REVIEW

Experimental rodent models of prostatitis: limitations and potential

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Prostatitis is a polyetiologiological inflammation of the prostate gland in men characterized by pelvic pain, irritative voiding symptoms, and sexual dysfunction. Histologically prostatitis is characterized by poly- and mononuclear cell infiltrates (neutrophils, lymphocytes, macrophages and plasma cells) in the stromal connective tissue around the acini or ducts. Prostatitis is an important worldwide health problem in men. The pathogenesis and diagnostic criteria for the condition are obscure, with the result that the development of management programs for this condition has been hindered. Animal model(s) might be useful in elucidating mechanisms involved in the molecular pathogenesis of chronic nonbacterial prostatitis and chronic pelvic pain syndrome. Given that prostatitis might have a multifactorial etiology, several animal models with unique features may prove helpful. This review examines a number of experimental rodent models of prostatitis and evaluates their advantages and limitations.

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Introduction

Available data confirms that prostatitis is commonly diagnosed in men, in the US, with 2 million office visits recorded annually.¹ Initial studies have estimated that up to 50% of men at some point in their life may suffer from prostate inflammation.² More recent studies have recorded incidences of prostatitis ranging from 3 to 16% in North America, Europe and Asia^{1,3–9} half of whom will have repeat episodes¹⁰ emphasizing that prostatitis is an important worldwide health problem. Unlike benign prostate hyperplasia and prostate cancer, prostatitis affects adult men of all ages and is generally considered to be the most common outpatient condition seen in urologic practice in men younger than 50 years old.¹ Moreover, prostatitis accounts for more office visits than either benign prostatic hyperplasia or prostate cancer.¹

The most recent classification of prostatitis includes acute and chronic bacterial prostatitis (category I and II), chronic pelvic pain syndrome (CPPS) (category III) and asymptomatic inflammation of the prostate (category IV).¹¹ The presence of white blood cells, commonly accepted uropathogens such as *Escherichia coli*, *Enterococci* spp, *Klebsiella* spp, *Proteus* spp and *Pseudomonas*

aeruginosa in the expressed prostatic secretions (EPS) and/or voided bladder, urine specimen and classic clinical symptoms can help to differentiate patients in categories I and II from patients in other categories. Asymptomatic inflammatory prostatitis (category IV) is diagnosed incidentally during patient evaluation of infertility, elevated serum prostate-specific antigen (PSA) or symptoms of benign prostatic hypertrophy. The majority of cases of prostatitis fall into category III also designated as chronic nonbacterial prostatitis/CPPS. Genitourinary pain with or without voiding symptoms in the absence of uropathogenic bacteria or other identifiable cause, such as malignancy, defines this category. Patients with chronic prostatitis/CPPS, however, are subcategorized into IIIA and IIIB subgroups. Pelvic pain lasting >3 months is an attribute of both subgroups but the presence of any number of white blood cells in the semen, postprostate massage urine specimen or EPS clearly distinguishes category IIIA from IIIB.

Infectious agents such as bacteria, *Chlamydia* or other microorganisms are implicated in 5–40% of cases of prostatitis.^{10,12,13} The remaining 60–95% cases of clinical prostatitis (categories IIIA and IIIB) may involve (i) hormonal imbalance;^{14,15} (ii) neurological dysfunction;^{16,17} (iii) α -adrenergic system abnormalities;^{18–20} (iv) urinary reflux into the prostate;^{21–23} (v) inappropriate cytokine release^{24–32} and/or (vi) an autoimmune response.^{33–37} The patients typically have repeat episodes of prostatitis and therapy is ‘hit or miss’. Quality-of-life may be significantly diminished.³⁸ Furthermore, there is a growing awareness that chronic prostate inflammation may play a role in the development of prostatic

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cancer.^{37,39–43} Recent analysis of follow-up prostate needle biopsies from 177 patients with clinical parameters suspicious for malignancy has revealed a strong association between chronic prostatic inflammation, and premalignant and malignant changes in prostatic epithelium.⁴⁴

Reliable experimental animal models of human diseases are critically important for the discovery of molecular pathways, genetic influences, environmental factors, and successful management strategies for humans. Animals experimentally affected by such diseases provide a unique opportunity to uncover disease-associated pathways, which are complicated or even impossible to define in man.⁴⁵ Currently, many human noncancer diseases have appropriate animal models that serve as sources for obtaining new research data and testing new therapeutic strategies.^{46–56} It should be noted that almost half the articles published on prostatitis and available on PubMed were published between the year 2000 and 2006. Nevertheless, the cause of category III and IV prostatitis has remained elusive. There is still no gold-standard diagnostic test for CPPS.⁵⁷ During the last three decades considerable attention has been focused on creating novel reliable animal models of prostatic inflammation, in hope of uncovering its etiology and pathogenesis. Several rodent models have been developed, each with its own unique characteristics and potential has been summarized in Table 1.

Human and rodent prostates are anatomically quite different. The rodent prostate is clearly divided into three or four lobes.^{58,59} The ventral lobe is the largest and rests on the ventral surface of the bladder. It is difficult to separate the dorsal and lateral lobes from each other in male mice or rats and both are harvested as one unit. The final lobe is the anterior prostate or coagulating gland. As it has been observed that neoplasms are usually derived from the dorsal-lateral lobe in rodents,^{60,61} it has been proposed that the dorsal-lateral lobe of the rodent prostate is analogous to the peripheral zone in the human prostate; however, this hypothesis currently has not been substantiated by histologic or biochemical evidence. Approximately, 30 human carcinoma cell lines putatively representing various stages of prostate cancer have been described and extensively used as powerful research tools.⁶² However, it should be noted that prostatitis is an inflammatory rather than a neoplastic disease. Consequently, cell cultures from normal or inflamed prostates are potentially limited in value in investigating prostatitis due to the restricted life span of nonmalignant prostate cells. The following is a review of studies conducted on several *in vivo* rodent models of prostatitis, including spontaneous, infectious models, hormone- and immune-induced, and some other models, with an attempt to assess their limitations and potential usefulness.

Spontaneous prostatitis models

Rat models of prostatitis

The development of spontaneous lobe-specific prostatitis is well documented for several rat strains. At approximately 12 weeks of age, 60–70% of male Lewis rats, developed spontaneous prostatitis in the lateral prostatic

lobe.⁶³ Sprague–Dawley male rats develop both ventral and lateral prostate inflammation at a significantly lower rate (16%) at 22–24 weeks age.⁶⁴ In 77% of Wistar rats, spontaneous age dependent nonbacterial dorso-lateral prostatitis develops.⁶⁵ By the age of 20 weeks, 88% of Copenhagen rats develop spontaneous lateral prostatic lobe inflammation; in contrast, prostatitis develops in only 6% of (Copenhagen × Fischer) 344 males.⁶⁶ Naslund *et al.*⁶⁷ reported that 12-week-old Lewis, Wistar, and Sprague–Dawley males had a 30, 0 and 0% incidence of spontaneous lateral prostate inflammation, respectively; the incidence of this lobe specific nonbacterial prostate inflammation was increased considerably in older (40- to 52-week-old) Lewis (72%) and Wistar (27%) males, but not in Sprague–Dawley rats (0%).

Mouse models of prostatitis

Data regarding the spontaneous prostate inflammation in mice is limited. Recently, Penna *et al.*⁶⁸ have shown that a strain of nonobese diabetic (NOD) mice develop spontaneous autoimmune prostatitis, which becomes apparent at ~20 weeks of age and remains stable. In this strain of NOD mice with type 1 insulin-dependent autoimmune diabetes, by the age 20–30 weeks the males exhibit prostate leukocyte infiltrates that are two to fourfold more numerous than seen in 8-week-old mice. A T-cell response to prostate antigens was detected in the spleens of unprimed 8-week-old NOD males. IFN- γ may contribute to the pathogenesis of spontaneous prostatitis in NOD mice, since the development of prostatitis was markedly delayed in INF- γ -deficient NOD males.⁶⁸ Unfortunately, the authors did not present the incidence rate and lobe specificity of spontaneous prostatitis in this mouse strain.

