and the neurokinin-1 receptor (NK1; the primary receptor for SP) as mediators of bladder inflammation. SP has been implicated in both neurogenic inflammation (1, 4, 7, 50) and experimental cystitis (7, 11, 13). In addition, NK1 receptor antagonists have been shown to reduce experimental inflammation (2–3, 29, 31). More recently, we presented evidence indicating that sensitized NK1 receptor knockout mice do not mount an inflammatory response to antigen challenge. The latter findings indicate that SP is upstream of a cascade of events that leads inflammation (41).

Pain is a hallmark of patients with interstitial cystitis (a painful bladder disorder of uncertain etiology), and a role for sensory nerves and NK1 receptors in interstitial cystitis has been proposed. Indeed, bladder biopsies from patients with interstitial cystitis exhibit increased nerve density (19), SP fibers (34), and NK1 receptors (32). In addition, desensitization of sensory C fibers decreases urinary bladder hyperreflexia, implicating a role for sensory peptides in this disorder (13).

An interesting hypothesis for increased pain observed in cystitis is that bacterial products can exacer-
bate the activity of sensory peptides. Among the possible mechanisms of LPS-peptide interaction, we found that LPS induced a time-dependent gene upregulation in the bladder (36). We also reported that intravesical inoculation of mice with LPS induced upregulation of peptide receptors such as bradykinin-1 (BK-1) (9) and NK1 (47). Moreover, we observed that LPS-induced cystitis was associated with activation of nuclear transcription factor-κB (NF-κB) (47), and inhibition of NF-κB with lactacystin blocks LPS-induced inflammation and NK1 receptor upregulation (47). However, the mechanisms by which LPS promotes SP-induced neurogenic inflammation are not clear and may occur at the level of the NK receptor (47), cytokine production (24, 33), or recruitment of inflammatory cells. SP appears to be involved in the response to LPS, because destruction of terminal sensory nerve endings before LPS administration abrogates tachykinin synthesis and downregulates TNF-α transcription and secretion (14). In addition, pretreating animals with an antagonist for the SP-specific NK1 receptor also downregulated the TNF-α response, whereas blockade of the NK2 receptor had no effect (14).

The first purpose of this study was to investigate whether LPS is absorbed by the bladder. The second was to determine the effects of prior exposure to LPS on SP-induced cystitis in vivo and in vitro release of inflammatory mediators [histamine, TNF-α, interferon-γ (IFN-γ), and transforming growth factor (TGF)-β1] by bladder tissue in response to SP.

MATERIALS AND METHODS

Induction of Cystitis

All animal experimentation described herein was performed in conformity with the “Guiding Principles for Research Involving Animals and Human Beings.” Eight-week-old female Balb C mice were used in this experiment according to an approved animal protocol (no. 00–109, Univ. of Oklahoma Health Sciences Center Animal Care and Use Committee).

Animals were anesthetized with ketamine HCl (40 mg/kg im) and xylazine (2.5 mg/kg im). A polypropylene catheter (24 gauge; 3⁄4 in.; Angiocath, Becton-Dickinson, Sandy, UT) was introduced transurethrally into the bladder and advanced 30 min. All mice were euthanized (pentobarbital sodium; 100 mg/kg ip) 24 h after the last instillation. Mice were then exsanguinated, and bladders were removed rapidly. One group of isolated bladders was placed in physiological salt solution for in vitro histamine and cytokine determination. A second group was placed in paraformaldehyde for confocal microscopy analysis. The third group was placed in buffered formalin for morphological analysis.

Uptake of LPS-Fluorescein Conjugate

Escherichia coli LPS strain 055:B5 (Sigma, St. Louis, MO) was conjugated to fluorescein by Molecular Probes (Eugene, OR) and provided without free reporter. LPS-fluorescein (100 μg/ml) was instilled into the bladder of anesthetized mice as described above. A group of mice was euthanized 1 h after instillation, and the bladders, rectums, and lung were removed. Tissues were fixed in paraformaldehyde, counterstained with Texas red-phalloidin conjugate to label the actin cytoskeleton, and examined by confocal microscopy.

