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A Mouse Model of Chronic Prostatic Inflammation Using a Human Prostate Cancer-Derived Isolate of *Propionibacterium acnes*

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Abstract

BACKGROUND—Prostatic inflammation has been linked to a number of prostatic diseases such as benign prostatic hyperplasia (BPH), prostatitis syndromes, and prostate cancer. Major unanswered questions include what pathogenic mechanisms, such as bacterial infections, may drive the accumulation of inflammatory infiltrates in the human prostate, and how inflammation might contribute to disease. To study this potential link in an in vivo system, we developed a mouse model of long-term bacteria-induced chronic inflammation of the prostate using a human prostatectomy-derived strain of *Propionibacterium acnes*.

METHODS—C57BL/6J mice were inoculated, via urethral catheterization, with vehicle control or a prostatectomy-derived strain of *P. acnes* (PA2). Animals were assessed at 2 days, 1, 2, or 8 weeks post-inoculation via histology and immunohistochemistry (IHC).

RESULTS—PA2 inoculation resulted in severe acute and chronic inflammation confined to the dorsal lobe of the prostate. Chronic inflammation persisted for at least 8 weeks post-inoculation. Inflammatory lesions were associated with an increase in the Ki-67 proliferative index, and diminished Nkx3.1 and androgen receptor (AR) production. Interestingly, the observed response required live bacteria and both IHC and in situ hybridization assays for *P. acnes* indicated a potential intracellular presence of *P. acnes* in prostate epithelial cells.

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CONCLUSIONS—To our knowledge, this is the first mouse model of long-term prostatic inflammation induced by *P. acnes*, and more generally, any prostatectomy-derived bacterial isolate. This model may serve as a valuable preclinical model of chronic prostatic inflammation that can be used to mechanistically study the link between inflammation and prostatic disease.

Keywords

prostate; *Propionibacterium acnes*; inflammation; mouse model

INTRODUCTION

Asymptomatic prostatic inflammation (i.e., “histological prostatitis”) is a nearly universal finding in the prostate of the adult male in the developed world, as evidenced by studies examining men who undergo biopsy for prostate cancer due to elevated prostate-specific antigen (PSA) levels and test negative for cancer [1–4], autopsy studies [5], and findings from transurethral resections for benign prostatic hyperplasia (BPH) [6]. Furthermore, prostatic inflammation has been linked to arguably all major diseases of the human prostate including BPH, prostatitis syndromes, and prostate cancer [7–10]. The major questions that remain to be answered regarding the pathogenic role of inflammation in prostatic disease are (1) what events, such as bacterial infections, drive the accumulation of inflammatory infiltrates in the human prostate, and (2) what are the mechanism(s) by which inflammation might contribute to prostatic disease.

Propionibacterium acnes (*P. acnes*) is an anaerobic, gram-positive bacterium that is found nearly ubiquitously on human skin. Although typically regarded as a human commensal, *P. acnes* is a highly pro-inflammatory bacterium that is implicated as the causative agent in the skin disease acne vulgaris, and in inflammatory conditions such as endocarditis, sarcoidosis, and post-surgical infections [11]. Interestingly, *P. acnes* has been identified in prostate-derived tissues and has been associated with prostate cancer in molecular and epidemiological studies [12–17]. Recently, *P. acnes* was reported as the predominant bacterium detected in tissues from patients with BPH [18] and prostate cancer [19]. Intriguingly, in the latter study, the ability to culture *P. acnes* was strongly correlated with prostatic inflammation in prostatectomy tissues [19]. Furthermore, in vitro studies demonstrated that *P. acnes* was capable of inducing a strong inflammatory response in prostate-derived cell lines [14,20,21]. However, the presence of this organism in the prostate has also been questioned as a potential contaminant, as it is a predominant member of the commensal skin microflora and often reported as a culture contaminant [22]. Providing evidence against this, multilocus sequence typing (MLST) analysis of prostatectomy-derived isolates of *P. acnes* demonstrated that prostate-derived *P. acnes* strains do not fall within the typical skin/acne sequence types (STs), but rather are characteristic of STs associated with opportunistic infections and/or urethral flora [23]. However, no study has firmly established a causal role for *P. acnes* in the acute or chronic inflammation often observed in the prostate.

