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Nociceptive and Inflammatory Mediator Upregulation in a Mouse Model of Chronic Prostatitis

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Abstract

Chronic nonbacterial prostatitis, characterized by genitourinary pain in the pelvic region in the absence of an identifiable cause, is common in adult males. Surprisingly, the sensory innervation of the prostate and mediators that sensitize its innervation have received little attention. We thus characterized a mouse model of chronic prostatitis, focusing on the prostate innervation and how organ inflammation affects gene expression of putative nociceptive markers in prostate afferent somata in dorsal root ganglia (DRG) and mediators in the prostate. Retrograde tracing (fast blue, FB) from the prostate revealed that thoracolumbar (TL) and lumbosacral (LS) DRG are the principal sources of somata of prostate afferents. Nociceptive markers (e.g., TRP, TREK and P2X channels) were upregulated in FB-labeled TL and LS somata for up to four weeks after inflaming the prostate (intra-prostate injection of zymosan). Prostatic inflammation was evident histologically, by monocyte infiltration and a significant increase in mast cell tryptase activity 14, 21 and 28 days after zymosan injection. Interleukin-10 and NGF were also significantly upregulated in the prostate throughout the four weeks of inflammation. Open field pain-related behaviors (e.g., rearing) were unchanged in prostate-inflamed mice, suggesting the absence of ongoing nociception, but withdrawal thresholds to lower abdominal pressure were significantly reduced. The increases in IL-10, mast cell tryptase and NGF in the inflamed prostate were cotemporaneous with reduced thresholds to probing of the abdomen and upregulation of nociceptive markers in DRG somata innervating the prostate. The results provide insight and direction for study of mechanisms underlying pain in chronic prostatitis.

INTRODUCTION

Chronic prostatitis affects adult men of all ages and is generally considered the most common outpatient urologic condition. More than 90% of such individuals report “urologic pain or discomfort in the pelvic region, associated with urinary symptoms and/or sexual dysfunction, lasting for at least 3 of the previous 6 months” [6]. The pain can be significant, even debilitating, and radiates to the back, abdomen and/or the colorectum, making sitting uncomfortable.

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The NIH defines four categories of prostatitis (I-IV): acute and chronic bacterial prostatitis (I and II), or chronic nonbacterial prostatitis (III), and asymptomatic inflammation of the prostate (IV) [12]. Most cases of chronic prostatitis fall into category III, referred to as “chronic prostatitis/chronic pelvic pain syndrome” (CP/CPPS), highlighting the uncertainty of whether symptoms originate from the prostate or elsewhere [23; 24], and, like other abdominal and pelvic pain syndromes (e.g., irritable bowel syndrome, painful bladder syndrome), has no identifiable cause. CP/CPPS includes genitourinary pain with or without voiding symptoms in the absence of uropathogenic bacteria or another identifiable cause (e.g., a malignancy). Diagnosed by the presence of pain in the absence of bacterial infection for > 3 months, CP/CPPS has an unknown, complex etiology that has hampered efforts to determine effective treatment strategies for ameliorating pain [11; 13; 22]. CP/CPPS is further categorized by the presence or absence of inflammatory markers in expressed prostatic secretions from patients, inflammatory (category IIIA) or non-inflammatory (category IIIB), based on amounts of pus cells (dead white blood cells) in urine, semen, and/or other fluids.

Although mechanisms underlying the pain associated with CP/CPPS are poorly understood, there is growing appreciation that persistent afferent (sensory) input from affected organs is important to the maintenance of pain and hypersensitivity as well as cross-organ sensitization in pelvic pain syndromes. For example, infusion of local anesthetic into the rectum [25; 34] rapidly relieves discomfort and pain in IBS patients, including referred abdominal hypersensitivity (tenderness). Such observations highlight the importance of organ afferent innervation and organ-centric contributions to afferent sensitization (e.g., pro-/anti-inflammatory cytokines, mast cells, growth factors, etc.), and ultimately central sensitization and hypersensitivity. The present study undertook characterization of a NIH IIIA-type mouse model of chronic prostatitis, focusing first on characterizing the innervation of the mouse prostate, which has not been extensively studied. Subsequently, we examined selected immunomodulatory molecules in the prostate to determine whether they were upregulated during inflammation and whether inflammation affected gene expression of putative nociceptive ion channels and receptors in prostate afferent somata in dorsal root ganglia.