Confocal Microscopy

Samples prepared for confocal microscopy were viewed as wet whole mounts in saline using a Nikon Diaphot 200 inverted microscope base and Bio-Rad 1024 confocal scanhead. The 15-mW, Kr-Ar mixed-gas laser light source was run in sequential scanning mode using Bio-Rad LaserSharp acquisition software. The 488-nm laser line was coupled to the 520DF32 band-pass filter block for fluorescein detection. The 568-nm laser line was coupled to the 605DF32 band-pass filter block for Texas red detection. Images were collected with LaserSharp 3.2 software, stored as TIFF files, and processed using Adobe Photoshop 4.0 for PC before final printing with a digital printer.

Quantification of Inflammation

The urinary bladder was evaluated for inflammatory cell infiltrates and the presence of interstitial edema. A cross section of bladder wall was fixed in formalin, dehydrated in graded alcohol and xylene, embedded in paraffin, and then four serial 5-μm sections obtained 8 μm apart were stained with hematoxylin and eosin and Giemsa. Histology slides were scanned using a Nikon digital camera (DXM1200, Nikon) mounted on a Nikon microscope (Eclipse E600, Nikon). Image analysis was performed using the MetaMorph Imaging System (Universal Imaging, West Chester, PA). A semi-quantitative score based on defined criteria was used to evaluate cystitis (21) and graded as follows: 0 (absence of inflammatory cells and edema); 1+, mild (infiltration of a low number of neutrophils in the lamina propria, little or no interstitial edema); 2+, moderate (infiltration of moderate numbers of neutrophils in the lamina propria, moderate interstitial edema); and 3+, severe (diffuse infiltration of moderate to large numbers of neutrophils in the lamina propria and severe interstitial edema).

In Vitro Studies

In vitro studies were conducted as described previously (36). Briefly, 24 h after the last instillation, three urinary bladders were isolated from each experimental group and placed in a physiological salt solution (PSS) of the following composition (in mM): 119 NaCl; 1 NaH2PO4; 4.7 KCl; 2.5 CaCl2; 0.5 MgCl2; 25 NaHCO3; and 11 glucose. Each experiment was repeated four times using a total of 12 mice/group. Tissues were minced and distributed among test tubes containing 1 ml PSS maintained at 37°C and aerated continuously with a mixture of 95% O2-5% CO2 (pH 7.4). Peptide inhibitors 1 μM N-(α-homopyrroloxyloxyhydroxysphingono)-leu-trp and 1 μM [2S]-1-[3-mercaptop-2-methylpropionyl]-L-proline were added to PSS to inhibit peptide degradation. PSS was replaced at 15-min intervals for a total of 1 h. At the end of the equilibration period, tissues were incubated with...
fresh PSS for one additional hour. This final incubation medium was analyzed to determine spontaneous release of cytokines and histamine. Tissues were then incubated with 1.0 ml substance P (10 μM), LPS (10 ng/ml), or saline for 1 h, after which the bath solution was collected for subsequent determination of cytokine and histamine release.

**Histamine Immunoassay**

Histamine content of the bath fluid was determined by immunoassay (Immunotech, Westbrook, ME; sensitivity of 0.2 nM for acetylated histamine; inter- and intra-assay variation <8%) as described before (39).

**Cytokine Determination**

TNF-α and IFN-γ. Concentrations of TNF-α and IFN-γ in the bath fluid were determined using ELISA with a sensitivity of 10 pg/ml. Briefly, 96-well plates (Nunc-Immuno Plate MaxiSorp; Nunc) were coated with purified anti-cytokine capture monoclonal antibody (anti-TNF-α or anti-IFN-γ; Pharmingen, San Diego, CA), diluted in coating buffer (0.1 M NaHCO₃, pH 8.2, 100 μl/well), and left overnight at 4°C. The plates were washed three times in washing buffer (0.05% Tween 20 in PBS), blocked with PBS containing 0.3% BSA at room temperature for 2 h, and subsequently washed again three times in washing buffer. Recombinant TNF-α or IFN-γ (Pharmingen) was serially diluted (1,000:1 pg/ml) in PBS with 0.3% BSA to derive a standard curve. Plates were incubated overnight at 4°C and then rinsed four times in washing buffer. For capture, biotinylated anti-cytokine-detected monoclonal antibody (Pharmingen) diluted in PBS with 0.3% BSA was added to the wells (100 μl/well; 2 μg/ml). Plates were incubated overnight at 4°C and then rinsed six times in washing buffer. ExtrAvidin alkaline phosphatase (1:3,500; Sigma) diluted in coating buffer (0.1 M NaHCO₃, pH 9.2, 100 μl/well), and left overnight at 4°C. The plates were developed at room temperature (up to 90 min). The reaction was stopped by the addition of 2 M NaOH, and absorbance was measured at 405 nm in an ELISA reader (Bio-Tek Instruments).

