Experimental Autoimmune Prostatitis Induces Chronic Pelvic Pain

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ABSTRACT

Pain is the hallmark of patients with chronic prostatitis (CP) and chronic pelvic pain syndrome (CPPS). Despite numerous hypotheses the etiology and pathogenesis remain unknown. To better understand CP/CPPS we used a murine experimental autoimmune prostatitis (EAP) model to examine the development, localization and modulation of pelvic pain. Pelvic pain was detected 5 days after antigen instillation and was sustained beyond 30 days, indicating the development of chronic pain. The pain was attenuated by lidocaine treatment into the prostate, but not into the bladder or the colon suggesting that pain originated from the prostate. EAP histopathology was confined to the prostate with focal periglandular inflammatory infiltrates in the ventral, dorso-lateral and anterior lobes of the mouse prostate. Inflammation and pelvic pain were positively correlated and increased with time. Morphologically, the dorso-lateral prostate alone showed significantly increased neuronal fiber distribution as evidenced by increased PGP 9.5 expression. Pelvic pain was attenuated by treatment with the neuromodulator gabapentin, suggesting spinal and/or supraspinal contribution to chronic pain. These results provide the basis for identifying mechanisms that regulate pelvic pain and the testing of therapeutic agents that block pain development in CP/CPPS.

KEYWORDS

Chronic pelvic pain syndrome, prostatitis, neuropathic pain, gabapentin, pelvic pain
INTRODUCTION

Prostatitis accounts for approximately 2 million outpatient visits per year in the United States, including 8% of all visits to urologists and 1% of those to primary care physicians (6). Chronic pelvic pain syndrome (CPPS), a non-bacterial category of prostatitis accounts for approximately 90% of all chronic prostatitis and is the most common urologic diagnosis in men less than 50 years of age in the United States (6). CPPS is clinically characterized by pain in the perineum, rectum, prostate, penis, testicles and abdomen of affected men (16). In cross-sectional studies, CPPS is associated with reductions in the patient’s quality of life similar to or greater than those associated with angina, congestive heart failure, Crohn’s disease and diabetes mellitus (19). Despite various hypotheses the etiology and pathogenesis of this disease remains unknown.

Numerous animal models of chronic prostatitis/ chronic pelvic pain syndrome (CP/CPPS) have been developed that utilize spontaneous, infectious, immune-mediated and hormone-associated methodology to induce prostatitis (32). Each of these models reflects key aspects of human chronic prostatitis but does not address the development of chronic pelvic pain, a distinguishing symptom underlying CPPS (30). We therefore examined the development of pelvic pain in a mouse model of experimental autoimmune prostatitis (EAP)(27). The EAP model utilizes rat prostatic antigen injection with adjuvant to induce autoimmune prostatitis in male non-obese diabetic (NOD) mice. A similar model has been previously characterized in NOD mice to be mediated by T cell activation leading to chronic inflammation of the prostate gland (27). This parallels observations in CP/CPPS where the expressed prostatic secretions (EPS) of some
patients contain cytotoxic T cells, a cell type more commonly associated with autoimmune inflammation and secondary remodeling of injured tissue (31).

The prostate gland receives regulatory autonomic innervation from both the sympathetic and parasympathetic nervous systems (20). Afferent innervation to the prostate appears to be localized to the sensory nerves from the L, and L, spinal segments with some small degree of innervation from T, to L, (20). Given the abundant innervation of the prostate gland, the pain of CPPS may result from neurogenic inflammation in the peripheral and central nervous systems (25). The expression of pain from the viscera is usually referred to the superficial areas of the body including the muscle and/or skin (17). Pelvic pain behavior in the EAP model was therefore studied in response to mechanical stimulation of the skin of the pelvic area. Evidence of central nervous system (CNS) remodeling has been shown by the finding that chemical irritation of the rat prostate or bladder causes c-fos expression at spinal cord levels L6 and S1 (13). One of the hallmarks of such remodeling is neurogenic inflammation. We therefore studied the role of peripheral and central mechanisms in persistence of pain by examining pain behavior following targeted therapeutic intervention with pharmacological agents.