To date, most published studies of prostatic infection conducted in mice have utilized uropathogenic strains of *E. coli*, are performed in rare strains of mice, and/or have only been

carried out for a few days or 2 weeks [24–27]. As a means to begin to address the potential role of *P. acnes* as a pro-inflammatory stimulus in the prostate, as well as to develop an in vivo system to study the role of long-term bacteria-induced prostatic inflammation in prostatic disease, we developed a model of *P. acnes* infection of the prostate in wild-type C57BL/6J mice. We report that inoculation with a human prostatic isolate of *P. acnes* (PA2) [23] is capable of inducing acute and chronic inflammation in the dorsal lobe of the mouse prostate, with persistence of the chronic inflammation for at least 8 weeks. Chronic inflammation is accompanied by increased epithelial proliferative fraction and decreased Nkx3.1 and androgen receptor (AR) protein levels, findings that have been reported previously in association with prostatic inflammation in another *E. coli* based model of short-term infection in the mouse prostate [26]. Our findings establish a causative relationship between prostatectomy-associated strains of *P. acnes* and prostatic inflammation. This mouse model of infection-induced chronic inflammation may serve as a valuable in vivo model to study inflammation-associated prostatic diseases such as BPH, prostatitis and prostate cancer.

METHODS

Animals

All procedures were performed on 8–10-week-old C57BL/6J wildtype mice under the guidelines of the Johns Hopkins Animal Care and Use Committee (ACUC) and with an approved animal protocol. Mice were housed in a pathogen-free environment, allowed free access to food and water, and were maintained on a 12 hr light/dark cycle. Mice were sacrificed via CO₂ asphyxiation and serum was collected by cardiac puncture. Seminal vesicles, urinary bladder, anterior, dorsal/lateral, and ventral prostate lobes were collected along with other reference organs.

Transurethral Catheterization and Inoculation of *P. acnes*

Mice were anaesthetized with ketamine/xylazine and then catheterized via the urethra using lubricated sterile polyethylene catheters (PE-10 tubing, BD Biosciences) 2.5 cm in length. Inoculation of a prostatectomy-derived strain of *P. acnes* (PA2) [23] into the bladder and prostate was performed with a PA2 dose of approximately 10⁷ colony forming units (CFU) in a 20 µl volume. PA2 belongs to CC36, a clonal complex of *P. acnes* that is associated with opportunistic infections [23]. Of interest, the PA2 strain was isolated from a radical prostatectomy specimen that contained both acute and chronic inflammation (Supplementary Fig. 1). Control animals were inoculated with an equal volume of sterile phosphate buffered saline (PBS, vehicle control) or heat-killed PA2 (heated at 60°C for 1 hr).

Prostate Histopathology and Assessment of Inflammation

Prostate lobes were dissected separately into anterior, dorsal/lateral, and ventral lobes and placed into formalin. All tissues were fixed in 10% buffered formalin for 48 hr before paraffin embedding. Hematoxylin and eosin (H&E) staining was performed using the Leica Microsystems AutoStainer XL (Buffalo Grove, IL). Slides were scanned at 20× using the Aperio ScanScope (CS model, Aperio, Vista, CA) and viewed using the freeware ImageScope Viewer Software (Aperio version 10.2.2.2353). Total dorsal prostate area, total

inflamed area, and grade of inflammation (mild, moderate, and severe), were evaluated in a manner such that the evaluator was blinded to the treatment group of the animal. Total percentage of inflamed area was calculated as the total area of inflamed prostate divided by the total area of dorsal prostate using the ImageScope Viewer Software. For inflammation grading, prostate tissues were considered positive for mild inflammation if scattered neutrophilic or mononuclear inflammatory cells were present and involved multiple glands/foci. Prostate tissues were considered to be moderately inflamed if clusters (but not follicles) of lymphocytes and/or macrophages were present and involved multiple glands/foci. Prostate tissues were considered to be severely inflamed if dense nodules/follicles of inflammatory cells were present.