MATERIAL AND METHODS

Animals

Experiments were performed on 6-11 week old male C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) housed in the AAALAC accredited facility at the University of Pittsburgh. Mice had access to water and food ad libitum. All protocols were approved by the Institutional Animal Care and Use Committee.

Surgical Procedures and Cell Labeling

All surgeries were performed under aseptic conditions. Anesthesia was initiated and maintained with 4% and 2% isoflurane, respectively. Primary afferent neurons innervating the prostate were identified in dorsal root ganglion (DRG) tissue sections or DRG cell cultures by content of the fluorescent retrograde tracer fast blue (FB; EMS-Chemie, Gross

Umstadt, Germany). At least seven days prior to harvesting DRG, mice were anesthetized with isoflurane, the prostate exposed aseptically by laparotomy, and the dorsal lobe injected with FB (1 mg/100 μ l; 10 μ l total distributed into 2-4 sites). Care was taken not to allow FB to spread to areas other than the injection site by removing any spillage using a cotton-tipped applicator and by rinsing the peritoneal cavity with sterile saline before suturing muscle and skin separately. After treating with buprenorphine (0.1 mg/kg; Bedford Labs, Bedford, OH) for postoperative analgesia, mice were allowed to recover in a warm environment under close observation.

In a group of six naïve mice, the distribution and number of FB-containing DRG somata was determined. Fourteen days after intra-prostate injection of FB, mice were deeply anaesthetized with isoflurane (5%) and perfused via the ascending aorta with 20 ml of cold sterile saline, followed by 20 ml of a mixture of 4% paraformaldehyde and 0.2% picric acid dissolved in 0.16 M phosphate buffer (pH 6.9; 37°C) and 50 ml of the same mixture at 4°C, the latter for approximately 5-7 min. Dorsal root ganglia were dissected out bilaterally and post-fixed for 90 min at 4°C in the same fixative and immersed in 10-20% sucrose in phosphate-buffered saline (PBS) (pH 7.4) containing 0.01% sodium azide and 0.02% bacitracin (both from Sigma-Aldrich, St. Louis, MO) (4°C) for 48 h. DRGs were embedded in Tissue-Tek O.C.T. compound (Sakura, Torrance, CA), frozen on dry ice, and sectioned in a cryostat at 12 μ m. The number of FB labeled DRG neurons innervating the prostate (DRG-p neurons) was determined by counting neuron profiles in every fifth section (i.e., 60 μ m between counted sections) in five sections per ganglion to avoid double counting of cells. Because ganglia were taken bilaterally, a mean of counted neuron profiles was determined for each pair of ganglia.

Induction of CP/CPPS

A variety of models of rodent prostatitis have been described [35], including murine transgenic models [9]. No model, however, has been established as best representing CP/CPPS and each is associated with advantages and disadvantages. Models that directly inflame or infect the prostate produce abdominal/pelvic hypersensitivity [2; 35], and we directly inflamed the prostate by intra-organ injection of zymosan. Vehicle or zymosan (0.1 mg in 10 μ l in a single injection, and also containing FB) was injected into the dorsal lobe of the prostate. In different groups of mice, prostates were removed 7, 14, 21 or 28 days after intra-prostate injection, weighed (the prostate was increased in size) and sectioned for histology and immunohistochemistry as described below.

DRG Cell Culture

Mice were deeply anesthetized (isoflurane) and perfused via the ascending aorta with ice-cold Ca²⁺/Mg²⁺-free Hank's Balanced Salt Solution (Invitrogen, Carlsbad, CA). Based on quantification and distribution of prostate afferent somata (see Results and Figure 1), T13-L1 and L6-S2 DRG were rapidly dissected out and separately prepared for culture as described previously [29; 30]. Dissociated cells were re-suspended in DMEM/F12 media (Life Biosciences, Grand Island, NY) containing 10% fetal calf serum and antibiotics (penicillin 50 U/ml and streptomycin 50 μ g/ml), and plated onto poly-D-lysine (5mg/ml) coated glass coverslips. No additional growth factors were added to the culture medium.

Cells were incubated overnight at 37°C and only FB-containing prostatic T13-L1 and L6-S2 DRG neurons were collected.