**TGF-β1.** TGF-β1 was quantified using an ELISA kit (Promega, Madison, WI) with a sensitivity of 25 pg/ml TGF-β1 and <2% cross-reactivity with TGF-β2 and TGF-β3. This system uses an antibody sandwich format and horseradish peroxidase-conjugated secondary antibody for the final chromogenic detection of bound TGF-β1. Briefly, 96-well plates were coated with anti-TGF-β-specific polyclonal antibody. After a washing, the amount of specifically bound polyclonal antibody was detected using a species-specific antibody conjugated to horseradish peroxidase as a tertiary reactant. The unbound conjugate was removed by washing, samples were incubated with a chromogenic substrate, and the color change was measured by an ELISA reader (Bio-Tek Instruments).

**Drugs and Solutions**

The following drugs were purchased from Sigma: SP, N-(α-mercapto-2-methylpropionyl)-L-proline, and [28]-[3-mercapto-2-methylpropionyl]-t-proline. All drugs were prepared in pyrogen-free saline immediately before use.

**Statistical Analysis**

Statistical analysis of the results was performed with Minitab software (Minitac, State College, PA) utilizing analysis of variance and Student’s t-test for paired and unpaired samples. Wilcoxon’s rank sum test was used for comparisons of inflammation (5). All values were expressed as means ± SE. A value of P < 0.05 was considered indicative of significant difference.

**RESULTS**

**LPS Uptake by Urothelial Cells**

One hour after intravesical instillation of LPS-fluorescein, specific fluorescence was detected within the bladder mucosa (Fig. 1A). Bladder tissues from eight
animals were reviewed, and it was apparent that LPS was internalized by some, but not all, urothelial cells (Fig. 1A). Fluorescence was also detected in the lungs (Fig. 1B) and rectums (Fig. 1C) of the same animals, suggesting systemic distribution of LPS-fluorescein.

Another group of mice was instilled with fluorescein-labeled LPS and euthanized 24 h later to verify that the LPS-fluorescein conjugate maintained its capacity to induce inflammation. Morphological analysis demonstrated that the histological response of the bladder to the LPS conjugate was identical to the response to unlabeled LPS (Fig. 2) (47). LPS-fluorescein induced intense vacuolization of bladder urothelial cells (Fig. 2A) compared with the appearance of the urothelium from bladders instilled with saline (Fig. 2B). However, the most striking difference was the presence of neutrophils within the bladder mucosa after instillation of LPS but not after instillation of saline (Fig. 2, C and D).

**LPS- and SP-Induced Cystitis**

Compared with mice instilled with saline (Fig. 3A), either SP (Fig. 3B) or LPS (Fig. 3C) induced bladder inflammation characterized by vasodilation, cell migration, and edema. In addition, inflammation of the bladders of mice that received intravesical LPS 24 h before instillation of SP was significantly greater than inflammation observed in response to intravesical SP in the bladders of mice that had previously received intravesical saline (Fig. 3D, Table 1).

**LPS Potentiates In Vitro SP-Induced Release of Histamine**

We have previously reported that LPS has the capacity to stimulate release of inflammatory mediators from isolated tissues (39) and that SP induces a time-dependent release of histamine from the urinary bladder (32). In the present study, we observed that in vitro incubation of bladder tissue from mice that received intravesical saline in vivo with either LPS or SP resulted in a significant release of histamine (Fig. 4). In vivo instillation of LPS into the bladder 24 h before in vitro testing caused an increase in spontaneous, and a twofold increase in SP-induced, histamine release ($P < 0.05$).
Cytokine Release

