Uropathogenic *Escherichia coli* Induces Chronic Pelvic Pain[▽]

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Received 20 August 2010/Returned for modification 8 October 2010/Accepted 6 November 2010

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a debilitating syndrome of unknown etiology often postulated, but not proven, to be associated with microbial infection of the prostate gland. We hypothesized that infection of the prostate by clinically relevant uropathogenic *Escherichia coli* (UPEC) can initiate and establish chronic pain. We utilized an *E. coli* strain newly isolated from a patient with CP/CPPS (strain CP1) and examined its molecular pathogenesis in cell culture and in a murine model of bacterial prostatitis. We found that CP1 is an atypical isolate distinct from most UPEC in its phylogeny and virulence factor profile. CP1 adhered to, invaded, and proliferated within prostate epithelia and colonized the prostate and bladder of NOD and C57BL/6J mice. Using behavioral measures of pelvic pain, we showed that CP1 induced and sustained chronic pelvic pain in NOD mice, an attribute not exhibited by a clinical cystitis strain. Furthermore, pain was observed to persist even after bacterial clearance from genitourinary tissues. CP1 induced pelvic pain behavior exclusively in NOD mice and not in C57BL/6J mice, despite comparable levels of colonization and inflammation. Microbial infections can thus serve as initiating agents for chronic pelvic pain through mechanisms that are dependent on both the virulence of the bacterial strain and the genetic background of the host.

Prostatitis is a common urologic disease that results in over 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 (5). The disease is classified into four categories, including acute bacterial prostatitis, chronic bacterial prostatitis, chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), and asymptomatic inflammatory prostatitis.

The third disease category, CP/CPPS, accounts for approximately 90% of all chronic prostatitis cases and is clinically manifested as chronic pain in the perineum, rectum, prostate, penis, testicles, and abdomen (5). Despite the predominantly nonbacterial nature of CP/CPPS, up to 8% of patients with CP/CPPS harbor uropathogens that have traditionally been deemed to be of no significance (25). Numerous studies have also identified bacterial DNA in prostate samples from CP/CPPS patients (9, 19, 20, 22, 25). CP/CPPS accompanied by uropathogens is differentiated from chronic bacterial prostatitis by the requirement for clinical symptoms of pelvic pain and the lack of recurrent urinary tract infections (UTIs).

It has been suggested that the virulence of major uropathogens such as UPEC is dependent on the expression of multiple virulence factors (10, 15). Phylogenetic analysis suggests that prostatitis-causing uropathogenic *Escherichia coli* (UPEC) strains largely belong to the B2 phylogenetic group and exhibit a wide variety of virulence traits, including non-hemagglutinin adhesin-siderophore receptor (*ihA*), type 1 fim-

briae (*fimH*), the salmochelin siderophore receptor (*iroN*), and outer membrane protease T (*ompT*) (1, 12, 16, 36).

Several animal models that induce histological inflammation of the prostate by spontaneous, immune-mediated, and/or infectious methodologies have been developed to recapitulate aspects of CP/CPPS (35). Infection-based models of prostatitis have relied on bacterial strains isolated from cystitis or acute prostatitis cases (7), of which UPEC is one of the most commonly isolated pathogens (2). While these previous models reflect key aspects of human acute prostatitis, they may not accurately represent potential pathogens in CP/CPPS and do not address the development of chronic pelvic pain, a distinguishing symptom in CP/CPPS (32).

We hypothesized that infection of the prostate by a UPEC isolate derived from a patient with CP/CPPS would initiate pathogenic processes that establish and sustain chronic pain. *In vitro* cell culture models and an *in vivo* murine prostatitis model were used to study the molecular pathogenesis of the CP/CPPS isolate in comparison to that of a prototypic clinical cystitis isolate.