In summary, readily available Lewis, Wistar and Copenhagen male rats and NOD male mice develop spontaneous (lobe-specific in rats) nonbacterial prostatitis with advancing age, providing a model for the study of prostatic inflammation.

Infectious prostatitis models

From the clinical point of view an animal model of chronic bacterial prostatitis is clearly desirable. Acute bacterial prostatitis in men has well-defined criteria and management.⁶⁹ On the other hand, chronic bacterial prostatitis is most often a consequence of acute bacterial prostate inflammation although the responsible pathogens may be fully sensitive to the antibiotics used for treatment, clearance of prostatic infection is often poor because of the limited ability of most antibiotics to achieve therapeutic levels within the prostate.⁷⁰ Moreover, it seems likely that rather than being an infectious disease, its etiology and pathogenesis are diverse and its management remains a continuing challenge.⁷¹

Rat models of prostatitis (categories I and II)

The effects of androgen deprivation (castration) on experimental bacterial prostatitis in 10-week-old Sprague–Dawley male rats have been evaluated by Kaplan *et al.*⁷² Approximately 5×10^5 *E. coli* organisms originally isolated from the prostatic secretions of a patient with

Table 1 Rodent models of prostate inflammation

Model	Species and strain	Inflamed site (s)	Phenotype
Spontaneous	Rat: Wistar, Lewis, Copenhagen	LL and VL	Age- and strain-dependent (Sprague–Dawley < Wistar < Copenhagen < Lewis) rat LL prostatitis ^{63,65–67} as early as 12 weeks of age with a peak after 20 weeks of age; PMNC and MNC prostate tissue infiltration
	Mouse: NOD	NA	Age-dependent prostatitis as early as 20 weeks of age with a T-cell response to prostate Ag(s) at 8 weeks of age is highly CD4 ⁺ cell adoptively transferable ⁶⁸
Infection	Rat: Wistar, Sprague–Dawley,	VL, LL, DL	Intraventral lobe ⁷² or transurethral ⁷³ prostatic introduction of <i>E. coli</i> present a model of acute (in 100%) prostatitis which is self-limiting and chronic (in ~50%) whole prostate ⁷² and ventral lobe prostatic inflammation ⁷³ with high percentage of eliminated animals due to their death or sterile prostate; ⁷⁷ evidence that an androgen deprivation might decrease bacterial growth and improve course of chronic bacterial prostatitis. ^{72,77} Single injection of <i>Chlamydia trachomatis</i> into vas deferens may generate acute self-limited (~80% of cases) prostatitis ⁷⁶
	Mouse: C3H/HeJ and C3H/HeOuJ	NA	Both C3H/HeJ and C3H/HeOuJ male mice were able to develop high acute prostate infection after inoculation intraurethrally with <i>E. coli</i> ; evidence that C3H/HeOuJ mice have signs of acute purulent (on the first week) and chronic (on 12 and 26 week) prostatitis after <i>E. coli</i> inoculation ^{80,81}
Immune Prostate Ag-induced	Rat: Wistar, Lewis, Copenhagen	Male sex accessory glands, DL, LL and VL	Single/multiple immunization of homogenate of rat male sex accessory glands, ⁸² rat prostate tissue, ⁹⁴ rat or human prostate acid phosphatase, ⁹² or rat prostate steroid binding protein (PSBP) ⁹⁴ with CFA induces accumulation of circulating Ab to prostate Ag(s) and low incidence rate (~30%) of MNC prostatitis for 1–2 months and is high cell adoptively transferable
	Mouse: C57BL/6 and NOD	NA	Single/multiple immunization of homogenate of rat male sex accessory glands, ¹⁰⁰ rat prostate tissue, ¹⁰¹ mouse prostate homogenate, ^{68,97} purified rat or mouse PSBP, ⁶⁸ and synthetic mouse PSBP peptides ⁶⁸ with CFA induces different incidence rate (from 30 ¹⁰¹ to 100% ⁹⁷) of mononuclear cell prostatitis, accumulation of circulating auto-Abs to prostate Ag(s), and is high cell adoptively transferable
Day 3 of life thymectomy (Tx-3)	Mouse: C3, 129, (C57BL/6N × A/J)F ₁ , (C3H/HeMs × 129/J)F ₁ and (SWR × NZB)F ₁	VL < DL = LL < AL	No any sensitization with tissue Ag(s). Tx-3 induces non-specific MNC prostate infiltration in ~60% of males on 50–210 day of age ^{102,107} with accumulation of circulating auto-Abs to prostate tissue in 70–97% of mice. ^{102–104,108} Tx-3 mice develop prostatitis by auto-sensitization to the Ag(s) normally expressing in the differentiated prostate and it is highly adoptive cell transferable and can be prevented by CD4 ⁺ spleen cells from normal males
Immune manipulation	Mouse: TRAMP and POET	LL, DL and VL	Simultaneous vaccination with tumor cells and treatment with CTLA-4 induces an enhanced immune response, at least in part, to normal prostate Ag(s) and prostate MNC infiltration. ¹²¹ Transitory prostatitis, without induction of prostate pathology, was induced by transfer of prostate antigen specific CD8 ⁺ cytotoxic cells ¹²⁴
Hormone	Rat: Wistar, Lewis	LL, LL/DL and VL	Strain, lobe-specific and age-dependent prostatitis. 17 β -estradiol given to male adults increases incidence and severity of spontaneous prostatitis that can be attenuated by testosterone but not DHT. ^{67,129} Testosterone treatment during adulthood induces LL and VL prostatitis only after estrogen neonatal imprinting. ⁶⁷ The model relays with hyperprolactinemia ¹⁴⁰ and accumulation of autoreactive cells in inflamed prostate ¹⁵¹
	Mouse: LuRKO	NA	Testosterone replacement from 21 day for 60 day of age induces severe PMNC and MNC prostate infiltration in 67% of hypogonadal LuRKO mice which may be related to the disturbance of sperm transport to ejaculated semen in the treated males ¹⁶²
Miscellaneous Stress	Rat: Sprague–Dawley	DL, LL < VL	Long-term (10 days) stress stimuli (starvation, low surrounding temperature, and small cage) induce prostatic inflammation ¹⁶⁸
Irritant	Rat: Sprague–Dawley	VP	Transurethral ethanol/dinitrobenzenesulfonic acid-mediated mucosal injury resulted in acute prostatitis that is peaked between 24 and 48 h ¹⁶⁹

Table 1 Continued

Model	Species and strain	Inflamed site (s)	Phenotype
Diet	Rat: Sprague–Dawley	LL, DL	Both 9 week oral administration of soy bean extract mixture ¹⁷⁰ and 11 week soy-free diet ⁶⁴ were able to induce LL/DL prostatitis in 80% of rat males that may suggest for role of the estrogen/androgen balance in initiating prostate inflammation
Mechanical	Rat: Wistar	VL	Partial mechanical obstruction of the urethra induces prostatic lymphocytic infiltration and interstitial edema, being most prominent on day 3 that might be due to intraprostatic urinary reflux ¹⁷¹

Abbreviations: AL, DL, LL, VL, anterior, dorsal, lateral, ventral (coagulating gland) lobes of prostate, respectively; CFA, complete Freund's adjuvant; DHT, dihydrotestosterone propionate; MNC, mononuclear cells; NA, not applicable; PMNC, polymorphonuclear cells; Tx-3, day 3 thymectomized.