Immunohistochemistry

Slides containing sections of formalin-fixed, paraffin-embedded (FFPE) mouse prostate tissues were deparaffinized in xylene and rehydrated through a series of graded ethanol. For androgen receptor (AR) and *P. acnes* antibody IHC, slides were steamed for 45 min (AR antibody) or 17 min (*P. acnes* antibody) in high temperature target retrieval solution for antigen retrieval (Dako Cytomation). For Ki-67 IHC, slides were steamed for 25 min in citrate buffer (Vector Laboratories). For Nkx3.1 IHC, slides were steamed in 1 mmol/L EDTA (pH 8.0) for 45 min. For *P. acnes* antibody staining, slides were digested with 1 mg/ml lysozyme (Sigma Aldrich) for 25 min at 37°C followed by digestion with achromopeptidase (30 U/ml, Sigma Aldrich) for 25 min at 37°C and blocking with 5% donkey serum for 10 min at room temperature. Slides were then incubated with primary antibodies for AR (Santa Cruz Biotechnology) at a dilution of 1:1,000 for 1 hr at room temperature, Ki-67 (Novocastra) at a dilution of 1:1,200 for 1 hr at room temperature, Nkx3.1 [28] at a dilution of 1:6,000 overnight at 4°C, or *P. acnes* antibody [14] at a dilution of 1:2,000 overnight at 4°C, followed by incubation with secondary antibody (Powervision, Leica Microsystems) for 45 min at room temperature. Staining was visualized using 3,3'-Diamino-benzidine (Sigma FAST 3,3'-Diamino-benzidine tablets), and slides were counterstained with hematoxylin.

In Situ Hybridization (ISH)

ISH was performed on FFPE mouse dorsal prostate tissues using the RNAscope 2.0 FFPE Brown Reagent Kit (Advanced Cell Diagnostics, Inc.) and a custom RNAscope target probe designed against the *P. acnes* 16S rRNA gene.

RESULTS

P. acnes Inoculation Induces Acute and Chronic Inflammation in the Mouse Prostate

Anaesthetized C57BL/6J mice between 8 and 10 weeks of age were inoculated transurethrally with PBS or 10^7 CFU of prostatectomy-derived *P. acnes* (PA2) [23] in a volume of 20 µl. Mice from each group (n = 2 for PBS controls, n = 5 for PA2 treated animals) were sacrificed 2 days, 1,2, or 8 weeks post-inoculation. Prostatic inflammation was never observed in PBS treated controls at any time point or tissue analyzed (Fig. 1). In contrast, in PA2 inoculated animals, the dorsal prostate lobe selectively developed acute and chronic inflammation in a distinct time-course. While at 2 days post-inoculation there was

an absence of any inflammation, at 1 week there was a dense accumulation of neutrophils primarily in glandular lumens along with dense clusters or follicles of mononuclear inflammatory cells in the stroma (Fig. 1). Although a sparse neutrophilic glandular infiltrate was still present in a subset of animals, by 2 weeks post-infection, the inflammatory infiltrates were primarily chronic inflammation (lymphocytes and macrophages) observed as dense follicles in the stroma. While this severe chronic inflammation began to subside somewhat with time, mild to moderate chronic inflammatory infiltrates persisted even at 8 weeks after *P. acnes* inoculation (Fig. 1). Interestingly, histological examination of genitourinary tissues revealed that the bladder, anterior prostate, and ventral prostate were unaffected by PA2 inoculation at all time-points analyzed. In order to determine whether the selectivity for inflammation in the dorsal lobe may be due to selectivity in the distribution of the inoculum, we performed transurethral inoculations with crystal violet dye to visualize its localization. Immediate sacrifice and examination revealed the presence of dye exclusively in the dorsal prostate lobes and the urinary bladder (Supplementary Fig. 2). Thus, it appears that the localization of inflammation in the dorsal lobe is reflective of the route of inoculation and not necessarily any lobe predilection to infection with *P. acnes*.