MATERIALS AND METHODS

Bacterial strains and cell lines. CP1 is an *E. coli* strain that was isolated at Northwestern University urology clinics using the Meares-Stamey four-glass collection technique (24) from the expressed prostatic secretion (EPS) and post-massage voided urine (VB3 fraction) of a patient with CP/CPPS. The patient presented with chronic pelvic pain with no concurrent UTI. The strain, designated CP1, was one of six separate *E. coli* isolates from the same patient collected over a 2-year time span that exhibited identical biotypes and antimicrobial susceptibility patterns. NU14 is an *E. coli* strain isolated from the urine of a woman with acute cystitis (14). Bacteria for *in vitro* assays and *in vivo* infections were prepared as previously described (34).

PD07i cells were previously derived from pediatric human bladder (18), while PEC-1 epithelial cells were derived from an adult healthy prostate and immortalized by introduction of human papillomavirus type 16 E6E7. RWPE-1 cells,

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[▽] Published ahead of print on 15 November 2010.

representing benign prostate epithelial cells, were obtained from the American Type Culture Collection (ATCC).

Phylogenetic analysis and virulence factor determination. The major *E. coli* phylogenetic group (A, B1, B2, or D) was determined by a three-locus PCR-based method (4). *E. coli*-associated virulence factor (VF) genes and variants were detected by using previously described multiplex PCR-based assays (13).

Animal infection. NOD/ShiLtJ and C57BL/6 (5 to 7 weeks old) mice were purchased from Jackson Laboratory (Bar Harbor, ME). All experiments were approved by the Northwestern University Animal Care and Use Committee. To infect animals, 10 μ l of phosphate-buffered saline containing 1×10^8 bacteria was introduced into the urethra of anesthetized mice by catheterization. To quantify bacterial colonization, mice were euthanatized, and the bladder or prostate was aseptically harvested, homogenized, and plated on eosin methylene blue agar.

Behavioral testing. Mice were tested prior to infection (baseline) and at postinfection days (PIDs) 1 to 7, 14, 21, and 28. Referred hyperalgesia and tactile allodynia were tested using von Frey filaments with forces of 0.04, 0.16, 0.4, 1, and 4 g (Stoelting) (21). Stimulation was confined to the pelvic area in the general vicinity of the prostate or the paw as previously described (31).

Histochemistry and inflammation scoring. Paraffin-embedded 5- μ m sections of the mouse prostate collected 3 days following CP1 infection were processed for histochemistry, and sections stained with hematoxylin-eosin (H&E) were examined and scored blindly using the histopathological classification system for chronic prostatic inflammation (26).

Bacterial infections. (i) Quantifying adherence and viable invasive bacteria. Cells seeded into 24-well dishes were infected with bacteria (multiplicity of infection [MOI], 10) as previously described (34). To measure bacterial adherence, cells were lysed with 0.5% trypsin (Gibco)–0.1% Triton X-100 (Calbiochem) and plated onto LB agar. Bacterial invasion was measured using the gentamicin protection assay (GPA) (34).

(ii) Intracellular proliferation. After infection with UPEC, cells were incubated with 100 μ g/ml gentamicin for 22 h. Cells were then lysed, and bacteria were plated onto agar. Proliferation was quantified as the change in the count (fold) at 24 h relative to the count at 2 h (CFU 24 h/CFU 2 h).

Microscopy. Epithelial cells seeded onto coverslips were incubated with NU14 or CP1 (MOI, 10) for 1.5 h at 37°C in 5% CO₂. To stain extracellular bacteria, live cells were incubated with a biotinylated rabbit anti-*E. coli* antibody (Abcam), followed by incubation with streptavidin-Alexa Fluor 594 (red) (Invitrogen) as previously described (3). Samples were subsequently fixed, permeabilized, and probed with the biotinylated rabbit anti-*E. coli* antibody, followed by anti-rabbit-Alexa Fluor 488 (green) (Invitrogen). Because these antibody incubations occurred after fixation and cell permeabilization, both intracellular and extracellular bacteria were stained green. Nuclei were stained by incubation with 4',6-diamidino-2-phenylindole (DAPI).