In addition to neurogenic inflammation restricted to a single organ, inflammatory crosstalk between pelvic organs that share innervation via the sacral spinal cord has been previously described (reviewed in (34, 35)). Early studies in cats showed that the majority of spinal neurons that responded to bladder stimulation also responded to colon stimulation, and vice versa (18), and that colon nerves modulate micturition (10-12). These findings of bladder-gut interactions were extended by a series of studies demonstrating that the uterus also modulates bladder function at the level of the spinal
cord (reviewed in (3, 4)). Similarly, chemical irritation of the bladder or prostate in rats yielded similar patterns of c-fos expression in the sacral spinal cord (13). Together, these studies demonstrate that neural crosstalk between pelvic organs can modulate pelvic organ physiologic function. In light of these studies and a more recent study demonstrating pelvic pain modulation by organ crosstalk between the colon and bladder (28), we examined whether colonic administration of a local anesthetic modulated pelvic pain in the EAP model.

In this study, prostate-specific autoimmunity was induced in mice by immunization with rat prostate homogenates and pelvic pain development, localization and modulation were examined. The EAP model of CP/CPPS developed pelvic pain that was chronic and localized to the prostate gland. Pain increased with time and was positively correlated with inflammation of the prostate gland. Finally, pelvic pain was amenable to treatment with therapeutic agents targeting the peripheral and central nervous systems.
METHODS

Animals. Adult male NOD/ShiLtJ (5-7 weeks old) mice were purchased from Jackson Laboratory (Bar Harbor, ME). All experiments were performed using protocols approved by Northwestern University Animal Care and Use Committee. The mice were housed in containment facilities of the Center for Comparative Medicine and maintained on a regular 12:12 hour light-dark cycle with food and water ad libitum.

Antigen preparation. The methods used to prepare antigen and immunize animals followed previous descriptions with modifications (27). Prostate glands from BB/Wor rats were used to prepare antigen extract. Pooled glands were homogenized in PBS at pH 7.2 with protease inhibitors, in an Ultraturrax homogenizer (Ivan Sorvall Inc., Norwalk, CT, USA). The homogenate was centrifuged at 10,000 × g for 30 min and the supernatant used as prostate antigen (PAg) homogenate. Protein concentration was determined and adjusted to a standard concentration of 10 mg/ml.

Immunization. Mice were injected with 1 mg of male prostate gland extract emulsified in an equal volume of TiterMax adjuvant (TiterMax USA, Inc, Georgia, USA) with a 26 gauge Hamilton syringe while maintaining the animals under isoflurane anesthesia. A total volume of 0.100 ml emulsion was injected subcutaneously in two different sites: base of the tail (0.050 ml) and shoulder (0.050 ml). Control animals received only TiterMax adjuvant.

Behavioral Testing. Mice were tested prior to rat prostate antigen (PAg) injection (baseline) and 5, 10, 15, 20, 25 and 30 days after PAg. Referred hyperalgesia and tactile allodynia was tested using von Frey filaments applied to the abdomen (14, 15) and the plantar region of the hind paw (5). Mice were tested early in the morning in individual Plexiglas chambers (6cm x 10cm x 12cm) with a stainless steel wire grid floor (mouse acclimation period of 20 min prior to testing). Standardized conditions for testing including fixed time-of-day, standard methodology, single experimenter testing of all animals and blinded testing of groups were utilized to combat the limitations of
behavior-based pain testing in animal models. Frequency of withdrawal responses to the application of von Frey filaments to the abdomen was tested using five individual fibers with forces of 0.04, 0.16, 0.4, 1.0 and 4.0 grams (Stoelting, USA). Each filament was applied for 1-2s with an inter-stimulus interval of 5s for a total 10 times, and the hairs were tested in ascending order of force. Stimulation was confined to the lower abdominal area in the general vicinity of the prostate and care was taken to stimulate different areas within this region to avoid desensitization or “wind up” effects. Three types of behaviors were considered as positive responses to filament stimulation: (1) sharp retraction of the abdomen; (2) immediate licking or scratching of the area of filament stimulation; or (3) jumping. Response frequency was calculated as the percentage of positive response (out of 10, e.g. 5 responses of 10 = 50%) and data was reported as the mean percentage of response frequency ± SEM.