Single-Cell RT-PCR and qRT-PCR

Single-cell RT-PCR and qRT-PCR assays were performed as described previously [29; 30]. Briefly, DRG neurons were dissociated and cultured as above and neurons retrogradely labeled from the prostate with FB (i.e., DRG-p neurons) were collected with large-bore (~50 µm) glass pipettes and expelled into microcentrifuge tubes containing reverse-transcriptase (RT) mix. RT-PCR was performed with 20U Superscript II (Invitrogen) for reverse transcription. RT reactions were performed in sequence at 65°C for 1.5 min, room temperature for 2 min, at 37°C for 20 min after adding Superscript II, and 65°C for 10 min. For each experiment, negative controls consisted of omitting reverse transcriptase or using a cell-free bath aspirate as template. The first-strand cDNA from a DRG-p neuron was used as a template in a PCR reaction containing 1×GoTaq reaction buffer (Promega, Madison, WI), 20 µM outer primers, 0.2 mM dNTPs, and 0.2mL GoTaq DNA polymerase (Promega). Reactions were started at 95°C for 10 min and then cycled 35 times at 94°C/30 sec, 52°C/30 sec and 72°C/30 sec before a final extension step at 72°C for 10 min. Each initial PCR product served as template in a subsequent PCR reaction using a nested primer pair, the products of which were electrophoresed on 2% agarose ethidium bromide gels and photographed. Only neurons producing detectable amplification of a housekeeping gene (GAPDH) were analyzed further. For qRT-PCR, the first-strand cDNA of cells expressing the target genes was pre-amplified (26 cycles) using the PCR conditions described above. The products were used as template for real-time PCR using SYBR® Green mastermix (Bio-Rad, Hercules, CA) in CFX Connect real time cycler (Bio-Rad). Gene transcripts were quantified by linear regression of individual amplification curves using LinReg PCR [27], and normalized to the quantity of internal reference standard gene transcript GAPDH.

Myeloperoxidase (MPO) Assay and Histology

The MPO assay was performed as described previously [29; 30]. Mice were overdosed with inhaled isoflurane and the dorsal lobe of the prostate was dissected, weighed, added to a beaker containing 1.0 ml 0.5% hexadecyltrimethylammonium bromide (HTAB; Sigma-Aldrich) and finely minced. The contents were transferred to a 15ml centrifuge tube with another 2ml HTAB and sonicated for 10 sec before being homogenized for 30 sec. Another 2ml HTAB was added and the tube was placed on dry ice until all samples were similarly prepared. The samples underwent three freeze/thaw cycles, were centrifuged twice and loaded, along with MPO standards (Calbiochem, San Diego, CA), onto a 96-well plate. The samples and standards were reacted with O-dianisidinedihydrochloride (Sigma-Aldrich) and read on a plate reader at 460nm every 20 sec for 15 min. The slope for each standard was calculated, plotted and used to calculate units of MPO activity/tissue weight for each sample. Histological assessments were made on paraffin-embedded cross-sections of prostate stained with hematoxylin and eosin (H&E) from vehicle- and zymosan-treated mice and evaluations were performed in a blinded fashion.

Mast Cell Typtase

Mast cell typtase was measured in proteins extracted from prostates (200 ug) from zymosan- and vehicle-treated mice 7, 14, 21 or 28 days after treatment. Mast cell tryptase was measured using the mast cell degranulation kit (EMD Millipore, Billerica, MA) according to the manufacturer's protocol.

Immunohistochemistry

Immunohistochemistry was performed as described previously [30]. Sections of prostates were incubated with rabbit anti-calcitonin gene-related peptide (CGRP) antibody (1:2000; Sigma-Aldrich) followed by Cy3-conjugated anti-rabbit IgG (1:200, Jackson Immunoresearch, WestGrove, PA) to immunostain peptidergic afferent (sensory) fibers. Monocytes and macrophages were stained in other prostate tissue sections using rat anti-F4/80 (1:1000; Abcam, Cambridge, MA) and Cy3-conjugated anti-rat IgG (1:200).