Statistical analyses. Results were expressed as means $\pm$ standard error of the means (SEMs) and analyzed for statistical significance by a single-factor analysis if variance (ANOVA) or two-way ANOVA with matching. Posttest analysis of multiple groups was performed using the Tukey-Kramer test, and a *P* value of <0.05 was considered statistically significant.

RESULTS

Bacterial strain isolated from CP/CPPS is an atypical UPEC strain. We sought to isolate, identify, and characterize bacteria that could contribute to the pathogenesis of CP/CPPS in men. Utilizing isolation techniques designed to localize pathogens from sites in the male urogenital tract (24), an *E. coli* strain was isolated from the prostate of a patient with CP/CPPS and designated CP1. We assessed CP1's phylogenetic background, virulence genotype, and expression of type 1 fimbriae (Table 1) in comparison to those characteristics of the prototypical cystitis strain NU14 (14).

CP1 belongs to phylogenetic group B1, setting it apart from the majority of UPEC isolates, including NU14, which belong to group B2. CP1 exhibited the pathogenicity island marker *malX*, adhesin genes for type 1 fimbriae (*fimH*), and a nonhemagglutinin adhesin-siderophore receptor (*iha*), siderophore receptors for yersiniabactin (*fyuA*) and ferric aerobactin (*iutA*), and uropathogenic-specific protein (*usp*).

TABLE 1. Characteristics of *E. coli* study strains

Characteristic	Strain NU14	Strain CP1
Clinical source	Cystitis	Prostatitis
Phylogenetic group	B2	B1
Virulence genes ^a		
<i>papACEFG</i>	+	–
<i>sfa/focDE</i>	+	–
<i>iha</i>	–	+
<i>fimH</i>	+	+
<i>hlyD</i>	+	–
<i>cnf1</i>	+	–
<i>fyuA</i>	+	+
<i>iroN</i>	+	–
<i>iutA</i>	–	+
<i>Iip</i>	+	–
<i>Iib</i>	+	–
<i>ibeA</i>	+	–
<i>traT</i>	+	–
<i>ompT</i>	+	–
<i>usp</i>	+	+
<i>malX</i> (PAI)	+	+

^a Virulence genes: *pap*, P fimbriae structural and adhesive subunits; *sfa/foc*, S and F1C fimbriae; *iha*, adhesin-siderophore; *fimH*, type 1 fimbriae; *hly*, hemolysin; *cnf*, cytotoxic necrotizing factor; *fyu*, yersiniabactin receptor; *iroN*, siderophore receptor; *iut*, aerobactin; *Iip* and *Iib*, *kpsM* group 2 capsule; *ibe*, invasion of brain endothelium; *traT*, serum resistance associated; *ompT*, outer membrane protease T; *malX*, pathogenicity island (PAI) marker; *usp*, uropathogenic-specific protein. Both strains were negative for *focG* (F1C fimbriae), *afa/dra* (Dr adhesions), *cdt* (cytolethal distending toxin), K1 (*kpsM* group 2 capsule), III (*kpsM* group 3 capsule), *rfe* (O4 lipopolysaccharide), and *cva* (colicin V).

NU14, in contrast, possessed genes for a larger number of virulence factors (Table 1), all of which were absent from CP1.

When CP1 was tested for expression of type 1 pili using the mannose-sensitive hemmagglutination assay (MSHA), it exhibited low titers (1:4) compared with those exhibited by NU14 (1:64), indicating that its type 1 pilus expression is low, despite the presence of *fimH* (data not shown). These results suggest that although CP1 possesses certain characteristics of classical UPEC, it lacks many others, distinguishing it as an atypical UPEC isolate.

CP1 invades and proliferates in prostate epithelial cells. Given that CP1 exhibited an apparent ability to persist in the prostate for extended periods of time, we hypothesized that bacteria may be able to invade and proliferate within prostate epithelial cells. We therefore examined bacterial adherence, invasion at 2 h after inoculation, and proliferation of bacteria at 24 h in epithelial cells derived from the prostate (PEC-1 and RWPE-1) or the bladder (PD07i).