chronic bacterial prostatitis were directly injected into one of the ventral prostatic lobes using a midline abdominal surgical incision. All prostatic lobes showed signs of marked acute inflammation by day 4 following injection. On day 18, however, the ventral prostates from intact rats had little evidence of inflammation but those from the castrated animals still showed a strong inflammatory reaction, although ventral prostate tissue from both were aseptic. In contrast to the ventral prostatic lobe, the dorso-lateral prostate in intact rats showed evidence of developing chronic bacterial prostatitis and tissue cultures remained positive for *E. coli*.⁷²

In the early 1990s, Nickel *et al.*⁷³ developed a novel protocol to induce acute and chronic bacterial prostatitis in 10- to 12-week-old Sprague–Dawley male rats. The retrograde inoculation of 2×10^7 colony-forming units of *E. coli* K-235 into the duct of the ventral prostatic gland via a calibrated catheter lodged in the proximal urethra-induced acute prostatitis within 3 days in all glands. However, approximately 50% of the animals overcame their infections spontaneously and did not develop chronic prostatitis during the next 6 weeks. Although it was not clear whether the dorso-lateral prostate was involved or not this model demonstrated two distinct histological phases of bacterial inflammation in the ventral prostate.⁷³ During the initial acute infection stage (days 1–14) an acute response to the bacterial challenge with florid infiltration of polymorphonuclear cells into bacteria-containing spaces was observed.⁷⁴ As the infections became chronic, heavy lymphocyte infiltration and fibrous tissue proliferation completely engulfed the ventral prostatic lobe. Although the inflammatory process in the human prostate remains localized to the gland itself, an intense serum immune reaction mounted against the specific *E. coli* is detectable during the chronic phase of disease at day 56 postinfection.⁷⁴

Infectious agents other than *E. coli* have been used to induce prostate inflammation in rats. Intraurethral injection of *Chlamydia* organisms or placement of a drop of a suspension of *Chlamydia* organisms on the meatus of the extruded penis resulted in transient urethritis and cystitis but not prostatitis in male guinea pigs.⁷⁵ However, a more aggressive approach, inoculating *Chlamydia psittaci* into the right vas deferens has been reported to induce prostate inflammation in 10- to 12-week-old male Wistar rats.⁷⁶ Although the specific site of inflammation in rat prostates was not stated, it was observed that 100% of the inoculated animals had purulent prostatitis both on day 7 and 14 post-

injection. By day 30, however, prostate tissue samples were negative for *C. psittaci* in more than 80% of infected rats.

Seo *et al.*⁷⁷ conducted studies of chronic bacterial prostatitis using the Nickel's model. Approximately, 62% of 12- to 16-week-old male Wistar rats develop chronic bacterial prostatitis by 4 weeks after instillation of 2×10^7 colony-forming units of *E. coli* Z-17 into the urethra. The other 38% of the rats developed severe acute inflammation in the prostate, or died of sepsis, or did not develop prostatitis. In contrast to a previous study,⁷³ only 8% of rats showed spontaneous resolution of chronic bacterial prostatitis, perhaps indicating that Wistar rats are particularly susceptible to bacterial prostatitis, or that *E. coli* Z-17 is particularly efficient in inducing prostate inflammation. Bacterial growth in prostate and urine samples and also prostate histological patterns (degree of inflammatory cell infiltration, acinar changes, interstitial fibrosis) were used to evaluate the effects of various androgen deprivation models on the course of chronic bacterial prostatitis. Although untreated, castrated, and 17 α -estradiol (50 mg/kg/day)-treated rats with chronic bacterial prostatitis shown similar ventral prostate histological patterns, 4 week administration of finasteride (10 mg/kg/day) or levofloxacin (100 mg/kg/day) was able to decrease inflammatory cell infiltration and acinar appearances of inflamed prostates. Again only the finasteride and levofloxacin could reduce significantly bacterial growth both in prostate tissue and urine cultures. In general, authors demonstrated that among other models of androgen deprivation, finasteride was 1.5- to twofold more effective modality to improve course of chronic bacterial prostatitis in the animal model without reducing the systemic testosterone levels.⁷⁷ More recent studies have evaluated the antibacterial effects of intraprostatic zinc injection⁷⁸ and synergistic effects of catechin treatment (extract of green tea) combined with the antibiotic ciprofloxacin in the experimental rat model of chronic bacterial prostatitis.⁷⁹

Accumulated evidence suggests that rodent models of experimentally induced prostate inflammation (categories I and II) share many striking similarities to the development and persistence of human chronic bacterial prostatitis, possible comparable to the natural history of the disease.⁷³ Furthermore, retrograde intraurethral introduction of infectious agent appears to be the most efficient means of inducing bacterial prostatitis in rodents. However, some disadvantages in this model are significant proportion (from 8 to 50%) of rats

subjected to intraurethral injection do not develop chronic prostatitis; for a variety of reasons, including spontaneous clearance of infection and death from sepsis.

Mouse models of prostatitis (categories I and II)

Prostate infection intensities in genetically different mouse strains after induction of prostatitis with uropathogenic strains of *E. coli* were recently assessed.⁸⁰ Although these did not include histologic assessment of prostate tissue, it was demonstrated that male BALB/c, C57BL/6J and (BALB/c × C3H/HeJ) F₁ mice 13 weeks of age showed no apparent prostate infection 5 days after intraurethral inoculation with *E. coli*. In contrast, C3H/HeJ and C3H/HeOuJ mice exhibited a high incidence of acute prostate infection at the same time point. More recently, acute purulent bacterial prostatitis in male C3H/HeOuJ mice 12-week-old after intraurethral inoculation with 2×10^6 *E. coli* 1677 was histologically confirmed on day 5 postinfection.⁸¹ Moreover, chronic prostatic inflammation was evident at 12 and 26 weeks postinoculation and was associated with reactive epithelial hyperplasia, glandular atypia and evidence of oxidative stress in prostate tissues.

Given the preliminary data available it is difficult to estimate the potential usefulness of this mouse model of acute and chronic bacterial (categories I and II) prostatitis. However, this model may be advantageous because the mouse strains are readily available, and the inoculated mice do develop chronic prostatitis, a feature that might be exploitable in studies evaluating the possible influence of chronic inflammation on the development of prostate cancer. At this point, because available data is limited, it is unclear whether the findings in this study are readily reproducible. Further studies are needed to ascertain whether this model will prove useful.

Immune-induced prostatitis models

Antigen-induced rat prostatitis models (category III)

An autoimmune model for antigen-induced prostatic inflammation in rodents was first described by Pacheco-Rupil et al.⁸² Male accessory gland (MAG) homogenate (prostate, seminal vesicles and coagulating glands) (15.0 mg total protein) with complete Freund's adjuvant (CFA) from Wistar rats was subcutaneously (s.c.) injected once on day 0 or twice on day 0 and 15 into syngeneic rats as antigenic material. An inflammatory reaction in the prostate was observed in three of eight rats (38%) on day 21 after a single inoculation of MAG. Repeating the antigen challenge resulted in a slight increase in the incidence of prostatitis (9/20, 45%) by day 30. No circulating antibodies were detected in rats after a single injection of MAG. However, more intense and prolonged immunization schedules induced humoral responses.⁸² Further variations in the doses and immunization schedules of MAG and various chemical modifications failed to increase the incidence of prostatitis. Typically, rat prostate morphology during the fifth week after antigen challenge was characterized by MNC infiltration into the interstitium, primarily centered around blood vessels close to the epithelium. In some

animals, the inflammatory process induced fibrosis with enlargement of the acini and accumulation of mast cells in the stroma of the inflamed prostate. The number of MNC in prostate inflammatory foci increased on day 14 and reached the highest levels on day 28 after the first immunization with MAG. The foci were constituted with both CD4⁺ and CD8⁺ T cells on the 14 and 28 day after the immunization. Only scarce macrophages were found in the prostate inflammatory foci of MAG-treated rats.⁸³