Tactile allodynia was tested on the plantar region of the hind paw using von Frey filaments with forces of 0.04, 0.16, 0.4, 1.0 and 4.0 grams. The median 50% withdrawal threshold (5) was assessed using the up-down method where testing was started with 0.04g filament applied perpendicularly to the plantar surface of the hind paw until the filament bent slightly. Filaments were tested in ascending order until a positive response was observed. A positive response to the filament was defined as either a sharp withdrawal of the paw or licking of the test paw. When a positive response was recorded the next weaker filament was applied, and if a negative response was observed, then the next stronger filament was applied.

Spontaneous behavior was recorded (Sony VAIO USB camera) for five minutes in a clear plastic open field chamber (18 x 29 x 12cm) and scored for rearing, grooming and cage crossing to assess general activity (26).

**Histochemistry**

Paraffin-embedded 5-µm sections were prepared from prostate samples fixed in 10% neutral buffered Formalin. Sections were stained with haematoxylin and eosin (H&E) at the Northwestern Pathology Core facility and examined using an upright microscope.
Inflammation scoring
The ventral (VP), dorsal and lateral (DLP) and anterior or coagulating gland (CG) lobes of the mouse prostate were collected from control (TiterMax) and antigen (prostate antigen, PAg) immunized animals (5 per group) at days 5, 10, 20 and 30 following injection. Individual prostate lobes were processed for histochemistry and H&E sections were examined and scored blindly using the histopathological classification system for chronic prostatic inflammation (23). Briefly, the anatomical location, extent and grade of inflammation were noted for each section using established criteria. The extent of chronic inflammation was graded from 0-3 with 0 representing no inflammation and 3 representing confluent sheets of inflammatory cells with tissue destruction or lymphoid nodule/follicle formation.

PGP 9.5 quantification. The ventral (VP), dorsal and lateral (DLP) and anterior or coagulating gland (CG) lobes of the mouse prostate were collected from control (TiterMax) and antigen (prostate antigen, PAg) immunized animals (3 per group) at day 30 following injection. Paraffin-embedded 5-µm sections were deparaffinized using standard methods and rehydrated in graded ethanols. Non-enzymatic Ag retrieval was performed by treatment with 0.01 M sodium citrate (pH 6.0) at 92°C for 10 min, and sections were blocked with blocking solution (10% fetal bovine serum in PBS) for 1 hour at room temperature, followed by overnight incubation at 4°C with rabbit anti-PGP 9.5 antibody (ab17039; Abcam). PGP 9.5 expression was detected using goat anti-rabbit Alexa-fluor 488 (Molecular Probes), mounted with diaminopropylindole mounting medium, and visualized using a fluorescence microscope. PGP 9.5 staining (green) was quantified using Volocity software (Improvision) to detect and count green pixel densities larger than 10 µm in a single dimension. Three random fields from a single 5-µm section of each prostate lobe were imaged and quantified and separate sections from the prostate lobe of three control (Titermax) and three antigen (PAg) treated mice were examined.

Lidocaine Treatment. Lidocaine drug therapy was administered as a 2% lidocaine solution in distilled water that was instilled into the bladder (25 µl), colon (50 µl) or
prostate (25 µl) via a 30 G Hamilton syringe needle (rounded tip needle 3.8 cm long for the colon) while the mouse was maintained under isoflurane anesthesia. Instillation into the prostate and bladder were preceded by localization of the prostate gland and the bladder in anesthetized mice 35 days after PAg or Titermax injection using ultrasound probes of the Vevo 770 (Visualsonics) high resolution in vivo micro-imaging system (36). Instillations into the corresponding organs were performed under real-time ultrasound guidance. All mice were tested for referred hyperalgesia and tactile allodynia using von Frey filaments before 45min after lidocaine treatment.

**Gabapentin Treatment.** Gabapentin is specifically recommended for the treatment of neuropathic pain (9) and acts on both excitatory and inhibitory spinal neurons (2). Gabapentin was used at a dose known to reverse pain in other mouse models (56mg/kg) and was administered as a solution in distilled water injected intraperitoneally (I.P.) (33). Sham controls were injected with distilled water (I.P.). All mice were tested 35 days after PAg or Titermax injection for referred hyperalgesia and tactile allodynia using von Frey filaments before, and following treatment at 1 and 24 hours.