CP1 was capable of adhering to both prostate and bladder cell lines to a level that was not significantly different from that for NU14 (Fig. 1A). However, upon attachment, CP1 appeared to be highly invasive in these cells (Fig. 1B and C) and invasive to a significantly greater extent than that observed for NU14 ($P < 0.003$), whether it was expressed as a percentage of adherent bacteria (Fig. 1B) or total number of invasive bacteria in the epithelial cells (Fig. 1C). Proliferation of bacteria at 24 h, expressed as a ratio over 2 h of invasion (Fig. 1D), was similar between CP1 and NU14. We performed immunofluorescence studies 2 h after infection to confirm the localization of the CP1 strain in the PEC-1 and RWPE-1 prostate epithelial cell lines. CP1 was observed to localize to both the extracellu-

FIG. 1. Genitourinary epithelial cell lines internalize CP1. PD071, RWPE, or PEC cells were incubated with NU14 (black bars) or CP1 (gray bars) for 2 h (MOI, 10). (A) Adherence of *E. coli* to urothelial cell lines was measured from colony counts of cell lysates plated on LB agar and is expressed as the percentage of total bacteria. NU14 and CP1 did not differ in adherence to urothelial cells. (B and C) Bacterial internalization by urothelial cells after 2 h of incubation was quantified by GPA and expressed as the percentage of adherent bacteria (B) or the number of invasive CFU (C). CP1 was internalized significantly more than NU14 (*, $P < 0.003$). (D) Bacteria within urothelial cells following 24 h of incubation were quantified by GPA; results are expressed as the ratio of the number of bacterial CFU at 24 h to the number of bacterial CFU at 2 h. Graphs represent the averages of three experiments $\pm$ SEMs. (E and F) Location and morphology of intracellular CP1. Fluorescence images of PEC-1 (E) and RWPE-1 (F) cells inoculated with CP-1 for 2 h. Before and after fixation and permeabilization, cells were incubated with anti-*E. coli* antibody. Intracellular bacteria stain green (arrows and inset in panel E), while extracellular bacteria are red/green (yellow). The nuclei of cells are stained by DAPI (blue). Scale bars, 5 μ m.

lar (yellow) and intracellular (green) regions of both PEC-1 (Fig. 1E) and RWPE-1 (Fig. 1F) cells. Intracellular bacteria appeared as single/multiple rods (Fig. 1E, inset) or as large clusters (Fig. 1F).

These results indicate that CP1 possesses the requisite mechanisms to efficiently attach to, invade, and proliferate within epithelial cells *in vitro*. These processes have not been described previously for prostate epithelial cells and are suggestive of *in vivo* persistence mechanisms of CP1 within the prostate gland.

CP1 colonizes the male urogenital tract in mice. We next examined whether CP1 was capable of establishing an infection *in vivo* in male C57BL/6J and NOD mice following intraurethral instillation, which in this model leads to a retrograde

infection of the bladder and the prostate (7). NOD mice were utilized in addition to the C57BL/6J mice on the basis of their predilection to develop induced autoimmune prostatitis and chronic pain (31). The prostate and bladder of mice were examined 24 h after instillation of CP1 to confirm colonization and then at various later time points to examine bacterial persistence in these tissues. Both NOD and C57BL/6J mice exhibited similar levels of colonization in the bladder (Fig. 2A) and prostate (Fig. 2B), with no significant differences between mouse strains.

To study bacterial persistence, we examined CP1 colonization density in the prostate and bladders of NOD mice at PIDs 5, 14, 28, and 35. CP1 colonized the prostate of 100% of mice at PIDs 5, 14 and 28 (Fig. 2D), but at PID 35, all mice were free of bacteria. Bacterial counts were $31,825 \pm 20,109$ CFU at PID 5 and $74,560 \pm 58,047$ CFU at PID 14, but colonization levels declined to 288 ± 137 CFU at 28 days. The bladders of these mice exhibited a more variable colonization, with 40%, 80%, and 40% of mice showing detectable bacteria at 5, 14, and 28 days, respectively (Fig. 2C). The bladders, too, were sterile by 35 days after infection. The consistent colonization of the prostate by CP1 in comparison to its variable bladder colonization suggests tropism to the prostatic microenvironment.