Studies have shown that antigen components of the MAG are capable of inducing T-cell proliferation *in vitro*⁸⁴ and the accumulation of auto-antibodies in the serum of immunized rats.⁸⁵ High percentage (~70–80%) of male syngeneic recipients exhibited inflammation in the prostate; however, no humoral immune responses were detected in these recipients after cell transfer of MNC from donors with MAG-induced prostatitis.⁸⁶ Galmarini et al.⁸⁷ used 3 months old male Wistar rats immunized with chemically modified MAG (MAG was coupled to diazonium derivatives of arsenilic and sulphonic acid which act as adjuvant) once, twice or three times on days 0, 30 and 45. Inoculation of MAG was necessary to confer higher titers of antibody. Histological changes in the target organs (coagulating glands and prostate > seminal vesicle) were evident in almost all animals examined at 35, 42 and 49 days after the first injection of modified MAG and were characterized by inflammatory reactions and small perivascular mononuclear infiltrates.⁸⁷ Thus, this model is consistent with a self-limiting mild to moderate prostate inflammation induced by both humoral and cell-mediated mechanisms.⁸⁸ It has been speculated that inflammatory damage induced in this model might be the result of generation of autoreactive cytotoxic cells, increased levels of radical oxidative species from prostatic infiltrating macrophages, and upregulated mast cell degranulation.^{89–91} These studies do not identify the specific prostatic proteins that are the targets on an immune response. The immunogenicity of prostatic acid phosphatase (PAP) as a target for the induction of an immune response in 6- to 8-week-old Copenhagen rat was investigated by Fong et al.⁹² They demonstrated that immunization with recombinant rat or human PAP leads to a significant antibody response, 4 weeks after the final boost, but does not generate cytotoxic T lymphocytes (CTL) or result in autoimmune prostatitis.⁹² In contrast, intravenous immunization with recombinant vaccinia virus expressing human PAP generated a CTL response and tissue-specific prostatitis in the absence of detectable PAP-specific antibody. Destructive prostatitis was detected in 80% of cases and interstitial inflammation was found in the remaining 20% of cases. These findings suggest that a cellular immune response to PAP, rather than antibody, mediates destructive autoimmune prostate inflammation. In this model, sensitization of the immune system to xeno-antigen leads to the generation of T lymphocytes that can cross-react with self-PAP or, alternatively, to the phenomenon of epitope spreading that results in tissue-specific inflammation and destruction of the prostate gland.⁹² The authors of this study did not specify the precise areas of the prostate that were involved in inflammation.

Although prostate steroid-binding protein (PSBP) has immunosuppressive activities,⁹³ it was identified as the

dominant antigenic target in another rat model of prostate inflammation.⁹⁴ PSBP is the major secretory protein in the rat ventral prostate, and so far it has not been found in any other organ. The authors used ventral prostate homogenate from pooled prostates of Lewis rats as a source of prostate antigen(s) to triple immunize 3–6 months old male syngeneic rats. This protocol did not induce prostate inflammation but resulted in the generation of high levels of auto-antibodies to PSBP. In contrast with the results using prostate homogenate significant focal inflammation of the ventral prostate was observed in four of 12 rats (33%) immunized with ion exchange chromatography-purified rat PSBP, a procedure that elicited both humoral and cellular responses against PSBP. Moreover, adoptive cell transfer experiments have shown that splenocytes from PSBP immunized donors were capable of inducing severe ventral prostate inflammation in five of eight rats (63%) even if the donors of the splenocytes did not have prostatitis.⁹⁴ Similar results were obtained when PSBP was used to immunize 3-month-old male Wistar rats.⁹⁵ The autoimmune response was characterized by delayed type hyper-sensitivity reaction accompanied by autoantibody production and increased degranulation of mast cells in the rat prostate gland. However, the authors emphasized that although PSBP was able to induce autoimmune prostatitis, the stronger autoimmune responses and the differences in the lesion observed in animals immunized with a prostate homogenate (which includes a wider spectrum of prostate antigen(s)) indicate that more than one auto-antigen was involved, as is typically the case of other autoimmune disorders.⁹⁵

Antigen-induced autoimmune prostate inflammation was used by Gilardoni *et al.*⁹⁶ as a background for long-term (20 weeks) hormonal treatment with testosterone (silastic membrane tubes, 45 mg/implant, placed 45 days after the first MAG immunization) in combination with a carcinogenic agent (7, 12 demethylbenz (α) anthracene (DMBA), 30 mg/kg, 28 days after the 1st immunization with MAG) in male Wistar rats. Histologic evaluation showed the presence of premalignant lesions (prostatic intraepithelial neoplasia) in five of 10 rats (50%) that were challenged with MAG's proteins. The development of prostate carcinoma in animals treated with testosterone and DMBA was seen only in animals in which prostate inflammation had previously been induced by MAG immunization. Controls animals exposed to testosterone and DMBA alone were found to have prostatic intraepithelial neoplasia, but did not develop carcinoma.⁹⁶ This model of category III prostatic inflammation has several advantages. The protocol for inducing it is fairly simple and the susceptible rat strains are readily available. It shows promise as a potential model for the investigation of possible relationship between prostatic inflammation and the development of prostate cancer. However, the disadvantage of this model includes: (i) prostatitis is induced by one or more proteins that have not so far been identified; (ii) only ~30–50% of immunized animals develop prostatitis; (iii) the autoreactive T-cell subset responsible for the autoimmune prostatitis is activated by immunization with an ill-defined mix of prostate proteins or PSBP. Finally, despite impressive findings in two elegant studies,^{92,94} these models have not been used and the findings have not been reported by others.

Antigen-induced mouse prostatitis models (category III)

Keetch *et al.*⁹⁷ reported in the mid 1990s one of the first mouse models of antigen-induced prostate inflammation. They reported that 100% of C57BL/6 male mice immunized once with prostate tissue extract developed autoimmune prostatitis by day 30. Only ventral lobes of the prostate from syngeneic mice were used for preparation of the extract and the extract was combined with CFA. Mice were immunized with different doses, up to a maximum of 0.75 mg protein extract and supplemented with pertussis toxin. A prominent interstitial infiltration of MNC was noted, predominantly surrounding blood vessels and prostatic acini. The inflammation was localized to the dorso-lateral prostate and the degree of inflammation was dose-dependent. The authors were able to induce prostatitis in syngeneic naive recipients by adoptive cell transfer. The autoimmune nature of the induced prostatitis was supported by the identification of circulating serum antibodies to unknown prostatic homogenate proteins with molecular weight 25 kDa. This study demonstrated that the C57bl/6 mouse strain was more susceptible to the induction of prostatitis than other strains such as SJA, AJ and Balb/c.⁹⁷