**Statistical analyses.** Results were expressed as mean ± SEM and analyzed for statistical significance by a single factor ANOVA or two-way ANOVA with matching. Post test analysis of multiple groups was performed using the Tukey-Kramer test and a value of p<0.05 was considered statistically significant.
RESULTS

EAP induces chronic pelvic pain in NOD mice. We examined the development of pelvic pain in a murine EAP model of CP/CPPS using referred pain to the skin of the pelvic region (17), as well as spontaneous behavioral changes (26) as indicators of visceral pain. To assess tactile sensitivity in the pelvic area, mice were stimulated with von Frey filaments at various times following immunization with PAg (5, 14, 15). Mechanical stimulation of the pelvic area of sham-immunized mice resulted in a response frequency that correlated with the applied force, and this response profile did not change during the 30-day course of the experiment (Fig. 1A). In contrast, although PAg-treated mice exhibited the same baseline response, the response frequency to pelvic stimuli was significantly greater at all filaments by post-injection day 10 (Fig. 1B; P<0.01). The increase in pelvic sensitivity was sustained until day 30 (P<0.001). On day five, the four largest filaments were significantly different for baseline (P<0.01), however the smallest filament was not. To assess the specificity of PAg-induced tactile sensitivity, we also quantified the 50% threshold sensitivity in the paw. PAg induced no changes in tactile sensitivity of the plantar region of the hind paw (Fig. 1C). These results suggest the development of chronic referred pain that is localized to the pelvic area.

To confirm that the effects of PAg were specific to pain behavior, we also quantified normal behaviors during free roaming (Table 1). PAg induced no significant differences in grooming, cage crossing, or rearing, suggesting that pelvic pain is evoked and not due to spontaneous pain. A significant change in weight was observed 5 days after PAg, but not at any other time point (Table 2). The absence of prolonged weight change indicates that PAg is not associated with dramatic changes in gross physiology (Table 2).

Pain in EAP is correlated with chronic prostate inflammation. Previous studies using an autoimmune prostatitis model in NOD mice have shown the presence of
inflammatory infiltrate in the prostate interstitium at 10 and 21 days after PAg immunization (27). We characterized the kinetics of onset as well as the nature of inflammation in individual prostate lobes of mice immunized with PAg. Inflammatory infiltrates in the ventral (VP), dorso-lateral (DLP) and coagulating gland (CG) were observed to increase significantly with time in the PAg-immunized animals (p=0.008) (Fig. 2A&C). Adjuvant-treated control mice exhibited low levels of inflammatory infiltrates that did not show any significant time-dependent changes (Fig. 2A&B). Inflammation in the PAG-immunized prostate gland was focal and periglandular in distribution but did not significantly differ between different lobes (Fig. 2A). In contrast, the bladder and colon of mice 30 days after PAg-immunization did not demonstrate any histological changes indicative of inflammation (data not shown), suggesting that the pathology is restricted to the prostate gland. We simultaneously quantified pelvic pain at 5, 10, 20 and 30 days after PAg or adjuvant injection and examined its correlation with prostate inflammation using the Pearson correlation test. Pain was positively correlated with chronic inflammation over the 30-day time-course in PAg-immunized mice ($r^2=0.8413$, $p=0.0414$) but not in the adjuvant treated mice ($r^2=0.4396$, $p=0.1685$). These results suggest that prostate-specific disease processes that lead to inflammation are likely to be associated with chronic pelvic pain.

**EAP pain is associated with increased prostatic nerve fiber density.** Although inflammation has been characterized in EAP, the potential for morphological changes in prostate innervation has not been examined. We characterized the density of prostatic neuronal processes by staining prostate sections with the pan neuronal marker PGP 9.5 (Fig. 3). PGP 9.5 immunoreactivity was evident in the prostate, and PGP 9.5 staining was independent of cell bodies, suggesting the labeling of neural processes (Fig. 3A & C). We next used Volocity software (Improvision) to quantify staining in prostate sections of EAP mice and control mice receiving TiterMax alone (compare Fig. 3A&B). Staining density was unchanged in the ventral prostate and coagulating gland of EAP mice compared to controls (Fig. 3D). However, PGP 9.5 staining was significantly
increased in the dorso-lateral prostate of EAP prostates relative to controls (Fig. 3D, P<0.05). These results suggest that EAP induces alterations in nerve fiber distribution significantly within the prostate, and this increased neuronal density may contribute to pelvic pain.