We also examined the consequence of infection on inflammation in the prostate. Both C57BL/6J and NOD mice exhibited mild-to-moderate, focal inflammation with infiltration of the prostatic stroma of the ventral prostate (VP), dorsolateral prostate (DLP), and coagulating gland (CG) by inflammatory cells (Fig. 2F). Quantification of the inflammation showed no significant differences in scores between the two strains (Fig. 2E).

We next sought to understand whether the ability to persist was unique to CP1 or was shared by NU14. NOD and C57BL/6J mice were infected intraurethally with NU14 or CP1, and colonization was assessed after 14 days in the bladder and the prostate (Fig. 3). In both C57BL/6J mice (Fig. 3A and B) and NOD mice (Fig. 3C and D), the levels of bladder and prostate colonization were significantly lower for NU14 than CP1. These results suggest that CP1 is adapted for persistence in the urogenital tract of male mice (compared with NU14).

CP1 elicits chronic pelvic pain in male NOD mice. Given that CP1 was isolated from a patient with chronic pelvic pain, we examined males versus females of two mouse strains for the presence of chronic pain upon CP1 infection. Male NOD or C57BL/6J mice (5 to 7 weeks old) were instilled with CP1 or saline into the urethra. Mechanical stimulation of the pelvic area with von Frey filaments of saline-instilled C57BL/6J or NOD mice resulted in a response frequency that did not change during the experiment (Fig. 4A, C, and E). Similar to saline-instilled mice, the response profile of CP1-infected C57BL/6J male mice also showed no significant change during the 28-day course of the experiment (Fig. 4B). In contrast, CP1-infected NOD mice exhibited responses to pelvic stimulation that were significantly greater by day 1, peaked at day 3, and remained significantly elevated until day 28 ($P < 0.05$; Fig. 4D and E). Notably, there were no detectable CP1-induced changes in tactile sensitivity of the plantar region of the hind paw or body weight (data not shown), indicating that CP1 induces no changes in gross physiology and that pain is specific to the pelvic region.

FIG. 2. CP1 colonizes and induces equivalent levels of inflammation in NOD and C57BL/6J mice. Mice were instilled via catheter with 1×10^8 CP1, and at 24 h postinfection, bladder (A) and prostate (B) colonization was determined ($n = 5$). CP1 exhibited similar levels of colonization in NOD and C57BL/6J mice. CP1 bladder colonization (C) and prostate colonization (D) of NOD mice was measured over a 2-week time course ($n = 5$ to 6/group). The dotted line represents the limit of detection. (E) Histological sections of prostate tissue from C57BL/6J (white bars) and NOD (black bars) mice infected with CP1 for 3 days were hematoxylin and eosin stained and scored blindly for inflammatory indicators in the VP, DLP, and CG (0, no inflammation; 1, mild inflammation; 2, moderate inflammation; 3, marked inflammation). CP1 induced equal amounts of inflammation in both mouse strains. (F) Inflammation was focal, with inflammatory cells (arrows) being detected in the VP, DLP, and CG of both NOD and C57BL/6J mice. The images are representative. Scale bars, 10 μ m.

Additionally, we examined whether pelvic pain was associated with bacterial colonization by examining pain behavior at day 35, a time point at which no detectable bacteria remained in the prostate or bladder. The pelvic pain response remained significantly elevated ($n = 13$, $P < 0.05$; percent increase in pain, 639.8 ± 124.4) and comparable to that on day 28 ($P > 0.16$; see Fig. 4E and Fig. 6C).

