Changes in urinary bladder cytokine mRNA and protein after cyclophosphamide-induced cystitis

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Malley, Susan E., and Margaret A. Vizzard. Changes in urinary bladder cytokine mRNA and protein after cyclophosphamide-induced cystitis. Physiol Genomics 9: 5–13, 2002. First published February 12, 2002; 10.1152/physiolgenomics.00117.2001.—Cyclophosphamide (CYP)-induced cystitis alters micturition function and produces reorganization of the micturition reflex. This reorganization may involve cytokine expression in the urinary bladder. These studies have determined candidate cytokines in the bladder that may contribute to the reorganization process. An RNase protection assay was used to measure changes in rat bladder cytokine mRNA [interferon-γ (IFN-γ), interleukin-1α/β (IL-1α/β), IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, and tumor necrosis factor-α/β (TNF-α/β)] after acute (4 h), intermediate (48 h), or chronic (10 day) cystitis. The correlation between bladder cytokine mRNA and protein expression was also determined by immunoassay. Although at each time point after cystitis significant changes in bladder cytokine mRNA were observed, the magnitude differed (acute > intermediate > chronic). Acute cystitis demonstrated the most robust changes (P ≤ 0.005; IL-1β, 330-fold increase; IL-2, 20-fold increase; IL-4, 8-fold increase; IL-6, 80-fold increase) in cytokine mRNA expression and TNF-α or TNF-β mRNA were only increased (2–10-fold) after acute cystitis. More modest increases in cytokine mRNA expression were observed after 48-h or 10-day cystitis. Cytokine protein expression generally paralleled that of mRNA. Increased cytokine expression after CYP-induced cystitis, alone or in combination with other inflammatory mediators or growth factors, may contribute to altered lower urinary tract function after cystitis.

inflammation; interleukins; tumor necrosis factor; neuroimmune interactions

CHRONIC PATHOLOGICAL CONDITIONS inducing tissue irritation or inflammation can alter the properties of sensory pathways leading to a reduction in pain threshold (allodynia) and an amplification of painful sensations (hyperalgesia) (6, 8, 19). Increased pain sensitivity can result from changes in peripheral nociceptor afferents (17, 34, 48, 50, 83) or from changes in the central nervous system mechanisms that process nociceptive inputs (9, 11, 41, 46). These changes have been linked with alterations in gene expression and synthesis of neurotransmitters (5, 18, 24, 25, 29). Recent experiments involving a chemically induced (cyclophosphamide (CYP)) urinary bladder inflammation have demonstrated alterations in neurochemical (74, 77–79) and electrophysiological (33, 85) properties of bladder afferent neurons in the L6–S1 dorsal root ganglia (DRG). In addition, changes in the magnitude and distribution of the protein product (Fos) of the immediate early gene, c-fos, have been demonstrated in the L6–S1 spinal cord in response to urinary bladder distension following chronic CYP-induced cystitis (77). These changes are reversed by pretreatment with capsaicin, a C-fiber neurotoxin (77). These changes suggest considerable reorganization of reflex connections in the spinal cord and marked changes in the properties of micturition reflex pathways following CYP-induced cystitis.

Possible mechanisms underlying the neural plasticity following chronic CYP-induced cystitis (33, 74, 77–79, 85) may involve alterations in neurotrophic factors (NTFs) and/or neural activity arising in the urinary bladder (75). In addition, neuroimmune activation, including the production of cytokines, occurs after injury to the central or peripheral nervous system, and cytokines are also likely to play a role in the development of pain, exacerbate pathology, or may contribute to repair strategies (1, 12, 31, 47, 60, 81, 82). The concept that target organs can influence the neurons that innervate them now is widely accepted and readily demonstrated during embryonic or postnatal development (22, 37, 39, 49, 65, 72, 73). Studies in the urethral-obstructed rat have demonstrated the influence of target organ-neuron interactions in the adult animal (21, 66–71). Biochemical studies have further suggested a role for nerve growth factor (NGF) in mediating some aspects of bladder afferent neuron plasticity following partial urethral obstruction (21, 66, 67, 71). Recent studies from this laboratory have demonstrated changes in the mRNA expression of a number of NTFs in the urinary bladder, including βNGF, brain-derived neurotrophic factor, glial-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4, after either CYP-induced cystitis or complete spinal cord transection in the rat (75).