**Lidocaine attenuates PAg-induced Prostate pain.** One clinical treatment that is reported to offer temporary relief of chronic pelvic pain is instillation of 2% lidocaine directly into the affected organ (24). This treatment modality presumably works by quelling C-fiber activity associated with the pathophysiology of the disease. We used a similar strategy to localize the source of pelvic pain in the EAP model by instilling 2% lidocaine into the prostate, bladder or colon 35 days after PAg immunization. Lidocaine instilled into the prostate significantly (p<0.05) reduced the response frequency to mechanical stimulation with von Frey filaments by approximately 46% (Fig. 4A), while animals injected with lidocaine into the bladder or colon exhibited no loss of pelvic sensitivity (compare Fig. 4B and C). The anesthetic effects were specific to pelvic pain because lidocaine instillation did not alter sensitivity to stimulation of the paw (Table 3). These data suggest that the pelvic pain in EAP localizes to the prostate.

**Pelvic pain is attenuated by gabapentin treatment.** The pain of CP/CPPS is increasingly believed to be neuropathic in origin and to be associated with CNS changes (7). The effect of CNS intervention on attenuating chronic pelvic pain in the EAP model was examined using the CNS-acting, anticonvulsant drug, gabapentin. Intraperitoneal instillation of gabapentin 1 hour before testing in PAg-immunized animals significantly reduced the response frequency to mechanical stimulation with von Frey filaments by approximately 30% (p<0.05). In contrast, animals injected with vehicle exhibited no loss of pelvic sensitivity (compare Fig. 5A and B). The pelvic pain in the gabapentin treated mice returned 24 hrs after injection (p>0.05) suggesting that the analgesic effects are not long-lived. The analgesic effects were specific to pelvic pain because gabapentin did not
alter sensitivity to stimulation of the paw (Table 3). These results suggest that chronic pelvic pain in the EAP model has neuropathic origins and may involve the CNS.
DISCUSSION

Pain is the hallmark of CPPS and is a characteristic clinical symptom in human patients (30). In this study, we report the development of chronic pelvic pain-related behavior in an EAP model of CP/CPPS and examine its localization, modulation and regulation. The pain-related behavior in the EAP model was localized to the pelvic region and became persistent, closely resembling the localization and chronic nature of the pain of CPPS in human patients. The pain-related behavior was amenable to therapeutic intervention locally using lidocaine and centrally using gabapentin suggesting multi-level regulation involving central and peripheral nervous systems. To our knowledge, our report is the first to show chronic pain in a CP/CPPS animal model and has broad implications for examining the mechanisms of pelvic pain in CPPS and for evaluating therapeutic intervention in this disease syndrome.

An autoimmune basis for CP/CPPS is a prominent theory for the etiology/pathogenesis of CP/CPPS that has been supported by evidence of autoimmune mediators in expressed prostatic secretions of CPPS patients (25, 31). Autoimmune prostatitis has been modeled in mice, and the immunological mediators as well as pathological changes developing in the prostate have been extensively studied (21). However, in contrast to other pelvic pain syndromes like interstitial cystitis (IC), the development of chronic pelvic pain has not been systematically characterized in EAP or other CP/CPPS models. We therefore examined pain-related behavior in the EAP model using behavior-based methods previously used to quantify pelvic pain in neurogenic cystitis (28). Using tactile
allodynia of the pelvic region as an indicator of pelvic pain, significant pain was shown
to develop by 5 days after PAg instillation and persist beyond 30 days in NOD mice.

Previous studies in the EAP model in NOD mice have shown histological changes in the
prostate within 10 days after instillation of rat prostate antigen (27). We observed that
inflammatory infiltrates accumulate in all the prostate lobes over time with no specificity
for any single lobe. More importantly, we observed a strong correlation between the
presence of inflammation and the development of pelvic pain. Given that the
inflammation is focal in nature these results suggest that foci of inflammation within a
largely unperturbed prostate are sufficient to elicit significant pelvic pain in this disease
model. Interestingly, recent studies in human patients have reported significant
correlation between average chronic inflammation and the total Chronic Prostatitis
Symptom Index score but not the pain subscore (22). We speculate that focal
inflammation in the prostate may be under-represented in such studies and may yet be
associated with chronic pelvic pain.