Pain Related Behaviors

Two approaches were taken to assess nociceptive behaviors following prostate inflammation. Open-field activity, as described previously [29; 30], was evaluated 4, 7, 10, 14, 21 and 28 days after intra-prostate injection of zymosan or a sham procedure (laparotomy to expose the prostate). Briefly, mice were placed individually in Plexiglas boxes 25 cm² × 40 cm high, and exploratory behaviors monitored photoelectrically (15 min periods). Photoelectric beams were spaced 1.5 cm apart, providing 0.75 cm spatial resolution. TruScan software (Coulbourn Instruments, Whitehall, PA) was used to analyze time spent in different parts of the box, path information, distance travelled and total movements simultaneously in the X-Y plane. In addition, the software also analyzed time spent in the vertical plane or standing position that requires stretch of the abdomen, a position assumed to be uncomfortable in the presence of ongoing abdominal nociception. Hypersensitivity to deep palpation is widely used to infer nociception arising from internal organs, and we evaluated abdominal/pelvic hypersensitivity to mechanical probing in the same mice at the same times after intra-prostate zymosan treatment. Sensitivity to abdominal probing was assessed by pressing the lower abdomen against a rod attached to a gram force meter, and measuring the force producing withdrawal/escape behaviors. To exclude nociception arising from superficial tissues, local anesthetic (1 % dibucaine) was applied topically to the area of probing 30 min before the start of the experiment. To further assess nociception, morphine (2.0 mg/kg) was given in a different group of zymosan-treated mice 30 min prior to testing. All behavioral assessment was performed in a blinded fashion to the experimenter.

Data analysis

Data are presented as mean ± SEM and were analyzed using SigmaStat (Version 3.1, Systat Software, San Jose, CA). Statistical analyses for differences in changes over time in multiple groups were performed using two-way ANOVAs followed by the Holm-Sidak post hoc test. Differences between groups were tested using Student's t test (when comparing two groups) or one-way ANOVA (when comparing more than two groups) followed by post hoc Bonferroni-protected pairwise comparisons. Statistical significance was set at $p < 0.05$.

RESULTS

Quantification of prostate afferent somata

We first examined the afferent innervation of the prostate in naïve mice. FB-positive neuron profiles were small to medium in size (e.g., ~15-30 μ m diameter), comparable to what we and others have reported for other visceral organs in mice (e.g., [3; 4; 15; 29; 30]. The rostro-caudal distribution and mean number of FB-labeled neuron profiles in DRG is presented in Figure 1, revealing a distribution associated with two peaks (T13-L1 and L6-S2), consistent with innervation of the prostate by lumbar splanchnic (TL) and pelvic (LS) nerves. Furthermore, the proportions of FB-labeled neuron profiles relative to the total number of somata in TL and LS DRG are consistent with what others have reported using a pseudorabies (PSV) tracer injected in the rat prostate [40] as well as consistent with what is generally reported for innervation of visceral organs by spinal nerves (i.e., ~7-10%).

Zymosan-Induced Morphological Changes and Inflammation

The extent of prostate inflammation was assessed by inspection of H&E-stained sections, quantification of MPO activity and mast cell tryptase, and CGRP immunostaining. No signs of inflammation were present in vehicle-treated mice whereas a single injection of zymosan (0.1 mg) produced prostatitis of 4 weeks duration. The size/weight of the prostate significantly increased relative to vehicle-treated prostates: vehicle, 42.6 ± 1.9 mg; zymosan 60.9 ± 2.3 mg, 65 ± 2.8 mg, 55.6 ± 2.1 mg and 58.2 ± 3.5 mg 7, 14, 21 and 28 days post-injection ($p < 0.05$ for all comparisons vs vehicle, $n=6$ group). In support, there was obvious thickening of both the epithelial layer and fibromuscular stroma as well as stromal infiltration of mononuclear inflammatory cells (Figure 2). These changes appeared to be greatest 14 days after zymosan injection and persisted out to 28 days post-treatment. CGRP immunostaining revealed an increase in peptidergic fiber density in zymosan-treated mice that peaked at day 14 post-treatment and appeared to largely resolve by 28 days post-zymosan injection. F4/80 immunostaining also revealed an increase in macrophage activation/infiltration throughout the entire time course in zymosan-treated mice. Inflammation was quantified by MPO assay, which did not significantly increase after zymosan injection, suggesting an inflammatory profile not mediated by polymorphonuclear cells (e.g., neutrophils) (Figure 3A). However, there was a significant upregulation ($p < 0.05$, zymosan vs vehicle) in mast cell tryptase following induction of CP/CPPS by zymosan starting at day 14 and continuing through day 28 (Figure 3B) and a four-fold upregulation of F4/80 (a well-characterized membrane protein associated with mature mouse macrophages; Figure 3C) throughout the course of study.