Interactions between cytokines, subsequent growth factor production, and altered pain sensation have also been demonstrated following injury to the central or peripheral nervous system (15, 42, 54, 55). For example, interleukin-1 (IL-1) has been shown to increase the...
stability and transcription of NGF mRNA in cultured rat fibroblasts (42). In addition, IL-1β and tumor necrosis factor (TNF-α) act synergistically to stimulate NGF from cultured rat astrocytes (26). It has been demonstrated that IL-1 is the responsible agent for the increase in NGF mRNA and protein expression by nonneuronal cells (Schwann and fibroblast-like cells) of the rat sciatic nerve following axotomy (42). Wolff et al. (83) have also speculated that TNF-α, by virtue of its ability to induce IL-1β and NGF, may contribute to the initiation of inflammatory hyperalgesia produced by localized inflammation of the rat hind paw. CYP-induced cystitis is also associated with changes in urinary bladder NGF mRNA and protein synthesis (75); thus we are particularly interested in examining changes in urinary bladder IL-1β and TNF-α mRNA and protein after cystitis. The expression of cytokines, alone or in combination with other cytokines, growth factors, or other mediators, may form a bidirectional communication network between the nervous system and the immune system (45).

The overall aim of the present studies was to determine candidate cytokines (pro-inflammatory and anti-inflammatory) in the urinary bladder that may contribute to altered properties and function of the lower urinary tract after CYP-induced cystitis. The present studies were designed to examine potential changes in cytokine mRNA [interferon (IFN)-γ, IL-1α/β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, and TNF-α/β] after CYP-induced cystitis. The time course of changes in both pro-inflammatory and anti-inflammatory cytokine expression were examined by using 1) acute (4 h) CYP-induced cystitis, or 2) intermediate (48 h) CYP-induced cystitis, or 3) chronic (10 day) CYP-induced cystitis. We also determined the correlation between urinary bladder cytokine mRNA expression and urinary bladder protein expression after CYP-induced cystitis by performing cytokine protein immunoassays.

**EXPERIMENTAL PROCEDURES**

**CYP-Induced Cystitis: Acute, Intermediate, or Chronic**

Chemical cystitis was induced in adult female Wistar rats by CYP, which is metabolized to acrolein, an irritant eliminated in the urine (13, 40, 80). CYP (Sigma ImmunoChemicals, St. Louis, MO) was administered in one of the following ways: 1) 4 h (150 mg/kg ip) prior to euthanasia of the animals to elicit acute inflammation (n = 12); 2) 48 h (150 mg/kg ip) prior to euthanasia to examine an intermediate inflammation (n = 12); or 3)administered every third day for 10 days to elicit chronic inflammation (n = 12, 75 mg/kg ip). All injections of CYP were performed under isoflurane (2%) anesthesia. Animals were killed by isoflurane anesthesia (3%) plus thoracotomy at the indicated time points, and the urinary bladder was harvested and weighed. The University of Vermont Institutional Animal Care and Use Committee approved all experimental procedures (protocol no. 99-059) involving animal use. Animal care was under the supervision of the University of Vermont’s Office of Animal Care in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care and National Institutes of Health guidelines. All efforts were made to minimize animal stress/distress and suffering and to use the minimum number of animals. Currently, no alternatives exist to the use of whole, live animals in the present study.

**Control Experiments**

Control animals (n = 15) received a corresponding volume of saline (0.9% ip) injected under isoflurane (2%) anesthesia.

**RNase Protection Assay**

Cytokine (IFN-γ, IL-1α/β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, and TNF-α/β) mRNA levels present in the bladder were determined by RNase protection assay (RPA; Pharmingen, San Diego, CA). Total RNA was prepared from freshly harvested tissue samples (n = 6 for each time point; n = 7 for control) using the Qiagen RNasy Maxi Kit (Qiagen, Valencia, CA). Individual bladders were homogenized using a Polytron homogenizer (Kinematica) in 7.5 ml of a guanidine thiocyanate-based buffer with 1% β-mercaptoethanol according to the muscle tissue isolation protocol with proteinase K digestion (Qiagen). Total RNA was eluted from a column and stored at −80°C. Sample RNA concentrations were determined spectrophotometrically.