While acute inflammation is often reported to be observed within 48 hr of inoculation in multiple rodent models of *E. coli*-induced bacterial prostatitis [24,29,30], we did not observe the onset of acute or chronic inflammation until 1 week post-inoculation. Interestingly, the dorsal prostate exhibited variable degrees of inflammation among PA2 infected animals. By 1 week post-inoculation, three out of five of the animals inoculated with PA2 showed evidence of a severe inflammatory response predominated by dense accumulations of luminal neutrophils and stromal mononuclear inflammatory cells (Fig. 2A). At 2 and 8 weeks following inoculation, while the overall fraction of animals showing any inflammation remained at three out of five animals, there was a greater fraction of animals having mild or moderate inflammation (Fig. 2A). Among animals with inflamed dorsal prostates, on average, 58.6% of the total dorsal prostate area was affected 1 week post-inoculation, 26.2% was affected 2 weeks post-inoculation, and 20.2% was affected 8 weeks post-inoculation (Fig. 2B).

***P. acnes*-Induced Prostatic Inflammation Requires Live Bacteria**

To determine if live bacteria are required to induce inflammation, we inoculated heat-killed PA2 *P. acnes* into C57BL/6J mice (n = 7). No evidence of an inflammatory response was observed in mice that had been inoculated with heat-killed PA2 at 1 week post-inoculation, indicating that live bacteria are required for the onset of the inflammatory response.

***P. acnes* Cells Were Found in the Intra- and Extra-Cellular Space in the Setting of Acute, But Not Chronic Inflammation**

In order to determine if the inflammation observed in the dorsal lobe of PA2 infected animals may relate to a continued presence of bacteria, we assayed for the presence of *P. acnes* in dorsal prostate tissues via IHC with a *P. acnes*-specific antibody [14] as well as *P. acnes*-specific RNA ISH. At both 1 and 2 weeks post-infection, *P. acnes* cells could be detected almost exclusively in areas of the dorsal prostate that contained prostate-infiltrating neutrophils (Fig. 3A–E). Interestingly, *P. acnes* cells were also detected in the intracellular

space of the prostate epithelium of acutely inflamed glands (Fig. 3B–E). However, by 8 weeks post-inoculation, whole *P. acnes* cells were no longer detected in the dorsal prostate lobe (Fig. 3F), despite the persistence of chronic inflammation in most animals. The anti-*P. acnes* antibody and the ISH probe produced highly similar staining results.

Prostate Glands Near *P. acnes*-Induced Inflammatory Lesions Have Increased Proliferative Index and Diminished Expression of Nkx3.1 and AR

To determine whether *P. acnes*-induced dorsal lobe inflammation was associated with an increase in cellular proliferation, we performed IHC with the proliferation-associated marker Ki-67. As shown in Figure 4, we only observed an increase in the number of cells positive for Ki-67 in inflamed prostatic ducts. This increase in Ki-67 positive cells in inflamed regions of the dorsal prostate was observed at 1, 2, and 8 weeks post-inoculation (Fig. 4).

Nkx3.1 is commonly decreased in human proliferative inflammatory atrophy (PIA) lesions [31], and was recently reported to decrease in the setting of *E. coli*-induced bacterial prostatitis [26]. As such, it was of interest to investigate the status of Nkx3.1 in *P. acnes*-induced prostatic inflammation. While Nkx3.1 is typically uniformly expressed in prostate epithelium in all lobes of the prostate, there was a marked decrease in Nkx3.1 production in inflamed glands of the dorsal lobe at both 1 and 2 weeks post-inoculation with PA2 (Fig. 5). Androgen receptor is likewise typically uniformly expressed in both epithelial and stromal cells in all lobes of the mouse prostate and is decreased in PIA lesions in the human prostate [32] as well as in the *E. coli* prostatitis model [26]. We observed a focal down-regulation of AR in epithelial cells that was restricted to inflamed glands of the dorsal lobe of animals both 1 and 2 weeks after infection with PA2 (Fig. 5). The down-regulation observed for both Nkx3.1 and AR was most apparent at 1 and 2 weeks, as it was specifically associated with severely inflamed dorsal prostate (Fig. 2A).