Prostatitis Increases Expression of Inflammatory Mediators

Real-time PCR was performed to measure expression of pro- and anti-inflammatory cytokine, growth factor mRNA content in the prostate. For relative quantification of gene expression, we normalized the amount of each inflammatory molecule gene transcript to that of the internal reference standard GAPDH. Two-way ANOVAs revealed no differences in GAPDH expression ($F(4,34)=1.2$, $p=0.43$), indicating that any change in gene expression of prostate inflammatory molecules (i.e., cytokines or growth factors) was not due to a time-dependent change in reference gene expression. Significant increases in mRNA content were

apparent for all mediators except IL-6 and artemin (Figure 4). IL-10 and NGF were upregulated ($p < 0.05$ vs. control) at all times through 28 days post-zymosan injection into the prostate. IL-10 upregulation appears to exhibit a trend to time-dependent fold reduction from 7 to 28 days after treatment, whereas NGF ($p < 0.05$) was consistently upregulated ~five-fold throughout. In contrast, TNF α was significantly upregulated only at day 7 ($p < 0.05$) after zymosan treatment and IL-1 β only later after treatment ($p < 0.05$; days 21 and 28).

Prostatitis Increases Expression of Putative Nociceptive Molecules in DRG-p Neurons

Consistent with inflammation of the prostate and upregulation of inflammatory mediators, gene expression of molecules that have previously been implicated in visceral nociceptive mechanisms (Acid-Sensing Ion Channel 3 [ASIC3], Transient Receptor Potential (TRP) channels V1 and A1, two-pore domain potassium channels TREK-1 and TREK-2, and ionotropic purinoceptors P2X2 and P2X3; see Discussion for citations) was significantly upregulated in both thoracolumbar (TL) and lumbosacral (LS) DRG-p neurons. Both gene transcript quantity (Table 1) and the proportion of DRG-p neurons expressing mRNA (Figure 5) of these molecules were generally increased in zymosan-treated mice ($p < 0.05$, vehicle- vs zymosan-treated groups). Increases in the amount of mRNA were typically apparent in both TL and LS DRG-p neurons 7 days after intra-prostate injection of zymosan and generally persisted throughout the four weeks of testing. ASIC-3, both TRP channels and TREK-1 mRNA was reliably increased in both TL and LS DRG-p neurons whereas TREK-2 and the P2X channels exhibited differential increases in TL and LS ganglia. The proportion of TL DRG-p neurons expressing these markers generally increased over the four weeks of testing whereas increases in the proportion of LS DRG-p neurons were limited to TRP channels.

Prostatitis Produces Mechanical Hypersensitivity

We previously employed assessment of open field activity in a model of chronic pancreatitis to infer the presence of ongoing nociception [30], and anticipated that mice with inflamed prostates would similarly exhibit changes in activity. Unexpectedly, vehicle- and zymosan-treated mice were indistinguishable in measures of open field activity or vertical rearing, suggesting the absence/insignificance of ongoing nociception in mice with inflamed prostates (Figure 6A). Zymosan-treated mice, however, showed an increase in evoked nociception; they were hypersensitive to deep abdominal pressure (Figure 6B). Relative to naïve and sham groups of mice, zymosan-treated mice responded (withdrew) to significantly less force (g) applied to the lower abdomen. Mechanical hypersensitivity to probing in the area of the lower abdomen was apparent 7 days after zymosan treatment and persisted throughout the 28 days of testing. We controlled for non-prostate-related causes of mechanical hypersensitivity (i.e., incision/laparotomy-related) by applying a 1% topical anesthetic (dibucaine) to both zymosan-treated mice and sham controls, which received a laparotomy prior to testing. We further assessed the nociception-related nature of this behavior by administration of the opioid analgesic morphine (2.0 mg/kg, given 30 min prior to testing) to zymosan-treated mice, which attenuated the mechanical hypersensitivity.