Standard multiprobe RPAs using a commercial rCK-1 template set (Pharmingen) for tissue cytokine transcript levels were performed. The assay, which employs a series of unique probes each targeted to a distinct region of their respective mRNAs, has the advantage of analyzing, simultaneously, the levels of several mRNA species in the same minute sample. Since the assay also incorporates probes for two housekeeping gene transcripts (GAPDH and L32) whose levels are invariant among tissues under different physiological states, normalizing the levels of the particular regulated target transcripts to the nonregulated housekeeping genes allowed direct comparisons of the target transcript levels among different experimental samples. Both of these features overcome the potential quantitative problems associated with inter- and intra-assay variability. Multiple antisense 32P-radiolabeled (3,000 Ci/mmoll Perkin-Elmer Life Sciences, Boston, MA) RNA probes synthesized from the rCK-1 template set were hybridized to total tissue RNA overnight, and the nonprotected RNA segments were digested with RNase; the protected RNA fragments were recovered, resolved on denaturing polyacrylamide gels, and exposed to autoradiographic film. For data analysis, the intensity of each autoradiographic band corresponding to the specific target transcripts was analyzed by semiquantitative image analysis using Alpha Imager V4.03 (Alpha Innotech) software. Background intensities were subtracted from bands of all target and housekeeping genes. Target gene expression levels were expressed as a function of tissue GAPDH levels and identical to transcript levels expressed as a ratio of tissue L32.

**Preparation of ELISA Samples**

Adult rats were killed as above, and the bladder (n = 6 for each time point; n = 8 for control) was rapidly disected and weighed. Individual bladders were solubilized in T-PER tissue protein extraction reagent (1 g tissue/20 ml; Pierce, Rockford, IL) with Complete (protease inhibitors cocktail tablets; Roche Diagnostics, Germany). Bladder tissue was disrupted with a Polytron homogenizer and then centrifuged (10,000 rpm for 5 min). The supernatants were used for IL-1β, TNF-α, IL-4, IL-6, and IL-10 quantification. Total protein was determined by the Coomassie Plus protein assay reagent kit (Pierce).
BLADDER CYTOKINE mRNA AND PROTEIN AFTER CYSTITIS

Principle of the R&D Systems Quantikine M Immunoassay Technique

Monoclonal antibodies against IL-1β, TNF-α, IL-4, IL-6, and IL-10 were adsorbed to microtiter (R&D Systems, Minneapolis, MN) plates. After addition of the sample or standard solution, the second antibody (polyclonal) was applied. Sample and standard solutions were run in duplicate. This antibody complex was detected with a horseradish peroxidase-labeled immunoglobulin. Enzyme activity was quantified by the change in optical density, using tetramethyl benzidine (TMB) as substrate. The IL-1β, TNF-α, and IL-4 standards provided with this system generated linear standard curves from 5 to 2,000 pg/ml (r² = 0.997, P ≤ 0.001), 5 to 800 pg/ml (r² = 0.996, P ≤ 0.001), and 5 to 1,000 pg/ml (r² = 0.955, P ≤ 0.001), respectively. The IL-6 and IL-10 standards provided with this system generated linear standard curves from 5 to 2,000 pg/ml (r² = 0.983, P ≤ 0.001) and 5 to 2,000 pg/ml (r² = 0.994, P ≤ 0.001), respectively. The absorbance values of standards and samples were corrected by subtraction of the background value (absorbance due to nonspecific binding). Samples were diluted to bring the absorbance values onto the linear portion of the standard curve. No samples fell below the minimum detection limits of the assay. Curve fitting of standards and evaluation of IL-1β, TNF-α, IL-4, IL-6, and IL-10 content of samples was performed using a least squares fit. No significant cross-reactivity or interference is observed to other cytokines with any Quantikine M immunoassay kit used in the present study according to the manufacturer.