NOD mice are a well-characterized model of type I diabetes. They are also prone to develop inflammatory reactions in multiple exocrine and endocrine glands including the salivary and lachrymal glands,⁹⁸ and the thyroid.⁹⁹ Rivero *et al.*¹⁰⁰ studied the induction of urogenital organ inflammation in NOD mice after xenoantigen challenge. They demonstrated that 10 days and 21 days after vaccination with 1.0 mg pooled MAG homogenate from Wistar rats with CFA, all mice showed inflammation in the dorso-lateral prostate, regardless of whether they had been exposed once or twice to MAG in CFA. Additionally, foci of moderately severe inflammation were also found in the ventral prostate, seminal vesicles and coagulating glands, although less frequently. Further experiments by the same group have shown that 1.0 mg Wistar rat prostate homogenate or 30 μ g of Wistar rat PSBP injected twice resulted in the induction of prostatitis in 80–100% of young male NOD mice but in only ~30% of C57BL/6 mice.¹⁰¹ Although precise sites of prostate inflammation were not specified in this study, histologic changes in the prostate were characterized by perivascular and stromal MNC infiltration accompanied by edema. The edema was more severe in mice immunized with prostate homogenate than in those immunized by PSBP. These experiments with NOD mice also demonstrated that immunized mice specifically responded to prostate xenoantigen(s) challenge by increased T-cell proliferation, increased release of INF- γ by upregulated T cells, and by increased production of circulating IgM and IgG autoantibodies. More recently, it was reported that NOD mice are characterized by the spontaneous development of autoimmune prostatitis which becomes apparent at about 20 weeks of age and is stably maintained thereafter.⁶⁸ In contrast to BALB/c and C57BL/6 mice, significant upregulation of PSBP-specific autoreactive T cells are detectable in NOD males before the spontaneous development of autoimmune prostatitis, indicating lack of tolerance to the self-antigen. There are indications that disease development was markedly delayed in IFN- γ -deficient mice and prostate lymphocyte infiltration could be transferred into NOD.S-CID recipients by CD4⁺ cells from 24-week-old NOD

males. Experimental autoimmune prostatitis was detected in NOD mice 30 days after priming of 8-week-old NOD males with mouse prostate homogenate, purified PSBP, and synthetic PSBP peptides. Moreover, both PSBP and prostate homogenate induced comparable levels of intraprostatic CD4⁺ cell infiltration of NOD mice.⁶⁸ The advantages of this mouse model of category III prostatitis are that (i) relevant mouse strains are readily available, and that there is a wide variety of immunological techniques available to study of immunological aspects underlying prostatic inflammation. On the other hand, the major disadvantage of this model is that the prostate tissue autoantigen involved in the induction of autoimmune prostatitis is not well characterized. Furthermore, NOD mice frequently develop diabetes and other organ-specific autoimmune diseases that may interfere to specify study of prostate inflammation.

T regulatory cell depleted mouse model

Taguchi *et al.*^{102–103} described another immune-mediated model of prostate inflammation in the mid-1980s. They reported that ~70% of C3H/HeMs × 129/J F₁ male mice that were thymectomized on day 3 (Tx-3) developed prostatitis which was found predominantly in the anterior prostate (coagulating gland) and to a much lesser degree in the ventral prostate.^{102,103} The autoimmune prostatitis was characterized by loss of secretory products in the lumen and massive lymphocyte infiltration in the stroma (beneath the epithelial cells). Marked inflammation in the prostate was accompanied by circulating autoantibodies to prostate epithelial cells in 42 of 60 cases (70%) of Tx-3 mice aged 50–150 days. The authors observed that performing a thymectomy on day 0 or 7 of life did not reproduce this phenotype and castration on day 0 (Orx-0) of life eradicated it. At the same time, seven of 13 Orx-0 + Tx-3 mice (54%) developed autoimmune prostatitis when they were given a testosterone pellet at 90 day of age. These data indicate that Tx-3 mice develop autoimmune prostatitis by autosensitization of the immune system to an unknown prostate antigen(s) normally expressed in the differentiated prostate gland. In the mid 1990s, Taguchi *et al.*¹⁰⁴ reported that depletion of the tissue-specific CD4⁺ T-suppressor cell population in Tx-3 mice results in autoimmune prostatitis after the onset of puberty. They demonstrated that the development of the prostate lesions in Tx-3 (C57BL/6N × A/J) F₁ male mice could be prevented by a single injection of CD4⁺ spleen cells from normal syngeneic mice, but not from normal females or Orx-0 mice. However, the splenocytes from Orx-0 mice could prevent prostatitis in Tx-3 recipients if the Orx-0 donors had received a dihydrotestosterone (DHT) pellet in adulthood, resulting in prostate maturation. The data of this elegant study indicate that immune tolerance to prostate antigen(s) is maintained by a tissue-specific T regulatory cell subset, which is activated by a specific auto-antigen(s) in the mature prostate. The autoimmune nature of prostatitis in the studied mice was also supportive by induction of prostate inflammation in nude recipients after adoptive cell transfer of autoreactive CD4⁺ cells from syngeneic Tx-3 mice with prostatitis. Moreover, the findings that even CD4⁺ cells from Orx-0 mice that were thymectomized as adults and treated thereafter with DHT were effective in preventing

prostatitis may suggest that activation of this T suppressor population takes place in the peripheral lymphoid organs.¹⁰⁴ It is clear that depletion of CD4⁺CD25⁺ T regulatory cells by 3 day thymectomy in this model is an underlying cause of prostatitis.^{105,106} More recently, Bagavant *et al.*¹⁰⁷ have reported that eight of 13 (62%) of Tx-3 lupus-prone (NZB × SWR) F₁ male mice developed prostatitis and antibody to PSAs was found in 11 of 15 cases (73%). These authors also reported that prostatitis was detected in 73% of Tx-3 males of another lupus-prone mouse strain (NZM2328).¹⁰⁸ It has been also shown that the transfer of 1 million CD25⁺ T cells to Tx-3 NZM2328 mice completely suppressed prostatitis. In summary, these data strongly suggest the role of T regulatory cells in this model of prostate inflammation.

The advantage of this mouse model of prostatic inflammation is that it appears to be a genuine disease that offers an opportunity to study the basic principles of self-tolerance. However, the disadvantages are that the model is too artificial and not specific. Indeed, these Tx-3 mice also develop thyroiditis,¹⁰⁹ oophoritis,¹¹⁰ gastritis,¹¹¹ orchitis¹¹² and insulinitis.¹¹³ Furthermore, the percentage mortality from surgery was not listed in the available publications; however, it is clear that complete thymectomy is a challenging exercise in the newborn mice.

Other immune induced mouse prostatitis models

The cytotoxic T lymphocyte antigen-4 (CTLA-4) is a second T cell counter-receptor for surface B7 molecule¹¹⁴ that plays a critical role in attenuating T-cell responses. CTLA-4 engagement may inhibit the initiation of T cell responses by raising the threshold of signals needed for full activation, or may also play a role in terminating ongoing T-cell responses.^{115,116} Anti-CTLA-4 antibodies that block CTLA-4/B7 interactions enhance *in vivo* T-cell responses to peptides, superantigens and parasites, and can exacerbate experimental autoimmune encephalomyelitis¹¹⁵ or induce the rejection of newly implanted and well established tumors in several transplantable murine tumor systems.^{117–120} Hurwitz *et al.*¹²¹ used CTLA-4 blockade to immunotherapy of primary prostate cancer in transgenic mouse model. They showed that significant accumulation of inflammatory cells in prostate interductal spaces when TRAMP mice were treated with anti-CTLA-4 and a granulocyte-macrophage colony-stimulating factor (GM-CSF) expressing tumor-derived vaccine account for a significant reduction of tumor incidence. The authors demonstrated that anti-tumor response is directed against antigen(s) expressed by both prostate adenocarcinoma and normal prostate, because immunization of nontransgenic mice with GM-CSF-expressing tumor cell vaccines under conditions of CTLA-4 blockade can also result in marked dorso-lateral prostatitis evident 28 days post-treatment. However, they found no evidence of significant inflammation or tissue damage in the dorso-lateral or ventral lobes of the prostates of nontransgenic mice immunized only with GM-CSF tumor vaccine.¹²¹