DISCUSSION

In a recent evaluation in multinational cohorts of CP/CPPS patients, pain was the most prevalent complaint, having greater impact on quality of life than urinary symptoms [36], yet pain mechanisms associated with prostatitis have not been widely studied. Accordingly, we undertook characterization of a NIH category IIIA model of chronic prostatitis in the mouse as a first step in building a foundation for study of pain mechanisms in CP/CPPS. We verified that the sensory innervation of the dorsal lobe of the mouse prostate is derived principally from thoracolumbar and lumbosacral DRG, confirming a previous report in the rat [19], and showed that putative nociceptive markers in sensory neurons innervating the prostate were significantly and persistently upregulated during prostatitis. Consistent with these findings, withdrawal thresholds to mechanical probing of the lower abdomen were significantly reduced throughout the four weeks of testing, revealing an opioid-reversible hypersensitivity/hyperalgesia. While the specific cause of the mechanical hypersensitivity and upregulation of nociceptive markers is unknown and was not investigated here, mast cell tryptase, cytokines and growth factors in the inflamed prostate are likely contributors.

While studying the effects of denervation of the rat prostate on its functional and structural integrity, McVary and colleagues [19] reported that sympathectomy (hypogastric neurectomy) and parasympathectomy (pelvic neurectomy) supported the dichotomy of function of the prostate innervation. We also found that both pelvic nerve and hypogastric/lumbar splanchnic nerve afferents innervate the mouse prostate, consistent with the innervation of other lower abdominal/pelvic midline organs (e.g., uterus, urinary bladder and colorectum [1; 8; 39]). In hollow organs where distension or stretch is the adequate noxious stimulus, the pelvic nerve innervation is most relevant for nociception [14]. In a solid organ like the prostate, which or whether one innervation is more important to nociception has not been determined and inspection of the changes reported here in selected putative nociceptive markers in thoracolumbar and lumbosacral DRG-p somata is not instructive. ASIC-3, TRPA1, TRPA1 and TREK-1 were all upregulated in both thoracolumbar and lumbosacral DRG-p somata, the principal differences between the innervations being upregulation in TREK-2 in thoracolumbar and P2X3 in lumbosacral DRG-p somata. These markers were selected for study based on documented modulation, either pharmacologically or genetically, of either or both urinary bladder and colorectal nociception in mouse models of organ hypersensitivity [5; 10; 15; 16; 18; 31; 33; 39]. We anticipated that prostate inflammation would lead to an increase in putative nociceptive markers in DRG somata and that the increases would be more evident in LS than in TL DRG based on previous studies. However, results revealed similar changes in both innervations. Shared among several of the markers is proton/acid sensitivity (e.g., TRPV1, TREK1, ASIC-3 and P2X3 channels) and/or mechanosensitivity (e.g., TRPV1, TRPA1 and TREK channels), but whether their upregulation reflects inflammatory processes in the organ or modulation of neuron excitability cannot be inferred from these data.

Evaluation of contents of expressed prostatic secretions from men with CP/CPPS has led to consideration of cytokine imbalance (pro- vs anti-), mast cell products and growth factors as mechanistically involved in the pathogenesis of CP/CPPS. Increased concentrations of IL-1 β and TNF α in expressed prostatic secretions have been reported in CP/CPPS patients and

IL-6 is significantly increased in their seminal plasma [24]. Similarly, we noted significant upregulation of pro-inflammatory cytokines TNF α at day 7 and IL-1 β at days 21 and 28 post-zymosan treatment; no changes were noted in IL-6. The anti-inflammatory cytokine IL-10 also has been reported to be increased in seminal plasma ~2.5 fold in CP/CPPS patients [24] and to correlate with pain severity. In the present study, IL-10 was significantly and persistently increased in the prostate after intra-prostate injection of zymosan. Mast cells are often seen in close proximity to nerves and thus have become a current focus of CP/CPPS research, with proposed roles as the main mediator and effector cell in disease progression, including neuronal activation and sensitization [28; 37], to which mast cells may contribute via release of NGF [7; 17]. Expressed prostatic secretions from men with CP/CPPS contain elevated mast cell tryptase, a constituent of mast cell granules released during degranulation, and NGF. In the inflamed mouse prostate, both mast cell tryptase and NGF were significantly increased. Like IL-10, it has been reported that NGF directly correlates with pain severity in men with CP/CPPS [20; 38]. NGF has long been established as a sensitizer of nociceptive afferents [21], and thus is presumably capable of sensitizing prostatic afferents. Mast cells express TrkA receptors at which NGF binds and causes degranulation [32], establishing a potential feedback mechanism that would promote sensitization mechanisms. Notably, the increased mechanical hypersensitivity documented here is consistent with the significant and sustained increases in IL-10, mast cell tryptase and NGF in the mouse prostate.