Statistics

All values are means ± SE. Comparisons of cytokine mRNA levels or protein concentration in urinary bladder samples after acute (4 h), intermediate (48 h), or chronic (10 days) CYP-induced cystitis were made using analysis of variance. When F ratios exceeded the critical value (P ≤ 0.05), the Dunnett post hoc test was used to compare the control means with each experimental mean.

RESULTS

Urinary Bladder Weight

Following acute (4 h), 2-day (48 h), or chronic administration (10 day) of CYP, bladder weight significantly increased (P ≤ 0.005) compared with that of control animals [250 ± 15 mg (4-h CYP), 230 ± 20 mg (48-h CYP), and 140 ± 10 mg (chronic CYP) vs. 70 ± 12 mg (control)]. As previously demonstrated (74, 75, 79) and confirmed again in this study, gross microscopic analysis of bladders from animals treated acutely (4 or 48 h) with CYP showed a few, scattered regions of mucosal erosion on the luminal surface. Chronic (10 day) administration of CYP increased the severity of the bladder changes, resulting in more extensive regions of mucosal erosion, ulcerations, edema, and in some instances petechial hemorrhages. Histological changes evident following chronic CYP treatment included edema of the lamina propria and plasma cell infiltrates in the lamina propria, submucosa, and perivascular tissue. We have previously demonstrated (77) that some of these cellular infiltrates include macrophages as detected with an ED1 antibody that recognizes an unidentified cytoplasmic antigen, unique to all phagocytic cells of monocyte/macrophage origin (56) and neutrophils (PMNs) as shown by significant increases in myeloperoxidase activity (77).

Changes in Urinary Bladder Cytokine mRNA Levels After CYP-Induced Cystitis

Acute (4 h) CYP-induced cystitis resulted in a significant (P ≤ 0.005) increase in urinary bladder cytokine mRNA for several factors examined, including IL-1β, IL-2, IL-4, IL-6, and TNF-α/β compared with the cytokine mRNA profile from control urinary bladder (Figs. 1 and 2). However, no changes were detected for IL-1α, IL-10, or IFN-γ mRNA compared with control urinary bladder. A similar significant (P ≤ 0.005) increase in IL-1β, IL-2, and IL-6 mRNA in the urinary bladder was also observed following 48-h or chronic (10-day) CYP-induced cystitis compared with control urinary bladder (Figs. 2 and 3). However, no changes were detected for IL-1α, IL-10, or IFN-γ mRNA compared with control urinary bladder. Forty-eight-hour CYP-induced cystitis differed from chronic CYP-induced cystitis only in the observation that IL-4 mRNA returned to control levels with chronic treatment.

Although at each time point following CYP-induced cystitis significant changes in urinary bladder cytokine mRNA were observed, the magnitude of the changes differed (acute > intermediate > chronic; Fig. 2). Acute (4 h) CYP-induced cystitis demonstrated the most robust changes (P ≤ 0.005) (IL-1β, 330-fold increase; IL-2, 20-fold increase; IL-4, 8-fold increase; IL-6, 80-fold increase) in cytokine mRNA expression and TNF-α or TNF-β mRNA were only increased (2- to 10-fold) following acute CYP-induced cystitis (Fig. 2). More modest increases in cytokine mRNA expression were observed for 48-h CYP-induced cystitis (IL-1β, 150-fold increase; IL-2, 4.5-fold increase; IL-4, 2-fold increase; IL-6, 80-fold increase) (Fig. 2). Smaller increases were observed in cytokine mRNA expression following chronic (10 day) CYP-induced cystitis (IL-1β, 55-fold increase; IL-2, 2-fold increase; IL-6, 25-fold increase) (Fig. 2). No changes in IL-1α, IL-10, or IFN-γ mRNA were observed at any time point following CYP-induced cystitis (Fig. 2).