We investigated whether LPS could prime the bladder to release proinflammatory cytokines. Cytokine release from tissues treated in vivo with saline during in vitro saline stimulation was the same as spontaneous release of the particular cytokine; therefore, net release was zero (Fig. 5, A–C). In bladders isolated from mice infused with saline in vivo, SP stimulated a modest release of TNF-α (Fig. 5A). In vivo LPS primed the bladder to TNF-α release. Indeed, LPS instillation led to an increase in basal release of TNF-α as well as a potentiation of SP- and LPS-induced release (Fig. 5A). Interestingly, in vivo pretreatment with SP significantly primed TNF-α release in response to LPS. We also investigated whether LPS could prime the bladder to release IFN-γ. In vivo treatment with LPS caused an increase in basal release of IFN-γ as well as a potentiation of SP- and LPS-induced release (Fig. 5A).

Table 1. Histological severity of LPS- and SP-induced cystitis in mice

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Stimulus</th>
<th>Edema</th>
<th>No. of Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Saline</td>
<td>0.10 ± 0.14</td>
<td>0.18 ± 0.14</td>
</tr>
<tr>
<td>None</td>
<td>LPS</td>
<td>2.70 ± 0.10*</td>
<td>2.30 ± 0.10*</td>
</tr>
<tr>
<td>None</td>
<td>SP</td>
<td>2.10 ± 0.20*</td>
<td>2.20 ± 0.20*</td>
</tr>
<tr>
<td>Saline</td>
<td>SP</td>
<td>2.00 ± 0.20*</td>
<td>2.10 ± 0.22*</td>
</tr>
<tr>
<td>LPS</td>
<td>SP</td>
<td>2.80 ± 0.10*</td>
<td>2.90 ± 0.12†</td>
</tr>
</tbody>
</table>

Values are means ± SE. LPS, lipopolysaccharide; SP, substance P. The histological severity of cystitis was graded by a score of 0–3. Inflammation was assessed 24 h after the last instillation, as described in MATERIALS AND METHODS (8 animals/group). Bladder tissue was fixed in formalin and paraffin embedded. Four serial sections (4 μm apart) were stained with hematoxylin and eosin for histological analysis in a blinded fashion. *P < 0.01 compared with the respective control (instilled with saline alone; Wilcoxon’s rank sum test). †P < 0.01, saline/SP treatment compared with LPS/SP treatment (Wilcoxon’s rank sum test).
increased release of IFN-γ in response to in vitro saline, SP, or LPS (Fig. 5B). In addition, in vivo intravesical instillation of SP increased IFN-γ release in response to LPS (Fig. 5B). Taken together, these results indicate a cross-potentiation between LPS and SP in inducing the release of proinflammatory cytokines.

Among the anti-inflammatory cytokines, we chose to determine the effects of LPS on TGF-β1 release from the urinary bladder. In vitro, SP stimulated a significant release of TGF-β1 from bladders treated in vivo with saline or SP, but, in vivo, LPS suppressed (by 70% reduction) in vitro release of TGF-β1 in response to SP (Fig. 5C). In addition, in vitro LPS failed to stimulate significant release of TGF-β1 from any tissues.

**DISCUSSION**

Our results indicate cross-communication between a bacterial toxin (LPS) and a sensory peptide (SP) during induction of experimental cystitis. In addition, we present evidence that intravesical LPS is internalized by the bladder urothelium and that it is also distributed systemically. In the present work, we did not explore the possibility that fluorescein conjugated to LPS may alter its uptake, distribution, and metabolism. However, conjugation of fluorescein to LPS did not alter the capacity of LPS to induce inflammation characterized by cell migration and edema formation.

Here, we provide evidence that both LPS and SP are capable of inducing and amplifying bladder inflammation. This interaction is characterized by an increase in the inflammatory cell infiltrate, potentiation of the release of histamine and proinflammatory cytokines, and downregulation of the release of TGF-β1, an anti-inflammatory modulator.

The present study confirms the previous observation that intravesical instillation of LPS leads to cell migration, edema formation (21, 47), and upregulation of proinflammatory genes (36). Others have shown that mucosally administered bacterial components activate IL-6 release and polymorphonuclear leukocyte (PMNL) migration (30). However, the major morphological effect that we observed with LPS instillation was migration of polymorphonuclear neutrophils (PMNs) into the urothelium (Fig. 2, C and D) and vacuolization of urothelial cells (Fig. 2A). These results are in agreement with Jezernik et al. (22), who showed that instillation of LPS into the mouse bladder is followed by desquamation of urothelial cells.

