Experimental autoimmune prostatitis induces chronic pelvic pain

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Rudick CN, Schaeffer AJ, Thumbikat P. Experimental autoimmune prostatitis induces chronic pelvic pain. Am J Physiol Regul Integr Comp Physiol 294: R1268–R1275, 2008. First published February 20, 2008; doi:10.1152/ajpregu.00836.2007.—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 model to examine the development, localization, and modulation of pelvic pain. Pelvic pain was attenuated 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. Experimental autoimmune prostatitis histopathology was confined to the prostate with focal periglandular inflammatory infiltrates in the ventral, dorsolateral, and anterior lobes of the mouse prostate. Inflammation and pelvic pain were positively correlated and increased with time. Morphologically, the dorsolateral prostate alone showed significantly increased neuronal fiber distribution, as evidenced by increased protein gene product 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.

chronic pelvic pain syndrome; prostatitis; neuropathic pain; gabapentin

Experimental autoimmune prostatitis (EAP) (27). The EAP model utilizes rat prostatic antigen injection with adjuvant to induce autoimmune prostatitis in male nonobese 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 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 L5 and L6 spinal segments, with some small degree of innervation from T11–L2 (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 (CNS) (25). The expression of pain from the viscer 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 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 L4 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 cross talk between pelvic organs that share innervation via the sacral spinal cord has been previously described (reviewed in Refs. 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 Refs. 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 cross talk between pelvic organs can modulate pelvic organ physiological function. In light of these studies and a more recent study demonstrating pelvic pain modulation by organ cross talk between the colon and bladder (28), we examined whether colonic administration of a local anesthetic modulated pelvic pain in the EAP model.

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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/PPS 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 nervous system and CNS.

METHODS

Animals. Adult male NOD/ShiLtJ (5–7 wk 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-h 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, Norwalk, CT). The homogenate was centrifuged at 10,000 g for 30 min, and the supernatant was 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) with a 26-gauge Hamilton syringe, while the animals were maintained 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 before rat PAg injection (baseline) and 5, 10, 15, 20, 25, and 30 days after PAg. Referred hyperalgesia and tactile allodynia were 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 (6 × 10 × 12 cm) with a stainless steel wire grid floor (mouse acclimation period of 20 min before 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 g (Stoelting). Each filament was applied for 1–2 s with an interstimulus interval of 5 s for a total of 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 were reported as the mean percentage of response frequency ± SE.

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 g. The median 50% withdrawal threshold (5) was assessed using the up-down method, where testing was started with 0.04-g filament applied perpendicular 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 5 min in a clear plastic open-field chamber (18 × 29 × 12 cm) and scored for rearing, grooming, and cage crossing to assess general activity (26).

Histology. Paraffin-embedded 5-μm sections were prepared from prostate samples fixed in 10% neutral buffered formalin. Sections were stained with hematoxylin and eosin (H&E) at the Northwestern Pathology Core facility and examined using an upright microscope.

Inflammation scoring. The ventral prostate (VP), dorsal and lateral prostate (DLP), and anterior or coagulating gland (CG) lobes of the mouse prostate were collected from control (TiterMax) and 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.

Protein gene product 9.5 quantification. The VP, DLP, and anterior or CG lobes of the mouse prostate were collected from control (TiterMax) and 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 ethanol. Nonenzymatic 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 h at room temperature, followed by overnight incubation at 4°C with rabbit anti-protein gene product (PGP) 9.5 antibody (ab17039; Abcam). PGP 9.5 expression was detected using goat anti-rabbit Alexa-fluor 488 (Molecular Probes), mounted with diaminopropylidodecyl mounting medium, and visualized using a fluorescence microscope. PGP 9.5 staining (green) was quantified using Velocity software (Improvement) to detect and count green pixel densities >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 was 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 45 min 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 (56 mg/kg) and was administered as a solution in distilled water injected intraperitoneally (IP) (33). Sham controls were injected with distilled water (IP). All mice were tested 35 days after PAg or Titermax injection for referred hyperal-
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 postinjection day 10 (Fig. 1B; \( P < 0.01 \)). The increase in pelvic sensitivity was sustained until day 30 (\( P < 0.001 \)). On day 5, 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 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Crossing</th>
<th>Rears</th>
<th>Grooming</th>
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<tr>
<td>Sham</td>
<td>110.2±3.1</td>
<td>72.1±3.7</td>
<td>1.8±0.4</td>
</tr>
<tr>
<td>PAg</td>
<td>129.2±9.4</td>
<td>83.2±4.2</td>
<td>1.4±0.2</td>
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Values are means ± SE of number of incidents. PAg, prostate antigen.