Cytokine Protein Determination in Urinary Bladder After CYP-Induced Cystitis

Using the changes in urinary bladder cytokine mRNA as a guide, we sought to determine whether cytokine protein (IL-1β, IL-10, IL-4, IL-6, and TNF-α) expression in the urinary bladder was similarly changed after CYP-induced cystitis at the specified time points (Fig. 4). We did not perform immunoassays for every urinary bladder cytokine because of limitations in commercial availability. IL-1β. Total urinary bladder IL-1β significantly (P ≤ 0.005) increased after acute (4 h; 4.5-fold increase), intermediate (48 h; 3.4-fold increase), or chronic (10 day; 3.3-fold increase) CYP treatment (Fig. 4) compared with total control urinary bladder IL-1β.
IL-10. Total urinary bladder IL-10 significantly ($P \leq 0.005$) increased after acute (4 h; 1.7-fold increase) CYP-induced cystitis (Fig. 4) compared with total control urinary bladder IL-10. No changes in urinary bladder IL-10 protein expressed were observed at the intermediate (48 h) or chronic (10 day) time point (Fig. 4).

IL-4. No changes in urinary bladder IL-4 protein expression were observed at any time point examined after CYP-induced cystitis compared with total control urinary bladder IL-4 (Fig. 4).

IL-6. Total urinary bladder IL-6 significantly ($P \leq 0.005$) increased after acute (4 h; 10.5-fold increase), intermediate (48 h; 2.5-fold increase), or chronic (10 day; 2-fold increase) CYP treatment (Fig. 4) compared with total control urinary bladder IL-6.

TNF-α. No changes in urinary bladder TNF-α protein expression were observed at any time point examined after CYP-induced cystitis compared with total control urinary bladder TNF-α (Fig. 4).

**DISCUSSION**

These studies demonstrate sudden and dramatic increases in urinary bladder cytokine mRNA following CYP-induced cystitis, and the magnitude of the response was greatest at the shortest time point examined (4 h following CYP). Four hours following CYP-induced cystitis there were significant increases in IL-1β, IL-2, IL-4, IL-6, and TNF-α/β mRNA compared with the cytokine mRNA profile from control urinary bladder. Increased expression of IL-1β and IL-6 mRNA was also accompanied by increased urinary bladder expression of IL-1β and IL-6 protein at the same time point. In contrast, increased urinary bladder TNF-α mRNA was not associated with increased TNF-α protein expression, and urinary bladder IL-10 protein was increased 4 h after CYP-induced cystitis despite a nonsignificant increase in IL-10 mRNA. Forty-eight hours after CYP-induced cystitis, increases in IL-1β, IL-2, IL-4, and IL-6 mRNA were still maintained; however, TNF-α/β mRNA returned to control urinary bladder levels. Increased urinary bladder expressions of IL-1β and IL-6 protein were similarly maintained. Urinary bladder IL-10 protein expression had returned to control levels at least by 48 h following CYP-induced cystitis. Ten days after CYP-induced cystitis, increases in IL-1β, IL-2, IL-4, and IL-6 mRNA were still maintained, although the magnitude of the change was reduced compared with acute (4 h) or intermediate (48 h) CYP-induced cystitis. Increased urinary bladder expressions of IL-1β and IL-6 protein were similarly...
maintained. These studies demonstrate that CYP-induced cystitis results in dramatic increases in IL-1β, IL-2, IL-4, IL-6, and TNF-α/β mRNA compared with the cytokine mRNA profile from control urinary bladder and that these changes are maintained, except for TNF-α/β mRNA, during intermediate as well as chronic CYP-induced cystitis. After cystitis, IL-1β and IL-6 protein expression in urinary bladder paralleled the mRNA pattern. There was a lack of correlation between IL-10 mRNA and protein and TNF-α mRNA and protein observed in the present studies. These differences may reflect (30, 38, 43, 64) 1) mRNA transcript instability, 2) decreased translation efficiency, or 3) aberrant posttranslational mechanisms.

Interstitial cystitis (IC) is a chronic inflammatory bladder disease syndrome characterized by urinary frequency and urgency and suprapubic and pelvic pain (20, 53). Although the etiology and pathogenesis of IC are unknown, numerous theories including infection, autoimmune disorder, toxic urinary agents, deficiency in bladder wall lining, and neurogenic causes have been proposed (20, 32, 35, 53, 61). We have hypothesized that pain associated with IC involves an alteration of visceral sensation/bladder sensory physiology.