In addition, mouse bladder infection produced by *E. coli* is characterized by neutrophil migration into the bladder mucosa and necrosis of superficial epithelial cells, sometimes resulting in erosion or ulceration of the epithelium (23). We did not investigate the time course of vacuolization, because our previous work indicated that the effects of LPS on cell migration and edema formation were observed 1 h after instillation. The effects of LPS were observed as early as 4 h and peaked at 24 h after LPS infusion (39). Therefore, in the present study, we did not perform morphometric studies at 1 h, other than confocal analysis of LPS uptake.

We do not have a clear understanding of the mechanisms involved in urothelium vacuolization after LPS instillation, although a role for bacterial toxins in inducing urothelial damage has been proposed (22, 45). In a different model of bladder inflammation, we found that antigen induces a decrease in endosomal fusion that is dependent on activation of SP receptors (16). It remains to be determined whether alteration of endosomal fusion underlies the vacuolization.

We have previously observed that either intravesical SP or nerve stimulation leads to bladder inflammation (6) and that blockade of NK1 receptors inhibits bladder endosomal fusion secondary to inflammation (16). SP induces a concentration-dependent cellular infiltration when administered intradermally in the mouse (38) and time-dependent release of inflammatory mediators (37) and gene expression (36) when instilled into the urinary bladder. In the present work, cystitis induced by intravesical SP was characterized by an intense PMNL infiltrate and edema (Fig. 3B). However, a significant finding of the present work was that prior exposure of the bladder to LPS potentiated SP-induced inflammation (Fig. 3D, Table 1). This indicates that

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**Fig. 4.** LPS-potentiates SP-induced release of histamine. Saline, LPS (100 μg/ml), or SP (10 μM) was instilled into the bladder, and mice were killed 24 h afterward. Bladder tissue was placed in physiological saline solution (PSS) maintained at 37°C for 1 h before manipulation. Spontaneous and induced histamine release from 3 isolated mouse bladders exposed to each treatment were determined by immunosay performed in quadruplicate. Values are presented as means ± SE. *P < 0.01, significantly different compared with the respective control (instilled with saline in vivo) by Student’s t-test. **P < 0.5, significant difference between spontaneous histamine release from the bladder of mice that received in vivo saline or LPS.
bacterial infection may dramatically augment neurogenic inflammation of the bladder, thereby suggesting a mechanism by which pain and inflammation associated with bacterial cystitis are amplified. Further evidence for the potential interaction of SP and LPS in vivo is provided by the observation that injection of LPS into the footpad of the mouse stimulated increased SP in the popliteal lymph node, which reached a maximum within 3 days of injection (35).

SP alone induced a modest release of TNF-α or IFN-γ from bladders of mice that were pretreated with saline. However, in vivo instillation of LPS resulted in increased in vitro release of these cytokines in response to SP. Increased cytokine release and inflammation as a result of interaction of SP and LPS have been described previously. SP or LPS alone failed to increase PMN adherence to A549 respiratory epithelial cells in culture, but the combination of these compounds stimulated a significant increase in PMN adherence (26). PMN adherence stimulated IL-1β and TNF-α release from these cells, in the presence or absence of either SP or LPS; however, exposure to a combination of SP and LPS significantly increased IL-1β and TNF-α release. Treatment of mice with either capsaicin to deplete neuropeptides (including SP) or the specific NK1 antagonist SR-140333 significantly reduced serum concentrations of TNF-α in response to subsequent intraperitoneal injection of LPS (15).

SP is involved in a variety of inflammatory processes and mediates stress-induced alterations in cytokine synthesis (10). SP greatly enhances LPS-induced TNF-α production in macrophages from cold-water-stressed mice (10). The present paper further links the nervous-endocrine-immune systems by describing influences of SP on the immune system. Additional evidence for communication between sensory nerves and bacterial products is the finding that capsaicin, known to deplete neuropeptides from sensory nerves, diminished stress-induced cytokine (TNF-α and IL-6) production to levels approximating those in control mice (52). Moreover, RP-67, 580, a specific NK1 receptor antagonist, eliminated cold-water stress-induced augmentation of IL-6 secretion from peritoneal macrophages (10).