DISCUSSION

While the exact causes of prostatic inflammation remain unclear, epidemiologic and histologic evidence support a role for bacterial infection. Pathogenic organisms that are known to infect and induce inflammation in the human prostate include *E. coli* and sexually transmitted organisms [9]. Recently, *P. acnes* was reported as the predominant bacterium detected in tissues from patients with BPH [18] and prostate cancer [19]. *P. acnes* is a highly pro-inflammatory bacterium that is implicated as the causative agent in the skin disease acne vulgaris and many other inflammatory conditions, including SAPHO (synovitis, acne, pustulosis, hyperostosis, osteitis) syndrome [11]. Interestingly, *P. acnes* (previously named *Corynebacterium parvum*) was studied in the 1970s and 1980s with the hope of harnessing its proinflammatory nature as an immunostimulatory agent in the treatment of cancer [33,34].

In recent reports, the ability to culture *P. acnes* was strongly correlated with prostatic inflammation in prostatectomy tissues [19]. Furthermore, in vitro studies have demonstrated that *P. acnes* is capable of inducing a strong inflammatory response in prostate-derived cell lines [14,20,21]. In order to explore a potential causal connection between *P. acnes* and prostate inflammation, we have developed a novel mouse model of *P. acnes* inoculation/

infection in the prostate. Using this model, we provide the first evidence that *P. acnes* can induce inflammation in the prostate in an in vivo mouse model. Inoculation of 10^7 CFU of prostatectomy-derived *P. acnes* (PA2) in a 20 μ l volume into the C57BL/6J mouse prostate resulted in severe acute inflammation exclusively in the dorsal prostate lobe. Administration of crystal violet dye using the identical volume and urethral catheterization methods revealed that the inoculum selectively localizes to the dorsal prostate, accounting for the observed exclusivity of the infectious/inflammatory sequelae in the dorsal lobe. While using higher volumes of the inoculum (200 μ l) has been reported to allow exposure of all prostate lobes, we wanted to avoid such high volumes since they were also associated with mechanical injury to the prostate, leading to infiltration of inflammatory cells, and induction of inflammatory cytokines even with instillation of just saline without any infectious inoculum [24].

In our model, we did not observe the onset of acute or chronic inflammation until 1 week post-inoculation, while acute inflammation is often reported to be observed within 48 hr of inoculation in other rodent models of *E. coli*-induced bacterial prostatitis [24,29,30]. This is consistent with in vitro observations of a delayed inflammatory response to *P. acnes* infection of prostate epithelial cells [21]. Intriguingly, we also observed evidence of intracellular *P. acnes* infection of mouse prostate epithelial cells. Since *P. acnes* is not typically known to be an intracellular pathogen, these observations will most certainly be the focus of future studies.

We observed variable degrees of inflammation in the dorsal prostates of PA2 infected animals. By 1 week post-inoculation, three out of five animals inoculated with PA2 showed evidence of a severe inflammatory response predominated by dense accumulation of luminal neutrophils and stromal mononuclear inflammatory cells. At 2 and 8 weeks following inoculation, the incidence of inflammation remained at three out of five animals; however the inflammatory response was less severe. Over time, the area of the prostate that was actively inflamed also decreased, although remodeled glands with thickened stroma were often found adjacent to these areas.

In addition to inducing an acute and chronic inflammatory response in the dorsal prostate, our model recapitulated molecular features of inflamed human prostate, including diminished expression of Nkx3.1 and androgen receptor in inflamed glands, as well as increased proliferation in epithelial cells of these glands. It has been previously reported that Ki-67 levels are increased and AR levels are decreased in association with human proliferative inflammatory atrophy (PIA), an inflammation-associated lesion in the prostate that is a suspected precursor lesion to prostatic intraepithelial neoplasia (PIN) and prostate cancer [32]. Furthermore, it has been proposed that Nkx3.1 may act as a tumor suppressor by protecting prostate epithelial cells from oxidative stress [35]. Consistent with this, Nkx3.1 expression is decreased in human prostate cancers [26]. Therefore, downregulation of this protein and enhanced proliferation of epithelial cells may provide an environment conducive to neoplastic transformation of epithelial cells and carcinogenesis. Further studies are currently underway to determine whether chronic inflammation induced by *P. acnes* can result in carcinogenesis in the prostate.

CONCLUSIONS

In conclusion, we have developed the first mouse model of chronic prostatic inflammation induced by a prostatectomy tissue-derived bacterial isolate. Furthermore, we provide the first causal evidence that *P. acnes* is capable of inducing inflammation in the mouse prostate. This preclinical in vivo model may prove valuable for studying the role of bacteria-induced chronic inflammation in prostate cancer initiation and/or progression. Finally, this mouse model of infection-induced chronic inflammation may also serve as a valuable in vivo model to study additional inflammation-associated prostatic diseases such as bacterial prostatitis and BPH.