While the immune response is of obvious importance, we were also interested in
examining whether there was any neurogenic contribution to pain in EAP. Interestingly,
there is considerable evidence for such a hypothesis in other immune mediated disease
like arthritis (8) and asthma (1). In the EAP model we observed a significant increase in
nerve fiber staining specifically in the dorso-lateral prostate. Thus pain in EAP could
partly be the result of the increased interaction of nerve fibers with inflammatory
mediators and neuropeptides released by pro-inflammatory cells in the prostate. These
mediators could presumably evoke neurogenic inflammation and pain as evidenced by
hyperalgesia and allodynia in EAP. The specificity of neuronal alterations to the dorsolateral prostate, is significant and suggests differences in prostatic innervation that may have important consequences for the development and progression of symptoms in EAP.

We confirmed the source of pelvic pain in EAP by instillation of lidocaine directly into the prostate and two separate organs the colon and bladder. A significant decrease in pelvic pain was detected only upon direct instillation into the prostate suggesting that pelvic pain in this model originates from the prostate gland. The inability of lidocaine to return pain responses to baseline may be accounted by our limited ability to directly instill the drug into prostatic lobes with the largest neurogenic response, particularly the dorso-lateral prostate. We speculate that peripheral neurons in the prostate that are sensitized to inflammation are quelled by lidocaine resulting in a significant inhibition of pelvic pain. The absence of negative modulation of pelvic pain upon instillation of lidocaine into the colon contrasts this model with that described for neurogenic cystitis where organ crosstalk was observed between the bladder and the colon (28). Our results suggest that despite significant overlap of spinal nociceptive neurons between pelvic soma and viscera (13), there may be distinct differences between the neural circuitry of the bladder and prostate at the level of convergence with other visceral organs. These differences may have important clinical implications with regard to the co-morbidity of prostate and bladder-specific pelvic pain with disorders of other organ systems.

The CNS has been suggested to play a role in mediating pain in CP/CPPS through sensitization or “wind up” of neurons at the spinal cord and brain (25). Experimental
evidence for such sensitization is provided by the finding that chemical irritation of the rat prostate and bladder causes c-fos expression at spinal cord levels L6 and S1 (13). Inhibition of CNS function using the neuromodulator gabapentin resulted in a significant attenuation of pelvic pain. Gabapentin does not completely abrogate pain responses suggesting that in addition to central sensitization mechanisms affected by gabapentin, other local pain pathways may also be involved in mediating pain behavior in EAP. Gabapentin is used to alleviate neuropathic pain in human patients and has been successfully used in the treatment of refractory genitourinary pain (29). Our results in the EAP model suggest that CNS agents like gabapentin bring about relief of pain in CP/CPPS through effects on the CNS and provide evidence for the hypothesis that chronic pelvic pain is neuropathic in nature. Thus the mechanisms of pelvic pain in CPPS may involve both the central and peripheral nervous systems and therapies aimed at abolishing pelvic pain may need to be multi-modal to achieve lasting therapeutic benefits.
PERSPECTIVES AND SIGNIFICANCE

Chronic pelvic pain syndrome (CPPS) accounts for approximately 90% of all chronic prostatitis and is the most common urologic diagnosis in men less than 50 years of age in the United States. The disease has no known etiology and is primarily characterized by pain in the perineum, rectum, prostate, penis, testicles and abdomen of affected men. While numerous animal models have been developed that recapitulate aspects of CPPS, our study is the first to examine chronic pain in an animal model of CPPS. We have characterized the development of pelvic pain in the EAP model, identified a role for the central and peripheral nervous systems in maintaining pelvic pain, and shown that pain can be localized to the prostate. These results provide the basis for identifying and isolating mechanisms that regulate pelvic pain and the testing of therapeutic agents that can block pain development in CP/CPPS.