We next sought to confirm that the pain in NOD mice

originated from the prostate by utilizing sex difference in the presence/absence of the prostate gland. We instilled female mice with CP1 or saline and examined the pain response over time. Female NOD mice challenged with CP1 exhibited no significant increase in response compared to that of saline-instilled mice ($P > 0.05$) (Fig. 4E). C57BL/6J female mice infected with CP1 also showed no significant increase in pelvic sensitivity (data not shown). These results suggest that pelvic

FIG. 3. Strains NU14 and CP1 differentially colonize the mouse bladder and prostate. C57BL/6J mice ($n = 5$ /group) and NOD mice ($n = 5$ /group) were infected with 1×10^8 NU14 or CP1 by catheterization, and at PID 14, colonization of C57BL/6J bladder (A), C57BL/6J prostate (B), NOD bladder (C), and NOD prostate (D) was quantified. Dotted lines represent the limit of detection. CP1 colonized the bladder and prostate at higher levels than NU14 in both C57BL/6J and NOD mice (**, $P < 0.001$; *, $P < 0.05$).

FIG. 4. CP1 induces chronic pelvic pain in male NOD but not C57BL/6J mice. Referred visceral hyperalgesia was measured as the response to mechanical stimulation of the pelvic region using von Frey filaments of five calibrated forces. Data are reported as the mean percentages of positive responses $\pm$ SEMs before instillation of bacteria (baseline) and at PID 1 to 7, 14, 21, and 28. (A) Saline-instilled male C57BL/6J mice ($n = 10$); (B) CP1-infected male C57BL/6J mice ($n = 10$); (C) saline-instilled male NOD mice ($n = 8$); (D) CP1-infected male NOD mice ($n = 25$). ANOVA indicated a significant increase in response frequency from that at the baseline for all filaments tested in CP1-infected NOD mice at PIDs 1 to 28 ($P < 0.05$), with no significant change in saline-instilled C57BL/6J or NOD mice or CP1-infected C57BL/6J mice. (E) The percent response at each PID was calculated as total responses to all fibers relative to baseline responses. CP1-infected male NOD mice exhibit chronic pelvic hyperalgesia that lasts for 28 days, whereas CP1-infected male C57BL/6J mice exhibit no significant response. Saline-instilled and CP1-infected female NOD mice ($n = 10$ for each group) exhibited no significant differences in comparison with one other or the baseline. The symbol key shown in panels A applies to panels B, C, and D.

pain is specific to NOD male mice and may be the result of pathogenic mechanisms specifically involving the prostate.

Male NOD mice develop spontaneous chronic pelvic pain. Previous studies have demonstrated that NOD mice spon-

taneously develop histological autoimmune prostatitis that gradually increases with advancing age (27). We hypothesized that NOD mice develop chronic pain as a consequence of the spontaneous prostatitis and tested tactile sensitivity from 14 to 28 weeks of age.

Mechanical stimulation of the pelvic area of mice resulted in an increased response frequency which became statistically significant at 20, 22, 24, 26, and 28 weeks of age, suggesting that NOD male mice develop spontaneous pain (Fig. 5A and B; $P < 0.05$). Although there was a significant decrease in tactile sensitivity of the plantar region of the hind paw at 18 weeks of age ($P < 0.05$), there was no significant change at any other time point ($P > 0.05$). There also were no detectable weight changes (data not shown) to suggest changes in gross physiology. These results indicate that male NOD mice spontaneously develop chronic pelvic pain at 20 to 28 weeks of age, in contrast to the CP1-induced pain that develops at 5 to 7 weeks of age.