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References

1. Gui-zhong LI, Libo M, Guanglin H, Jianwei W. The correlation of extent and grade of inflammation with serum PSA levels in patients with IV prostatitis. *Int Urol Nephrol*. 2011; 43(2):295–301. [PubMed: 20820917]
2. Stimac G, Reljic A, Spajic B, Dimanovski J, Ruzic B, Ulamec M, Sonicki Z, Kraus O. Aggressiveness of inflammation in histological prostatitis—correlation with total and free prostate specific antigen levels in men with biochemical criteria for prostate biopsy. *Scott Med J*. 2009; 54(3):8–12. [PubMed: 19725275]
3. Ugurlu O, Yaris M, Oztekin CV, Kosan TM, Adsan O, Cetinkaya M. Impacts of antibiotic and anti-inflammatory therapies on serum prostate-specific antigen levels in the presence of prostatic inflammation: A prospective randomized controlled trial. *Urol Int*. 2010; 84(2):185–190. [PubMed: 20215823]
4. Fujita K, Hosomi M, Tanigawa G, Okumi M, Fushimi H, Yamaguchi S. Prostatic inflammation detected in initial biopsy specimens and urinary pyuria are predictors of negative repeat prostate biopsy. *J Urol*. 2011; 185(5):1722–1727. [PubMed: 21420119]
5. Delongchamps NB, de la Roza G, Chandan V, Jones R, Sunheimer R, Threutte G, Jumbelic M, Haas GP. Evaluation of prostatitis in autopsied prostates—Is chronic inflammation more associated with benign prostatic hyperplasia or cancer? *J Urol*. 2008; 179(5):1736–1740. [PubMed: 18343414]
6. Nickel JC, Downey J, Young I, Boag S. Asymptomatic inflammation and/or infection in benign prostatic hyperplasia. *BJU Int*. 1999; 84(9):976–981. [PubMed: 10571623]
7. Chughtai B, Lee R, Te A, Kaplan S. Role of inflammation in benign prostatic hyperplasia. *Rev Urol*. 2011; 13(3):147–150. [PubMed: 22110398]
8. De Marzo AM, Platz EA, Sutcliffe S, Xu J, Gronberg H, Drake CG, Nakai Y, Isaacs WB, Nelson WG. Inflammation in prostate carcinogenesis. *Nat Rev Cancer*. 2007; 7(4):256–269. [PubMed: 17384581]
9. Sfanos KS, De Marzo AM. Prostate cancer and inflammation: The evidence. *Histopathology*. 2012; 60(1):199–215. [PubMed: 22212087]