**Fig. 2.** Changes in urinary bladder cytokine mRNA following CYP treatment: acute (4 h), intermediate (48 h), or chronic (10 day). Changes in cytokine mRNA are expressed relative to the housekeeping gene, GAPDH. Each experimental paradigm resulted in significant changes in urinary bladder cytokine mRNA; however, the magnitude of the changes differed (acute > intermediate > chronic). Acute CYP (4 h) treatment exhibited the largest increase ($P \leq 0.005$) in cytokine mRNA levels relative to control urinary bladders. More modest increases were observed for intermediate or chronic CYP treatment. No changes in urinary bladder were observed for IL-1α, IL-10, or IFN-γ mRNA at any time point examined. Absence of error bars associated with some data is indicative of very low variance. *$P \leq 0.005$. O.D., optical density.

**Fig. 3.** Same as for Fig. 1. Cytokine mRNA expression for control animals, animals treated with CYP following the 48-h protocol, or animals treated chronically following the 10-day protocol.
Altered visceral sensations from the urinary bladder (i.e., pain at low or moderate bladder filling) that accompany IC (20, 32, 35, 53, 61) may be mediated by many factors including changes in the properties of peripheral bladder afferent pathways such that bladder afferent neurons respond in an exaggerated manner to normally innocuous stimuli (alldynia). These changes may be mediated, in part, by inflammatory changes in the urinary bladder. Among potential mediators of inflammation, neurotrophins (e.g., NGF) have been implicated in the peripheral sensitization of nociceptors (15, 16, 19, 43, 47, 68). Pro-inflammatory cytokines also cause sensitization of polymodal C-fibers (19) and facilitate A-β input to the spinal cord (3, 84). The present studies suggest that cytokines produced in the urinary bladder after CYP-induced cystitis may also contribute to this sensitization process. Sources of cytokines in the CYP-induced cystitis model may include a variety of cell types (resident and/or infiltrating) in the urinary bladder: lymphocytes, macrophages, mast cells, microglial cells, urothelial cells, urinary bladder smooth muscles cells, and fibroblasts (4, 27, 52).

Changes in synthesis and/or release of cytokines by target organs may also have an impact on the changes in lower urinary tract function as well as reorganization of reflex connections in the spinal cord after CYP-induced cystitis. Saban et al. (57–59) were the first to demonstrate gene regulation during inflammatory bladder responses in the mouse (lipopolysaccharide-, substance P-, or antigen-induced inflammation) using cDNA expression array technology. Consistent with the results of the present study using the CYP-induced cystitis model in the rat, Saban et al. (57–59) have also demonstrated urinary bladder upregulation of IL-1β, IL-6, and TNF mRNA and a variety of other mediators not included in the RPA used in this study.

The present study extends these observations by examining changes in cytokine mRNA expression in the urinary bladder beyond 48 h to include chronic inflammation (10 day) as well as by demonstrating concomitant changes in urinary bladder cytokine protein expression. Interestingly, several cytokines (IL-1, IL-6, and IL-8 among others) have also been reported to be upregulated during active states of inflammatory bowel disease (12, 14, 44, 62, 63). These cytokines are elevated during active ulcerative colitis and Crohn’s disease and correlate with the severity of the inflammation (62, 63). Thus inflammatory processes in visceral organs and associated changes in neurochemistry (10, 36, 74, 76, 78) and reflex organization (77) may involve cytokines.