While several models have addressed the generalized response to prostate antigen(s), these models did not examine antigen specific T-cell populations during responses.^{92,94,95} To address the problem of inability to

monitor specific T-cell populations in prostate inflammation, the prostate ovalbumin-expressing transgenic (POET-1) mouse model was developed. POET-1 mice express a membrane-bound ovalbumin fusion protein (mOVA) at high levels in the ventral and dorso-lateral prostate lobes following adolescence.^{122,123} The relative specificity of the mOVA for high-level prostate expression was demonstrated by Lees *et al.*¹²⁴ with a system studying the effects of the CD8⁺ T-cell response against a prostate tissue antigen. The effectiveness of activated T cells in infiltrating and damaging POET prostate was evaluated using adoptive transfer of either CD8⁺ or CD4⁺ T cells that recognize of mOVA in prostate tissue.¹²⁵ Additionally, endogenous antigen-specific CD8⁺ T cells were studied with respect to their ability to recognize and respond to a prostatic self-antigen. Ovalbumin specific cytotoxic CD8⁺ cells were activated by autologous prostate antigen and trafficked to the prostate, but did not induce inflammation unless present in overwhelming numbers (~65% of CD8⁺ T-cells). Activation of antigen-specific CD8⁺ T cells *in vitro* (peptide pulsed antigen presenting cells) or *in vivo* (infection with a replication deficient type 5 adenovirus that encodes mOVA) induced only transitory prostate inflammation, without induction of prostate pathology, regardless of helper-inducer CD4⁺ T cell availability.¹²⁴ Cumulatively, these data suggest that the prostate is resistant to CD8⁺ immune-mediated epithelial destruction, regardless of the context in which T-cell activation occurs. These observations indicate that immunologically induced prostatitis requires specific antigen(s) presentation and/or manipulation of immune co-stimulatory signaling, specific immunocompetent cell depletion, or specific T-cell activation. These mouse models address the basic principles of cell immune tolerance and self-recognition. Based on current knowledge, these models do not appear promising in furthering our understanding of prostatic inflammation.

Hormone-associated prostatitis models

Rat models of prostatitis

The prostate gland is an androgen-dependent organ in males. Therefore, androgens play a key role in the regulation of prostatic growth, function and disease. Supra-physiologic doses of exogenous testosterone do not stimulate additional prostate growth; however, it should be noted that normally occurring neonatal hormone surges may permanently imprint the prostate and determine its future growth in adulthood.¹²⁶ Moreover, severe diffuse prostatitis was induced in 80% of ventral and 100% of lateral prostates in young adult (12-week-old) Wistar rats by neonatal treatment with 100 µg of 17β-estradiol (days 2 and 5 of life) followed several months later (weeks 10–12) by testosterone administration (2.0 mg/day) for 2 weeks in adulthood.⁶⁷ Grossly both prostates lobes appeared edematous and diffuse sheets of lymphocytes and monocytes, as well as extensive areas of fibrosis, were detected throughout the stroma of the affected lobes. Neither neonatal administration of 17β-estradiol alone, nor testosterone treatment in adulthood alone was able to initiate prostatitis.⁶⁷ Additionally, more recently it has

been shown that perinatal (from dam's gestation day 18 to parturition and then the pups from postnatal day 1–5) exposure to 17β-estradiol was sufficient to increase the incidence of stromal and lumen inflammation both in the ventral and lateral prostate, as well as hypoplasia and stromal fibrosis. Perinatal tamoxifen (partial estrogen agonist) administration also resulted in similar phenotype responses and prostate inflammation but without fibrosis in the ventral prostate 90 days post-treatment in Wistar rats.¹²⁷ Although, a similar protocol resulted in permanent alteration of the prostate growth with lobe-specific changes in androgen receptor expression in the adult prostate, there was no indication of prostate inflammation in neonatal estrogen imprinted Sprague-Dawley rats.¹²⁸ These data clearly indicate the role of genetic susceptibility to prostatitis observed in different rat strains. Indeed, spontaneous nonbacterial age-related prostatitis is commonly observed in Lewis and Wistar rats, and does not occur in Sprague-Dawley rats.⁶⁷

Treatment of 10- to 13-month-old intact Lewis and Wistar male rats for 30 days with 17β-estradiol has shown 1.5- to 3-fold increases in the incidence and two- to sixfold in the severity of spontaneously occurring inflammation in the lateral prostate lobes.⁶⁷ Histological sections of these prostates showed severe, multifocal, predominantly chronic inflammation. Sex steroid hormone ablation (castration) had effects comparable to those observed in rats administered estrogen. Concomitant administration of 2.5 mg/kg/day of testosterone but not the administration of dihydrotestosterone propionate (DHT) reduced the incidence and severity of spontaneous lateral lobe prostatitis induced by 17β-estradiol. Interestingly, treatment with 17β-estradiol or castration worsened lateral lobe prostatitis, whereas injections of live cytomegalovirus or heat killed *E. coli* into the ventral prostate did not initiate ventral prostatitis in these animals.⁶⁷ Similar findings were observed in a study of the sub-acute (2–4 weeks) effects of 17β-estradiol and DHT on the lateral lobe of prostate in 2- to 3-month-old male Wistar rats.¹²⁹ The late effect at 21 weeks of these hormone administrations was intense lateral prostate inflammation; accompanied by a fibromuscular proliferation that consisted with fibroblast, smooth muscle cells and collagen. However, even the administration of the same hormones on a chronic base failed to initiate leukocyte infiltration and/or stromal proliferation in ventral prostate of young adult Wistar rats.¹²⁹

A lateral lobe specific upregulation of proinflammatory genes was observed during estradiol-induced prostate inflammation in young adult Wistar rats.¹³⁰ Based on the pattern of cytokine production of CD4⁺ T-helper-inducer cells, responses to infection, allergy and autoimmune conditions have been divided into two classes, designated Th1 and Th2.¹³¹ The Th1 response has been associated with a variety of chronic inflammatory conditions and autoimmune disorders and the Th1 cytokine pattern is characterized by IFN-γ production and macrophage activation. The Th2 response is characterized by production of IL-4, IL-5 and IL-10, which contribute to mast-cell activation, eosinophil influx, and the immediate hypersensitivity response. Although both Th1 and Th2 type immune responses are responsible for proinflammatory cytokine patterns, they tend to down-regulate one another. It has been demonstrated that treatment of 8-week-old male non-castrated Wistar rats

with estrogen resulted in an upregulation of a non-specific cohort of pro-inflammatory transcripts such as MIP-2, iNOS, IL-6, IL-1 β and TNF- α in the lateral prostate after as little as 4 days of treatment, well before any histological evidence of inflammatory cell infiltration was observed.¹³⁰ Further examination of a broader set of cytokines at 4 weeks, exhibited histological signs of inflammation accompanied by upregulation of IL-4, IL-5, IL-10, eotaxin and iNOS transcripts, but with no change in the expression of IL-2, IL-12, COX-2 or IFN- γ . These expression patterns suggest that the inflammatory process in the lateral prostate lobes of estradiol-exposed rats resembles that of the Th2 helper-cell response.¹³⁰

A fundamental aspect of inflammation is infiltration of the interstitial tissue by leukocytes which elaborate matrix metalloproteinases (MMPs) that facilitate clearance of foreign agents and remove damaged cells and extracellular matrix.¹³² To examine the role of MMPs in the pathogenesis of sex hormone induced prostatitis, Wilson *et al.*¹²⁹ replicated the estrogen-induced lateral lobe-specific prostate inflammation rat model of Robinette *et al.* They demonstrated matrix metalloproteinases (MMP-2 and MMP-9) activities associated with the chronic prostatitis in the lateral prostate lobe. They noted that these could be significantly reduced by treatment with testosterone, but not DHT.¹³³