Numerous rodent models of prostatitis have been developed, including spontaneous prostatitis, autoimmune prostatitis, infection- and hormone- induced prostatitis, and others associated with diet, stress, etc [35]. Most models have been employed to study prostatic histopathology, inflammatory mechanisms or cancer, not the principal complaint of CP/CPPS patients – pain. Visceral pain is generally difficult to evaluate directly and so we evaluated ongoing nociception and deep, provoked nociception. We hypothesized that mice with inflamed prostates would spend less time pursuing exploratory behaviors (open field measurements of vertical and horizontal movements). That this approach is sufficiently sensitive was established in a model of chronic inflammation of another parenchymous organ, the pancreas [29; 30], in which both horizontal and vertical exploratory behaviors were significantly reduced and also opioid sensitive. Mice with inflamed prostates, however, did not show significant differences in any exploratory behavior relative to vehicle-treated controls, which we interpret as an absence of ongoing nociception. In contrast, mice with inflamed prostates were hypersensitive to mechanical stimulation of the lower abdomen, exhibiting significantly reduced thresholds for withdrawal from probing. In related work, Quick and colleagues [26] described reduced thresholds to von Frey-like monofilament probing of the abdominal skin (“tactile allodynia”) in a mouse model of bacterial prostatitis. We did not examine sensitivity to stimuli in the area of referral (i.e., abdominal skin), from which we blocked input using topical local anesthetic to examine more directly deep pressure. To evaluate whether the reduced thresholds to probing we noted were nociception-related, we showed that low dose morphine reversed the hypersensitivity in zymosan-treated mice, but had no effect on withdrawal thresholds of sham-treated or naïve mice. Importantly, morphine was efficacious as a single treatment given at a time when prostatitis and

hypersensitivity were well established, thus validating the utility of this behavioral methodology.

In summary, we focused in the present study on the innervation of the mouse prostate, documenting upregulation of nociceptive markers in TL and LS DRG-p somata. We also found that these changes are likely associated with inflammatory changes in the prostate, illustrated by concurrent changes in cytokines, mast cell tryptase, and NGF in the inflamed prostate. These results are cotemporaneous with reduced thresholds to mechanical probing of the lower abdomen. Taken together, these findings provide targets for subsequent, mechanistic investigations focused on functional consequences of the changes. For example, CP/CPPS patients complain of bladder dysfunction and future studies will focus on studying the effect of prostate inflammation on bladder function.

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Figure 1.

Distribution of neuron profiles in thoracic-sacral dorsal root ganglia (DRG) retrogradely labeled from the dorsal lobe of the mouse prostate with fast blue (FB). Labeling in T13-L2 DRG represents the hypogastric/lumbar splanchnic nerve innervation; labeling in L5-S2 DRG represents the pelvic nerve innervation. Illustrated above are representative photomicrographs of retrogradely labeled neurons in DRG.

Figure 2.

Photomicrographs of the mouse prostate 14 days after intra-prostate injection of vehicle (veh) and 7, 14, 21 and 28 days after intra-prostate injection of zymosan (zym). (A) Hematoxylin and Eosin (H&E) staining reveals evidence of inflammation, including infiltration of inflammatory cells (macrophages, see F4/80 staining below) into the periglandular, fibromuscular stroma (S), which thickens (hyperplasia) during progression of the inflammation, and shedding of luminal epithelial cells (E) into the prostatic glandular lumen (*). CGRP immunohistochemistry (arrows) is presented in the middle row, revealing the typical increase and reversal in density of innervation during progression of the inflammation. F4/80 immunohistochemistry is presented in the bottom row, showing activation/infiltration of macrophages during progression of the inflammation. Calibration, 50 μ m.

Figure 3.