Chronic pain in male NOD mice can be induced by CP1 but not NU14. We hypothesized that the chronic pain that occurs in male NOD mouse was specific to CP1, when the findings for CP1 were compared with those for NU14 (previously shown to elicit acute pain in female C57BL/6J mice [30]). As previously described, CP1-infected NOD mice exhibited chronic pelvic pain (Fig. 6B and C; $P < 0.05$), whereas CP1-infected C57BL/6J mice developed no pain (Fig. 6A and C). NU14-infected NOD mice exhibited pain on PID 3 only (Fig. 6E and F; $P < 0.05$), while NU14-infected C57BL/6J mice exhibited an acute pain response that peaked on PID 3 ($P < 0.05$) and gradually declined over the 28-day time course (Fig. 6D and F). There were no CP1- or NU14-induced changes in tactile sensitivity of the plantar region of the hind paw or detectable weight changes (data not shown), indicating that the chronic pain response develops only in NOD mice and only in response to atypical UPEC strain CP1.

DISCUSSION

Although CP/CPPS is associated with bacteria only in about 8% of cases (25), infection has often been postulated to be an initiating factor (29). We present the first experimental evidence that a bacterial isolate from a patient with CP/CPPS can initiate and sustain the development of chronic pelvic pain, a distinguishing characteristic of CP/CPPS. In doing so, our *in vitro* and *in vivo* animal models suggest that bacterial characteristics as well as the immunogenetic background of the host are important determinants in the development of chronic pelvic pain.

Our molecular and phenotypic characterization of CP1 demonstrated that it lacks a number of the virulence-associated traits that typify acute prostatitis isolates and UPEC strains generally. The *E. coli* species is divided into four main phylogenetic groups, commonly designated A, B1, B2, and D (8). Most *E. coli* strains responsible for UTIs or other extraintestinal infections belong to group B2 or, less frequently, group D (28). Our studies place CP1 within group B1, whose members normally lack extraintestinal virulence factors (11) but when selected for in clinical disease states can exhibit high levels of virulence (12). In addition to its phylogenetic background, CP1's virulence gene repertoire also is atypical in comparison

FIG. 5. Male NOD mice spontaneously develop pain. Referred visceral hyperalgesia was measured as responses to mechanical stimulation of the pelvic region using von Frey filaments of five calibrated forces. Data are reported as the mean percentages of positive responses $\pm$ SEMs at 14, 16, 18, 20, 22, 24, 26, and 28 weeks of age. (A) Responses to pelvic stimulation from 14 to 28 weeks of age ($n = 10$). ANOVA indicated a significant increase in response frequency at all filaments tested at 20, 22, 24, 26, and 28 weeks of age compared to that at 14 weeks of age ($P < 0.05$). (B) Percent response at each time point was calculated as total responses to all fibers relative to the responses at 14 weeks of age. (C) The tactile sensitivity (50% threshold) was significantly decreased at week 18 only ($P < 0.05$).

to that of acute prostatitis isolates, which characteristically are enriched for *hly*, *cnf1*, *sfa*, and *iroN* (36), all of which are absent in CP1 (Table 1). The atypical nature of CP1 may reflect specific adaptations for chronic prostate colonization that differentiate it from the strains obtained from acute prostatitis. Characteristics that still place CP1 in the larger UPEC family include its possession of virulence genes such as *usp*, *iut*, and *iha*, which are common among UPEC strains. Future studies will determine whether the characteristics exhibited by CP1 are representative of CP/CPPS strains generally.

It has been hypothesized that chronic bacterial prostatitis is characterized by the presence of biofilms (6). *In vitro* studies on prostatitis bacteria have demonstrated a greater tendency for the development of biofilm-like structures that are assumed to adhere to the epithelium of the ductal system (33). Our studies with CP1 are the first to suggest that in addition to classical mechanisms of biofilm formation, prostatitis strains possess the ability to invade and persist within the cytoplasm of prostate epithelial cells (Fig. 1). The potential to invade and proliferate inside cells would dramatically enhance the ability of these bacteria to resist host immune mechanisms and would provide a unique ecological niche.