10. Nelson, W.; Sfanos, K.; DeMarzo, A.; Yegnasubramanian, S. Prostate inflammation and prostate cancer. In: Klein, EA.; Jones, JS., editors. Management of prostate cancer, current clinical urology. New York, NY: Humana Press; 2013. p. 103-115.
11. Jakab E, Zbinden R, Gubler J, Ruef C, von Graevenitz A, Krause M. Severe infections caused by *Propionibacterium acnes*: An underestimated pathogen in late postoperative infections. *Yale J Biol Med*. 1996; 69(6):477–482. [PubMed: 9436290]
12. Sfanos KS, Sauvageot J, Fedor HL, Dick JD, De Marzo AM, Isaacs WB. A molecular analysis of prokaryotic and viral DNA sequences in prostate tissue from patients with prostate cancer indicates the presence of multiple and diverse microorganisms. *Prostate*. 2008; 68(3):306–320. [PubMed: 18163428]
13. Alexeyev OA, Marklund I, Shannon B, Golovleva I, Olsson J, Andersson C, Eriksson I, Cohen R, Elgh F. Direct visualization of *Propionibacterium acnes* in prostate tissue by multicolor fluorescent in situ hybridization assay. *J Clin Microbiol*. 2007; 45(11):3721–3728. [PubMed: 17881550]
14. Fassi Fehri L, Mak TN, Laube B, Brinkmann V, Ogilvie LA, Mollenkopf H, Lein M, Schmidt T, Meyer TF, Brüggemann H. Prevalence of *Propionibacterium acnes* in diseased prostates and its inflammatory and transforming activity on prostate epithelial cells. *Int J Med Microbiol*. 2011; 301(1):69–78. [PubMed: 20943438]
15. Severi G, Shannon BA, Hoang HN, Baglietto L, English DR, Hopper JL, Pedersen J, Southey MC, Sinclair R, Cohen RJ, Giles GG. Plasma concentration of *Propionibacterium acnes* antibodies and prostate cancer risk: Results from an Australian population-based case-control study. *Br J Cancer*. 2010; 103(3):411–415. [PubMed: 20606679]
16. Sutcliffe S, Giovannucci E, Isaacs WB, Willett WC, Platz EA. Acne and risk of prostate cancer. *Int J Cancer*. 2007; 121(12):2688–2692. [PubMed: 17724724]
17. Galobardes B, Smith GD, Jeffreys M, Kinra S, McCarron P. Acne in adolescence and cause-specific mortality: Lower coronary heart disease but higher prostate cancer mortality. *Am J Epidemiol*. 2005; 161(12):1094–1101. [PubMed: 15937017]
18. Alexeyev O, Bergh J, Marklund I, Thellenberg-Karlsson C, Wiklund F, Gronberg H, Bergh A, Elgh F. Association between the presence of bacterial 16S RNA in prostate specimens taken during transurethral resection of prostate and subsequent risk of prostate cancer (Sweden). *Cancer Causes Control*. 2006; 17(9):1127–1133. [PubMed: 17006718]
19. Cohen RJ, Shannon BA, McNeal JE, Shannon T, Garrett KL. *Propionibacterium acnes* associated with inflammation in radical prostatectomy specimens: A possible link to cancer evolution? *J Urol*. 2005; 173(6):1969–1974. [PubMed: 15879794]
20. Drott J, Alexeyev O, Bergstrom P, Elgh F, Olsson J. *Propionibacterium acnes* infection induces upregulation of inflammatory genes and cytokine secretion in prostate epithelial cells. *BMC Microbiol*. 2010; 10(1):126. [PubMed: 20420679]
21. Mak TN, Fischer N, Laube B, Brinkmann V, Metruccio MM, Sfanos KS, Mollenkopf HJ, Meyer TF, Brüggemann H. *Propionibacterium acnes* host cell tropism contributes to vimentin-mediated invasion and induction of inflammation. *Cell Microbiol*. 2012; 14(11):1720–1733. [PubMed: 22759266]
22. Sfanos KS, Isaacs WB. An evaluation of PCR primer sets used for detection of *Propionibacterium acnes* in prostate tissue samples. *Prostate*. 2008; 68(14):1492–1495. [PubMed: 18651578]
23. Mak TN, Yu S-H, De Marzo AM, Brüggemann H, Sfanos K. Multilocus sequence typing (MLST) analysis of *Propionibacterium acnes* isolates from radical prostatectomy specimens. *Prostate*. 2012 Online, AOP [ahead of print]. 10.1002/pros.22621
24. Boehm BJ, Colopy SA, Jerde TJ, Loftus CJ, Bushman W. Acute bacterial inflammation of the mouse prostate. *Prostate*. 2012; 72(3):307–317. [PubMed: 21681776]
25. Elkahwaji JE, Zhong W, Hopkins WJ, Bushman W. Chronic bacterial infection and inflammation incite reactive hyperplasia in a mouse model of chronic prostatitis. *Prostate*. 2007; 67(1):14–21. [PubMed: 17075821]
26. Khalili M, Mutton LN, Gurel B, Hicks JL, De Marzo AM, Bieberich CJ. Loss of Nkx3. 1 expression in bacterial prostatitis: A potential link between inflammation and neoplasia. *Am J Pathol*. 2010; 176(5):2259–2268. [PubMed: 20363913]