Quantification of myeloperoxidase (MPO), mast cell tryptase activity and F4/80 in intra-prostate vehicle- (veh) and zymosan-treated mice (7, 14, 21 and 28 days post treatment). (A) MPO activity (units/mg tissue) was unchanged by zymosan treatment whereas mast cell tryptase activity (units/mg tissue) (B) increased significantly 14, 21 and 28 days post-zymosan treatment. (C) F4/80 mRNA content (represented as fold control GAPDH) in the prostate increased significantly ~four-fold during zymosan-induced prostatitis. * $p < 0.05$, (zymosan vs veh); $n = 6$ /treatment group.

Figure 4.

Expression of cytokines and growth factors in the dorsal lobe of the prostate during zymosan-induced prostatitis. The time course and magnitude of changes in pro- and anti-inflammatory cytokine and growth factor mRNA content in the prostate during zymosan-induced inflammation was measured by real-time PCR and is represented as fold control (GAPDH). * $p < 0.05$ vs. control; two-way ANOVA with Bonferroni posthoc test; $n=8$ /group.

Figure 5.

Expression of putative nociceptive markers in neuronal somata innervating the prostate. The time course and magnitude of changes in nociceptive markers in somata from vehicle (veh) and zymosan (zym)-treated mice was measured by real-time PCR. Individual neurons back-labeled from the prostate were collected after plating and processed for single cell PCR analysis. Each group represents cells from 4 mice with 20 cells/group (veh: TL, 28 cells and LS, 26 cells; zym-7d: TL, 24 cells and LS, 25 cells; zym-14d: TL, 28 cells and LS, 27 cells; zym-21d: TL, 26 cells and LS, 29 cells; zym-28d: TL, 27 cells and LS, 24 cells). * $p < 0.05$ (vs. veh) two-way ANOVA with a Holm-Sidak posthoc test.

Figure 6.

Assessment of open field activity revealed that the number of rearing events in the vertical plane did not significantly differ between any treatment group: naïve, vehicle (veh), sham or zymosan (zym) - treated mice (A). Withdrawal thresholds (g) to probing of the lower abdomen are presented in B. Intra-prostate injection of zymosan produced a significant and persistent reduction in the force required to produce withdrawal to probing (* $p < 0.01$ vs sham). Withdrawal thresholds in untreated, naïve mice and mice receiving a laparotomy to replicate accessing the prostate (sham group) were unaffected over the course of the experiment. Morphine (morph, 2.0 mg/kg, s.c.) given 30 min prior to testing significantly

reversed the mechanical hypersensitivity to probing ($\dagger$ $p < 0.05$ vs zym). All data analyzed by two way ANOVAs with a Holm-Sidak posthoc test to compare treatment groups (n=6/group).

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Proportions of neurons expressing nociceptive markers in DRG somata retrogradely labeled with fast blue from the prostate

Percentages of dorsal root ganglion (DRG) neurons expressing putative nociceptive markers. Only DRG somata innervating the prostate were collected and processed for single cell PCR analysis. Each group represents cells from 4 mice with 20 cells/group.

Table 1

Thoracolumbar (TL) DRG					
	veh	zym-7d	zym-14d	zym-21d	zym-28d
ASIC-3	52%	66%	58%	82% *	78% *
TRPV1	62%	74%	69%	78% *	65%
TRPA1	48%	52%	50%	72% *	68% *
TREK-1	34%	48%	55% *	65% *	66% *
TREK-2	65%	51%	68%	78% *	75% *
P2X2	35%	46%	50% *	62% *	60% *
P2X3	58%	61%	80% *	82% *	84% *
Lumbosacral (LS) DRG					
	veh	zym-7d	zym-14d	zym-21d	zym-28d
ASIC-3	77%	76%	68%	84%	80%
TRPV1	54%	64%	48%	82% *	85% *
TRPA1	44%	51%	41%	70% *	74% *
TREK-1	64%	66%	63%	72%	62%
TREK-2	77%	68%	70%	80%	72%
P2X2	55%	52%	50%	60%	58%
P2X3	86%	76%	92%	88%	86%

Mice were treated with vehicle (veh) or zymosan (zym). See text for details.
Vehicle, veh; zymosan, zym.

* p<0.05 vs. vehicle by Chi-square test.