Currently, there is only one report that supports the autoimmune nature of sex-hormone-induced prostatitis.¹³⁴ Although these investigators used an unusually small number of *in vitro* activated splenocytes for the adoptive cell transfer, they reported that rat lobe-specific prostatitis can be induced in naive syngeneic recipients by adoptive transfer of spleen CD3⁺ T cells from 17 β -estradiol-treated male rat donors. It remains unclear whether this model of prostate inflammation is of autoimmune origin, since there is considerable evidence that 17 β -estradiol is capable of inhibiting physical and histological signs of experimental autoimmune encephalomyelitis (EAE), a mouse model of human multiple sclerosis.¹³⁵ EAE is a classical Th1 type cell-mediated autoimmune disease.¹³⁶ The immunoregulatory effects of 17 β -estradiol in preventing EAE appear to be mediated by direct or indirect inhibition of encephalitogenic Th1 cells, macrophages and dendritic cells. The most dramatic effect of 17 β -estradiol involves strong inhibition of TNF- α by Th1 cells and antigen presenting cells, and it is noteworthy that 17 β -estradiol also enhanced production of TGF- β 3, which has been shown to have protective effects against EAE when used in combination with other agents.¹³⁷ A novel finding is that 17 β -estradiol upregulates the expression of Foxp3 and CTLA-4, which contribute to the activity of CD4⁺CD25⁺ T regulatory cells.^{138,139}

Lateral lobe prostate inflammation in 17 β -estradiol-treated male Wistar rat model has been clearly shown to correlate with increased serum prolactin, elevated pituitary weight, and a greater than twofold increase in lateral prostate DNA concentration.¹⁴⁰ These effects of 17 β -estradiol were blocked by administration of bromocryptine (a dopamine D2 agonist that inhibits pituitary prolactin release). Inflammation was restored in the bromocryptine-treated estradiol-implanted rats by administering exogenous ovine prolactin. Recently, it has been found that there is a significant association between

spontaneous inflammation in the dorsolateral lobes of prostate and prolactin positive pituitary gland adenomas in Fisher-344 rats; this finding supports the hypothesis that prolactin plays an important role in the pathogenesis of prostatic inflammation.¹⁴¹ It has been also shown that prepubertal (postnatal days 22–32) exposure to various compounds that increase prolactin secretion, including pimozide (a dopamine antagonist), bisphenol A (a monomer of polycarbonate plastic reported to have weak estrogenic activity), and 17 β -estradiol, albeit through different mechanisms, can increase the incidence of inflammation in the lateral prostate the Wistar rats.^{142,143}

Shyr *et al.*¹⁴⁴ used daily injections of bromocryptine to depress maternal plasma and milk PRL levels in lactating rats. Such treatment during only postpartum days 2–5 resulted in a marked suppression of tuberoinfundibular dopamine DA turnover in the median eminence and an elevation of the plasma prolactin concentration in the offspring when measured at 4–5 weeks of age.¹⁴⁴ Further, Stoker *et al.* have shown that suppressed suckling-induced prolactin release in Wistar dams induced by the herbicide atrazine was associated with lateral prostate inflammation of the adult offspring by about 17 weeks of age. The most critical period for this effect was again postnatal days 1–4.¹⁴⁵

It is important to note that estrogenization limited to the neonatal period results in both lateral and ventral prostatic inflammation in adulthood, producing a marked prolactin-dependent infiltration of CD4⁺ and CD8⁺ lymphocytes and a prolactin-independent macrophage accumulation in the adult ventral prostate lobe of Sprague–Dawley rats.¹⁴⁶ Collectively, these findings suggest that hyperprolactinemia may contribute in the 17 β -estradiol-induced immune response.

Altogether, these studies suggest that the experimental estradiol-induced rat model of prostate inflammation is mediated by hyperprolactinemia. Curiously, although prolactin receptors are more readily detected and quantified in the ventral rat prostate than in the lateral prostate.¹⁴⁷ The exact mechanism by which hyperprolactinemia induces inflammation in the adult prostate remains to be determined.¹⁴⁸ It is possible that the inflammatory responses seen in the lateral prostate of adult rats in this model are related to an altered immune function, because studies have shown that hyperprolactinemia or manipulation of prolactin in adults can either enhance or suppress humoral and cellular immune responses.¹⁴⁹

To investigate the contribution of the immune system in this model of prostate inflammation, Vykhovanets *et al.*^{150,151} developed a novel protocol to isolate rat intraprostatic lymphocytes. They reported that the lymphocyte populations within whole prostates of healthy Sprague–Dawley rats are unusual in that they contain three subsets of roughly equivalent volume: $\alpha\beta$ TCR⁺ T cells, CD161a⁺ $\alpha\beta$ TCR⁺ natural killer (NK) cells, and CD161a⁺ $\alpha\beta$ TCR⁺ NKT cells.¹⁵⁰ In contrast with spleen, liver, blood, iliac and mesenteric lymph nodes, whole rat prostate contain unexpectedly high numbers of NK cells, T cells bearing NK cell marker (NKT cells) which are predominately CD8⁺ NKT cells, and CD45RC⁺CD4⁺ T cells. Subsequent studies replicating the estrogen-induced prostatitis model of Naslund *et al.*⁶⁷ showed that (i) both rats with spontaneous and

sex-hormone-induced prostatitis show a decreased proportion of intraprostatic NK like cells ($CD161a^{+}\alpha\beta TCR^{-}$ real NK cells and $CD161a$ -bearing (NK-marker) $\alpha\beta TCR^{+}$ T cells i.e. NKT cells); (ii) downregulation of the intraprostatic NK-like cells and a low NKT/T cell ratio are reliable markers of sex-hormone-induced lateral prostate inflammation in this model; (iii) decreased proportions of NKT cells in inflamed prostate appear to be due to a decrease in the proportion of $CD4^{+}$ NKT cells; (iv) the inflamed lateral prostates show an accumulation of total $CD4^{+}$ T helper-inducer cells, $CD45RC^{+}CD4^{+}$ T cell subset and decreased levels of NK cells.¹⁵¹ Further analysis of pooled ventral and dorso-lateral lobe intraprostatic lymphocytes revealed that healthy prostates in both Sprague-Dawley and Wistar rats had an unexpectedly low ratio of $CD4^{+}/CD8^{+}$ T cells.^{150,151} In contrast, the $CD4/CD8$ ratio was increased in inflamed prostates. A low $CD4/CD8$ T cell ratio has also been observed in the normal human prostate.¹⁵²

Altogether these findings may suggest that prostates in rats that are resistant to inflammation show a cellular profile characterized by high levels of cytotoxic $CD8^{+}$ T cells, a high NKT/T-cell ratio, and low $CD4/CD8$ T cell ratio. NKT cells are novel T-cell lineage expressing NK cell markers ($CD161a/NKR-P1A$ in rats).¹⁵³ Upon activation, NKT cells produce large amounts of $IFN-\gamma$ and $IL-4$ ¹⁵⁴ and play an important role in rat autoimmunity^{155,156} NKT cells have been shown to have immunoregulatory functions with a Th2 type immune response deviation in the Wistar-Furth rat whole pancreaticoduodenal transplantation model.¹⁵⁷ Of note, in young Sprague-Dawley rats which are resistant to spontaneous prostatitis,⁶⁷ the intraprostatic lymphocytes are almost equally divided between NK cells and NKT cells, whereas in similarly aged Wistar rats NK cells predominate.^{150,151}