Previous studies from several laboratories have suggested that NTF expression in the urinary bladder may underlie the changes in neurochemistry (74, 76, 78) and electrical (33, 85) phenotype of bladder afferent neurons after urinary bladder dysfunction. The concept of trophic interactions between nerve cells and their targets is clearly demonstrated during embryonic or postnatal development (22, 37, 39, 49, 65, 72, 73). Early target removal and loss of NTFs can have devastating effects on cell survival as well as on the chemical and electrical properties of afferent and efferent neurons (22, 37, 39, 49, 65, 72, 73). Recent experiments from several laboratories including our own have demonstrated the influence of target organ-neuron interactions in the adult animal (21, 66–71, 75). Partial urethral obstruction leads to increased resistance to urine flow and in turn to increased bladder workload and ultimately to bladder hypertrophy (66, 68, 69, 71). This is also accompanied by hypertrophy of afferent neurons in the L6–S1 DRG and efferent neurons in the major pelvic ganglia (66, 68, 69). The hypertrophied bladder following partial urethral obstruction exhibits mark-
edly increased levels of NGF, and autoimmunization against NGF reduces, but does not completely abolish, the major pelvic ganglion neuronal hypertrophy (67, 69, 71). This suggests that NTFs released in the hyper trophyed bladder are partly responsible for the change in neuronal morphology. Recent studies from our laboratory have demonstrated that CYP-induced cystitis or chronic spinal cord injury also alters the expression of NGF and NGF mRNA in the urinary bladder as well as a variety of other NTFs (brain-derived neurotrophic factor, glial-derived neurotrophic factor, neurotrophin-3, neurotrophin-4) (75).

The present study adds cytokines to the list of potential mediators that may contribute to altered micturition reflexes after cystitis. A significant body of literature exists to support the concept that cytokines (e.g., IL-1β and IL-6) are key signals that are released in the periphery to signal the central nervous system that infection/inflammation has occurred (15, 42, 45, 54, 55). Because of the significant increases in IL-1β mRNA and protein and IL-6 mRNA and protein in the urinary bladder and the maintenance of the responses through acute, intermediate, and chronic CYP-induced cystitis, these cytokines, in particular, may contribute to neuroplasticity in lower urinary tract reflexes after cystitis. IL-6 has numerous biological activities and is generally considered to be pro-inflammatory (27, 52). Some of these diverse biological activities include promotion of neuronal survival, protection against neuronal damage, induction of neuronal differentiation, modulation of neurotransmitter/neuromodulator synthesis, and modulation of pain (27, 52). The last activity is of particular interest to the present study involving a model of cystitis. It has been reported that intracerebroventricular injection of IL-6 induces thermal hyperalgesia via a mechanism involving prostaglandins (51). In addition, intrathecal IL-6 injection produces alldynia and hyperalgesia after peripheral nerve injury and intrathecal anti-IL-6 antibody treatment decreases alldynia (2). IL-1β has also been implicated in inflammatory hyperalgesia (23, 54). Several lines of evidence further suggest that IL-1β and IL-6 cause hyperalgesia by releasing cyclooxygenase products through stimulation of cyclooxygenase-2 (28, 60) and phospholipase A2 (7) or by inducing arachidonic acid release (16), respectively. In contrast, the increase in IL-10, an anti-inflammatory cytokine, may be related to a compensatory drive to counterbalance the upregulation of pro-inflammatory cytokines (81), most notably, IL-1β and IL-6. However, this increase is not sustained at the intermediate or chronic time points examined.

In summary, these studies have demonstrated significant alterations in urinary bladder cytokine mRNA, following CYP-induced cystitis examined at three time points (acute, intermediate, and chronic). This study has demonstrated that acute inflammation of the urinary bladder induces greater changes in bladder cytokine mRNA and protein expression compared with intermediate or chronic CYP-induced cystitis. Our demonstration that the changes in cytokine expression are not maintained, but rather decrease, in the chronic model of cystitis, suggests that the chronic changes in micturition reflexes associated with cystitis may be influenced to a greater degree by other chemicals [i.e., NTFs (75)] released in the urinary bladder. However, it is also possible that cytokines produced in the urinary bladder, alone or in combination with other cytokines (26, 83) or NTFs (55) also upregulated in the urinary bladder (75), may play a role in changes in the neurochemical (74, 76, 78), electrophysiological (33, 85), and organizational (77) properties of the lower urinary tract following CYP-induced cystitis. Future studies will determine the contribution of IL-1β and IL-6 to neural plasticity after cystitis by performing addition or subtraction experiments.

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