Epithelial invasion and proliferation have been previously reported for cystitis UPEC strains such as NU14 (3, 23). However, the CP1 strain appears to be more invasive, possibly accounting for its enhanced persistence at 14 days *in vivo* in the murine infection model (Fig. 3), a time point at which NU14 has almost been cleared from the bladder and prostate. CP1 colonization of the prostate and the resulting inflammatory response appear to be independent of the genetic background of the mouse strain, since both NOD and C57BL/6J mice were equally susceptible and yielded similar histopathologic findings, including inflammatory changes. The consistency of prostate colonization over time is suggestive of tropism to the prostate through mechanisms that remain to be defined.

Given the similarities in colonization and elicited inflammation in the NOD and C57BL/6J mice, it was surprising to note the sustained development of chronic pain in the NOD mice, while the C57BL/6J mice remained asymptomatic. The NOD mouse, a strain genetically prone to develop different organ-specific autoimmune diseases (17), has previously been shown by our group to develop chronic pelvic pain in response to induction of autoimmunity (31). In this study, we show that male NOD mice spontaneously develop pelvic pain that is significantly elevated by 20 weeks of age and that is sustained to at least 28 weeks. Thus, male NOD mice develop chronic pelvic pain that is initiated by 20 weeks and established with advancing age. These results agree with those from previous studies that demonstrate spontaneous development of autoimmune prostatitis in the NOD male mouse by 20 weeks of age (27). In contrast, 5- to 7-week-old NOD males infected with CP1 develop pelvic pain that peaks in 3 days and that is sustained thereafter. We propose that the CP1 infection of 5- to 7-week-old NOD male mice initiates and accelerates the known process of autoimmunity in NOD mice, a characteristic that is less likely in C57BL/6J mice. Preliminary studies suggest that differential chemokine and immune responses in the NOD and C57BL/6J mice may mediate the symptomatic variations observed (data not shown). Interestingly, the differential responses are not simply a function of the host but also a func-

FIG. 6. Differential pain response induced by CP1 and NU14 infection. Referred visceral hyperalgesia was measured as responses to mechanical stimulation of the pelvic region using von Frey filaments of five calibrated forces. Data are reported as the mean percentages of positive responses $\pm$ SEMs before instillation of bacteria (baseline) and at PIDs 3, 7, 14, 21, and 28. (A) CP1-infected male C57BL/6J mice ($n = 8$); (B) CP1-infected male NOD mice ($n = 10$). The symbol key shown in panel A applies also to panel B. ANOVA indicated a significant increase in response frequency at PIDs 3 to 28 compared with that at the baseline for all filaments tested in CP1-infected NOD mice ($P < 0.05$) but no significant change in CP1-infected C57BL/6J mice. (C) The percent response at each PID was calculated as total responses to all fibers relative to baseline responses. (D) NU14-infected male C57BL/6J mice ($n = 10$); (E) NU14-infected male NOD mice ($n = 5$). The symbol key shown in panel D also applies to panel E. ANOVA indicated a significant increase in response frequency at PIDs 3 to 14 compared with that at the baseline for all filaments tested in CP1-infected C57BL/6J mice ($P < 0.05$), whereas CP1-infected NOD mice exhibited a significant increase at PID 3 only. (F) The percent response at each PID was calculated as total responses to all fibers relative to baseline responses.

tion of the bacterial strain. That is, infection of the NOD male mouse by NU14 is insufficient to activate mechanisms leading to chronic pain, a reminder that host-pathogen interactions are key to mediating the pathogenesis of chronic pelvic pain.

In summary, we provide experimental evidence for an infectious etiology of chronic pelvic pain. Using a clinical bacterial isolate from a patient with CP/CPPS, we recapitulate the symptoms of chronic pain in a murine strain that is predisposed to the development of autoimmunity. Our results implicate characteristics of both the host and the pathogen to be key features in determining the development of symptoms. These results have potentially important implications in understanding the pathogenesis of CP/CPPS.

ACKNOWLEDGMENTS

This research was supported by grant 1K01DK079019A2 (to P.T.) from the NIH/NIDDK.

We thank Eduardo Sarmiento and Fe Sarmiento for technical expertise.

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Editor: S. M. Payne