$CD45RC^{-}CD4^{+}$ T cells have been termed regulatory T cells because of their ability to prevent autoimmune thyroiditis and diabetes in rats.¹⁵⁸ In contrast, the $CD45RC^{+}CD4^{+}$ T cell subset may actually promote autoimmune diabetes, wasting and graft-versus-host disease in rats.^{159,160} The balance between these two subsets may be critical for providing adequate cell-mediated immunity versus the development of autoimmune disease. In healthy prostates of both Wistar and Sprague-Dawley rats,^{150,151} about 7–8% of the $CD4^{+}$ T cells are $CD45RC^{+}CD4^{+}\alpha/\beta TCR^{+}$ T cells. This value doubles in prostates from old Wistar rats with spontaneous and sex-hormone-induced prostatitis, suggesting an accumulation of autoreactive $CD45RC^{+}CD4^{+}\alpha/\beta TCR^{+}$ T cells and the loss of the regulatory $CD45RC^{-}CD4^{+}\alpha/\beta TCR^{+}$ T cells. Moreover, the spleens of young intact Sprague-Dawley rats naturally contain ~5-fold lower levels of $CD45RC^{+}CD4^{+}\alpha/\beta TCR^{+}$ T cells than Wistar rats that are susceptible to spontaneous prostatitis.^{150,151} However, it is interesting to note that the proportion of these cells is decreased about twofold in the spleens and lymph nodes of old Wistar rats that have spontaneous and sex-hormone-induced prostatitis. In view of the fact that the ventral prostate is always inflammation free in this model, these data suggest that (i) $CD45RC^{+}CD4^{+}\alpha/\beta TCR^{+}$ T cells may migrate from the spleen to the lateral prostate during the development of inflammation; (ii) lower levels of intraprostatic NKT

cells and higher levels of $CD45RC^{+}CD4^{+}\alpha/\beta TCR^{+}$ T cells in the lymphoid tissue may reflect a genetic susceptibility of Wistar rats to age-related spontaneous prostate inflammation.¹⁵¹ More recent studies have shown that although surface prolactin receptor expression on lateral lobe intraprostatic $CD4^{+}$ T cells was upregulated twofold after castration in 3-month-old Lewis rats after 30 days of daily administration of 17β -estradiol it was not accompanied by detectable circulating autoantibodies to lateral prostate tissue (Vykhovanets EV *et al.*, unpublished data).

Since the 1980s the estrogen-induced lateral lobe-specific prostate inflammation model has been the most widely studied rodent model of chronic nonbacterial prostatitis and may represent category III prostatitis. It is useful model because the rat strains involved are readily available, and the induction of lobe-specific prostate inflammation in those rats is highly reproducible. It is an inexpensive model and shares some striking similarities to the development and persistence of human chronic nonbacterial prostatitis. Its etiology may be comparable to that in humans, who often develop this condition at a time in life when androgen levels are downregulated. On the other hand, it is still unclear whether this model represents experimentally induced prostatitis, or whether the prostatitis is spontaneous and genetically predetermined, and simply accelerated by androgen deprivation and estrogen treatment.¹⁶¹ Another disadvantage of this model is that it is so far restricted to rats, and it is unclear whether mice may be used for this estrogen/androgen derivation model of prostatitis. As most commercially available reagents are developed for use in mice (especially in immunology) studies of this sort in rats are hampered.

Mouse models of prostatitis

Recently Pakarainen *et al.*¹⁶² reported that four of six (67%) testosterone-treated LH receptor knockout (LuRKO) mice exhibited marked inflammation in the prostate, characterized by the abundant presence of lymphocytes in stromal tissue and between tissue layers in prostate glands. The 8 week long testosterone replacement regimen started at the prepubertal age of 21 days to restore both anatomical abnormalities and accessory sex organ function in these hypogonadal LuRKO male mice.^{163–165} Additionally, in some LuRKO mice testosterone replacement also induced severe inflammation in the coagulating glands, *vasa deferentia*, and epididymis.¹⁶² Long-term administration of testosterone in Lobund-Wistar and Noble rat strains has been shown to induce invasive prostate adenocarcinomas.^{61,166} It has been demonstrated that testosterone alone blocks 17β -estradiol-induced prostate inflammation, but prostatitis has not been reported to occur in animals treated with testosterone alone. Therefore, the exact mechanisms by which testosterone induces inflammation and/or infection in the urogenital organs of LuRKO mice still remain elusive. It seems likely that this pathology is related to the disturbance of sperm transport to ejaculated semen in the testosterone-treated LuRKO males.¹⁶² This mouse transgenic model may hold some promise for future studies. Both hormone-induced and immunologically induced prostatitis may potentially be at work in this model.

Miscellaneous prostatitis models

Rat models of prostatitis

Gatenbeck *et al.*¹⁶⁷ have observed that long-term (10 days) stress stimuli (starvation, low surrounding temperature and small cage) were associated with inflammatory histopathological changes in prostatic tissue in male 9- to 10-week-old Sprague–Dawley rats. These stress stimuli were associated with marked nonbacterial ventral prostatic lobe inflammatory changes that were sparse in the dorso-lateral prostate.¹⁶⁸ Lang *et al.*¹⁶⁹ employed transurethral prostatic instillation of dissolved dinitrobenzenesulfonic (DNBS) acid in 50% ethanol in phosphate-buffered saline to develop novel model of abacterial prostatitis in 8- to 10-week-old male Sprague–Dawley rats. They demonstrated that ethanol/DNBS-mediated mucosal injury in the ventral prostate resulted in acute inflammation, which peaked between 24 and 48 h. In contrast with tumor necrosis factor- α , the interleukin-1 β appears to play a role both in the early and late phases of this local inflammation. Sharma *et al.*⁶⁴ reported that four of five (80%) of 13- to 14-week-old male Sprague–Dawley rats maintained on soy-free diet for 11 weeks developed severe inflammation only in the lateral prostate, while rats maintained on soy-containing diets or commercial rat chow did not develop any sign of prostatitis. Increased severity and incidence of lateral lobe prostatitis in rats maintained on the soy-free diet coincided with significantly diminished urinary excretion of various phytoestrogens. The authors speculated that dietary soy might play a protective role against the development of prostatitis in rats. Findings contradictory to the above were subsequently reported by Kwon *et al.*,¹⁷⁰ who studied prostatitis in 8-week-old male Sprague–Dawley rats. In this model, prostatitis was induced by 9 week of oral administration of soy bean extract mixture (isoflavone 300 mg/kg/day: 60% genistein and 20% daidzein) and was not accompanied by any changes in serum androgen levels.¹⁷⁰ Severe prostatic inflammation developed in more than 80% (15/18) of rats, characterized by neutrophil and lymphocyte infiltration in the dorso-lateral but not in the ventral prostatic lobes. The authors speculated that the prostatitis they observed might be attributable to the weak estrogenic effects of isoflavonoids compounds.¹⁷⁰ The differences in the findings in these two studies may indicate that long-term consumption of different doses of soy derivatives may have different effects on the prostate, related to the estrogenic effects of isoflavone. Thus, although the simplicity of this model is very attractive, the use of soy diet on its derivatives for the production of experimental prostatitis in rats may be problematic.

In another study, Takechi *et al.*¹⁷¹ demonstrated that lymphocytic infiltration and interstitial edema in the ventral prostate could be induced by partial mechanical obstruction of the urethra, being most prominent on day 3 in 12-week-old male Wistar rats. After the release of the nylon ligature from urethra, these prostatic inflammatory changes gradually disappeared. The authors speculated that intraprostatic urinary reflux and penetration of prostatic stromal tissue by low molecular weight substances in the urine may have been responsible for the development of prostatitis in this model.

The above studies attempted to determine which etiologic factors might contribute to the pathogenesis of

chronic nonbacterial prostatitis. Estimation of the advantages or limitations of these models is difficult because subsequent studies using these models were not carried out.

Conclusions

Significant progress in unraveling the underlying mechanisms of prostatitis has been made since the time of Young (1906).¹⁷² It is sobering to consider, however, that a recent prostate biopsy study demonstrated lack of correlation between prostatitis-like symptoms and identifiable chronic inflammation.¹⁷³ Acknowledging that prostatitis may have a polyetiological nature, several animal models with unique features are available for study. Further study of these models is warranted in view of increasing evidence that chronic prostate inflammation may play a role in the development of human prostate cancer.

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