27. Vykhovanets EV, Resnick MI, MacLennan GT, Gupta S. Experimental rodent models of prostatitis: Limitations and potential. *Prostate Cancer Prostatic Dis.* 2007; 10(1):15–29. [PubMed: 17199136]
28. Chen H, Bieberich CJ. Structural and functional analysis of domains mediating interaction between NKX-3. 1 and PDEF. *J Cell Biochem.* 2005; 94(1):168–177. [PubMed: 15523673]
29. Rippere-Lampe KE, Lang M, Ceri H, Olson M, Lockman HA, O'Brien AD. Cytotoxic necrotizing factor type 1-positive *Escherichia coli* causes increased inflammation and tissue damage to the prostate in a rat prostatitis model. *Infect Immun.* 2001; 69(10):6515–6519. [PubMed: 11553597]
30. Quintar AA, Doll A, Leimgruber C, Palmeri CM, Roth FD, Maccioni M, Maldonado CA. Acute inflammation promotes early cellular stimulation of the epithelial and stromal compartments of the rat prostate. *Prostate.* 2010; 70(11):1153–1165. [PubMed: 20564470]
31. Bethel CR, Faith D, Li X, Guan B, Hicks JL, Lan F, Jenkins RB, Bieberich CJ, De Marzo AM. Decreased NKX3. 1 protein expression in focal prostatic atrophy, prostatic intraepithelial neoplasia, and adenocarcinoma: Association with Gleason score and chromosome 8p deletion. *Cancer Res.* 2006; 66(22):10683–10690. [PubMed: 17108105]
32. De Marzo AM, Marchi VL, Epstein JI, Nelson WG. Proliferative inflammatory atrophy of the prostate: Implications for prostatic carcinogenesis. *Am J Pathol.* 1999; 155(6):1985–1992. [PubMed: 10595928]
33. Lichtenstein AK, Kahle J, Berek J, Zigelboim J. Successful immunotherapy with intraperitoneal *Corynebacterium parvum* in a murine ovarian cancer model is associated with the recruitment of tumor-lytic neutrophils into the peritoneal cavity. *J Immunol.* 1984; 133(1):519–526. [PubMed: 6373934]
34. Currie GA, Bagshawe KD. Active immunotherapy with *Corynebacterium parvum* and chemotherapy in murine fibrosarcomas. *BMJ.* 1970; 1(5695):541–544. [PubMed: 5435189]
35. Ouyang X, DeWeese TL, Nelson WG, Abate-Shen C. Loss-of-function of *Nkx3. 1* promotes increased oxidative damage in prostate carcinogenesis. *Cancer Res.* 2005; 65(15):6773–6779. [PubMed: 16061659]

Fig. 1.

Dorsal prostate of C57BL/6J mice 1,2, or 8 weeks after inoculation of either PBS (control) or PA2 *P. acnes* via urethral catheterization. Accumulations of neutrophils were observed in the glandular lumens (arrows) at 1 and 2 weeks post-inoculation. Accumulations of chronic inflammatory cells in the stroma (arrowheads) were observed at all time points starting at 1 week post-inoculation. Bottom row is enlarged view of boxed area in panel above.

Fig. 2. Prevalence (A) and extent (B) of inflammation in mouse dorsal prostate lobes after PA2 *P. acnes* infection.

Fig. 3.

Detection of *P. acnes* in mouse dorsal prostate. IHC (**A,B**) and ISH (**C**) for *P. acnes* at 1 week post-inoculation. IHC for *P. acnes* 2 weeks post-inoculation (**D,E**) and 8 weeks post-inoculation (**F**). Note positive staining for *P. acnes* cells in prostate-infiltrating neutrophils (arrows pointing to representative examples) and prostate epithelium (arrowheads pointing to representative examples).

Fig. 4. Increased proliferative index of inflamed dorsal prostate. IHC for Ki-67 1, 2, and 8 weeks post-inoculation with PA2 *P. acnes*. Note increased Ki-67 nuclear staining in epithelium of inflamed glands (arrowheads) versus non-inflamed glands (arrows).

Fig. 5. IHC for Nkx3.1 and AR in *P. acnes* infected mouse dorsal prostate at 1 (top row) and 2 weeks (bottom row) post-inoculation. Note diminished Nkx3.1 and AR staining in epithelial cells of inflamed glands (arrowheads) versus non-inflamed glands (arrows).