It is apparent from the present work that SP and LPS both can affect cytokine release in the urinary bladder. It was not within the scope of this work to determine the origin of the cytokines. However, both bladder mucosa and inflammatory cells can produce cytokines. Spontaneous bacterial cystitis in humans has been associated with increased urinary concentrations of cytokines, including IL-1α, IL-1β, TNF-α, and IL-6 (12). Although LPS stimulation of macrophages and monocytes within the bladder wall probably contributes to urinary cytokines (25), urothelial cells have the capacity to produce cytokines, and in vivo and in vitro exposure of urothelial cells to whole bacteria, LPS, or bacillus Calmette-Guerin stimulates synthesis of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α, and TGF-β (14, 17, 20, 25, 42, 43).

Further evidence of modulation of cytokine release by SP is provided by the observation that nanomolar concentrations of SP stimulated a significant increase in IFN-γ release from cells isolated from the spleens and granulomas of mice with schistosomiasis that were primed in vitro with suboptimal concentrations of antigen (8). Modulation of cytokine production by SP may also play a significant role in protection against infection. In mice injected with lethal doses of Salmonella, it was found that SP mediated production of both IL-12
and IFN-γ (24). In this model, Salmonella induced rapid and dramatic increases in SP-receptor mRNA and IFN-γ (24). In this model, Salmonella induced rapid and dramatic increases in SP-receptor mRNA and IFN-γ (24).

Although it is clear that each of these cytokines can have dramatic effects on inflammation, little is known about the kinetics, magnitude, or possible synergistic contributions of SP- and LPS-induced cytokine release. LPS has been shown to increase expression of cytokine mRNA (30, 36), but the synergistic effects of LPS and SP on cytokine mRNA have not been investigated. Leukocytes are also a potential source of cytokines, and increased numbers of PMNs were observed in the bladders of mice that received intravesical LPS and SP. Instillation of LPS before SP exposure suppressed TGF-β production. Similar results were described by Marriott and Bost in cultured macrophages (33). Therefore, it is possible that LPS deregulates the fragile balance of pro- and anti-inflammatory cytokines, leading to an exacerbation of immune-mediated tissue damage.

We have previously observed that intravesical LPS stimulates the NF-kB cascade and expression of NK1 receptors in mice, effects that were blocked by the proteosomal inhibitor lactacystin (47). Additional support for modulation of LPS effects by NK1 receptors is the finding that NK1 receptor antagonists reduce detrusor hyperreflexia caused by bacterial cystitis (28) and restore bladder endosomal fusion secondary to antigen-induced cystitis (16). The present work and previous studies have offered support for NK1 receptors after LPS-induced inflammation and suggests that NK1 receptor antagonists may be useful in the treatment of detrusor hyperreflexia.

Although there is considerable evidence that NK1 receptors seem to play a critical role in the transmission of noxious stimuli (6, 27), NK1 receptor antagonists were described to be of low efficacy in the clinical treatment of pain (18). The apparently low efficacy of NK1 receptor antagonists for treatment of pain in humans has been attributed to inadequate clinical trials (44). Another possibility is differential involvement of the tachykinins according to the model of neuropathic pain. Indeed, the intrathecal effect of NK1, NK2, and NK3 receptor antagonists was tested in pain induced by a mechanical stimulus in both mononeuropathic (sciatic nerve ligature) and diabetic rats. NK1 and the NK2 receptor antagonists were antinociceptive in both models, and NK1 antagonists were more effective in diabetic rats. The tachykinin NK3 receptor antagonist was weakly effective in diabetic rats only (12). Finally, recent studies indicate that NK3 antagonists also prevent hyperalgesia and associated spinal cord SP release in monoradicthritic rats (51). Taken together, these results indicate the need for more extensive evaluation of the role of NK receptors in pain and inflammation.

We postulate that urinary tract infections or exposure of the bladder wall to bacteria-producing LPS may play a role in the pathogenesis of cystitis by a mechanism that involves augmentation of neurogenic inflammation. Further research is required to determine the role NK receptors play in modulating interaction between bacterial cystitis and neurogenic inflammation.

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