Table 2. Body mass during experimental autoimmune prostatitis

<table>
<thead>
<tr>
<th>Time, days</th>
<th>Sham</th>
<th>PAg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25.8±0.4</td>
<td>25.2±0.3</td>
</tr>
<tr>
<td>5</td>
<td>26.6±0.4</td>
<td>23.6±0.8*</td>
</tr>
<tr>
<td>10</td>
<td>27.2±0.6</td>
<td>26.5±0.4</td>
</tr>
<tr>
<td>15</td>
<td>28.2±0.4</td>
<td>28.0±0.3</td>
</tr>
<tr>
<td>20</td>
<td>28.6±0.5</td>
<td>28.7±0.4</td>
</tr>
<tr>
<td>25</td>
<td>28.9±0.6</td>
<td>29.3±0.4</td>
</tr>
<tr>
<td>30</td>
<td>29.2±1.0</td>
<td>29.4±0.3</td>
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Values are means ± SE in grams. *\( P < 0.05 \).
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 VP, DLP, and CG were observed to increase significantly with time in the PAg-immunized animals (\(P = 0.008\)) (Fig. 2, A and C). Adjuvant-treated control mice exhibited low levels of inflammatory infiltrates that did not show any significant time-dependent changes (Fig. 2, A and 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.

Fig. 2. Experimental autoimmune prostatitis (EAP) in NOD mice induces chronic inflammation of the prostate. A: NOD mice were injected with PAg (PAg) or adjuvant (control (C)), and the ventral prostate (VP) lobe, dorsolateral prostate (DLP) lobe, and coagulating glands (CG) of the prostate were removed at 5, 10, 20, and 30 days after immunization. Hematoxylin- and eosin-stained sections were scored blindly, and the extent of chronic inflammation was graded from 0 to 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 of the lobes of the prostate. The images shown are representative, and the scale bar represents 50 μm. 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 means ± SE of 5 mice per group at each time point.
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. 3, A and C). We next used Volocity software (Improvision) to quantify staining in prostate sections of EAP mice and control mice receiving TiterMax alone (compare Fig. 3, A and B). Staining density was unchanged in the VP and CG of EAP mice compared with controls (Fig. 3D). However, PGP 9.5 staining was significantly increased in the DLP 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 $\sim 46\%$ (Fig. 4A), whereas animals injected with lidocaine into the bladder or colon exhibited no loss of pelvic sensitivity (compare Fig. 4, B 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. IP instillation of gabapentin 1 h before testing in PAg-immunized animals significantly reduced the response frequency to mechanical stimulation with von Frey filaments by $\sim 30\%$ ($P < 0.05$). In contrast, animals injected with vehicle exhibited no loss of pelvic sensitivity (compare Fig. 5, A and B). The pelvic pain in the gabapentin-treated mice returned 24 h 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 multilevel 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 exam-
ning 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, 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 detected in the EAP model (Fig. 4). Prostate lidocaine attenuated 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 ± SE. 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).

Table 3. Paw sensitivity determined by 50% threshold

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>PAg 35 Days</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>Lidocaine prostate</td>
<td>1.92±0.33</td>
<td>1.53±0.34</td>
<td>1.50±0.39</td>
</tr>
<tr>
<td>Lidocaine bladder</td>
<td>1.79±0.19</td>
<td>1.57±0.47</td>
<td>1.94±0.36</td>
</tr>
<tr>
<td>Lidocaine colon</td>
<td>2.01±0.51</td>
<td>1.88±0.39</td>
<td>1.72±0.48</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>1.78±0.38</td>
<td>1.64±0.41</td>
<td>1.57±0.48</td>
</tr>
<tr>
<td>Distilled water</td>
<td>2.15±0.29</td>
<td>2.27±0.41</td>
<td>2.29±0.39</td>
</tr>
</tbody>
</table>

Values are means ± SE in grams.

Fig. 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 ± SE. 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).

Fig. 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 ± SE. 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.
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 PAg (27). We observed that inflammatory infiltrates accumulate in all of 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 underrepresented 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 diseases, like arthritis (8) and asthma (1). In the EAP model, we observed a significant increase in nerve fiber staining specifically in the DLP. Thus pain in EAP could partly be the result of the increased interaction of nerve fibers with inflammatory mediators and neuropeptides released by proinflammatory 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 DLP 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 for by our limited ability to directly instill the drug into prostatic lobes with the largest neurogenic response, particularly the DLP. 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 cross talk 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 comorbidity 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 L4 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 CNS and peripheral nervous system, and therapies aimed at abolishing pelvic pain may need to be multimodal to achieve lasting therapeutic benefits.

**Perspectives and significance.** CPPS accounts for ~90% of all CP and is the most common urological diagnosis in men <50 yr 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 CNS and peripheral nervous system 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.

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