ACKNOWLEDGEMENTS

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REFERENCES


FIGURE LEGENDS

Figure 1. PAg induces chronic pelvic pain in male NOD mice. Referred visceral hyperalgesia was measured as responses to mechanical stimulation of the pelvic region and hind paw using von Frey filaments of 5 calibrated forces. Data are reported as the mean percentage of response frequency ± SEM (e.g. 5 responses of 10 = 50%) before (baseline) or at 5, 10, 15, 20, 25 and 30 days after PAg immunization. A) Responses to pelvic stimulation of sham-injected male NOD mice receiving adjuvant injection (n=10). B) Responses to pelvic stimulation of male NOD mice injected with PAg (n=20). ANOVA indicated a significant increase in response frequency from baseline at all filaments tested in PAg-treated mice at days 10-30 (p<0.001), with no significant differences in baseline between controls and PAg-treated mice. C) PAg induced no significant change in tactile sensitivity (50% threshold) of the plantar region of the paw (p>0.05 at all time points).

Figure 2. EAP in NOD mice induces chronic inflammation of the prostate. A) NOD mice were injected with PAg (PAg) or adjuvant (C) and the ventral lobe (VP), dorsolateral (DLP) lobe and coagulating glands (CG) of the prostate were removed at 5, 10, 20 and 30 days after immunization. H&E stained sections were scored blindly and the extent of chronic inflammation was graded from 0-3 with 0= no inflammation, 1=mild, 2=moderate and 3= marked inflammation. PAg-immunized mice demonstrated increasing inflammatory infiltrates that were focal and periglandular and increased with time in all the lobes of the prostate. The images shown are representative and the scale bar represents 50 microns. In contrast to adjuvant-immunized mice (B), PAg-immunized mice (C) demonstrated a significant increase (p<0.05) in inflammation scores in all prostate glands over time. Data are shown as mean ± SEM of 5 mice per group at each time-point.
Figure 3. **EAP results in increased nerve fiber density.** Mouse prostate sections were stained with the neuronal marker PGP 9.5 (green, scale bar 100 µm). Nerve fiber distribution (white arrows) was observed to be more profuse in the dorso-lateral prostates from EAP mice (A) compared to control mice (B). A magnified image from the same section as panel (A) with white arrows indicating PGP 9.5 staining (C). PGP 9.5 staining was quantified in mouse prostate sections using Volocity to detect and count green pixel densities larger than 10 µm in a single dimension (D). Data reflect mean ± SEM for sections of 3 animals within the dorso-lateral prostate (DLP), the ventral prostate (VP), and the coagulating gland. The mean nerve fiber density for individual animals was determined by quantifying PGP 9.5 staining in 3 random fields.

Figure 4. **Prostate lidocaine attenuates PAg-induced pelvic pain.** Referred visceral hyperalgesia was measured as responses to mechanical stimulation of the pelvic region and hind paw using von Frey filaments of 5 calibrated forces. Data are reported as the mean percentage of response frequency ± SEM. Responsiveness was characterized at baseline, 35 days following PAg injection, and 45 min following ultrasound guided administration of 2% lidocaine. Instilling lidocaine into the prostate (A, n=8) reduced pelvic pain responses (p<0.05), whereas bladder (B, n=5) or colon lidocaine (C, n=5) had no significant effect (p>0.05).

Figure 5. **Gabapentin attenuates PAg-induced pelvic pain.** Referred visceral hyperalgesia was measured as responses to mechanical stimulation of the pelvic region and hind paw using von Frey filaments of 5 calibrated forces. Data are reported as the mean percentage of response frequency ± SEM. Responsiveness was characterized at baseline, 35 days following PAg, and 1 h following administration of distilled water (A) or gabapentin (B). Gabapentin (B, n=5) reduced pelvic pain responses 1 h after injection (p<0.05), distilled water controls (A, n=5) had no significant effect. The pelvic pain returned 24 h after gabapentin injection.
## TABLES

**Table 1.** Spontaneous Behaviors in EAP (*p*<0.05).

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Table 2. Body mass during EAP (*p<0.05).

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Table 3. Paw sensitivity determined by 50% Threshold (g, *p<0.05).

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<td>2.15±0.29</td>
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<td>2.29±0.39</td>
</tr>
</tbody>
</table>
Figure 1 Rudick et al.,
Figure 2 Rudick et al.,
Figure 3, Rudick et al., 100 μm

Prostate Lobe

DLP

VP

CG

PGP 9.5 staining (arbitrary units)

Control

PAG

*
Figure 4 Rudick et al.,

A  Prostate

B  Bladder

C  Colon

Response Frequency (%)

Von Frey (g)
Figure 5 Rudick et al.,

A. Control

B. Gabapentin