Immunological Aspects of the Prostate Gland and Related Diseases

Mariana Maccioni, Virginia Elena Rivero, Ruben Dario Motrich, Gerardo Gatti, Juan Pablo Mackern Oberti, Virginia Andreani and Clelia Maria Riera*

Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

Abstract: It is surprising that despite the prevalence of prostate disease, little is known about the immunobiology of the prostate and its contribution to disease. The prostate is the target of many diseases like infection of the prostate gland, chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), benign hyperplasia and cancer. In this review we will focus on the relationship of the immune system with the prostate gland in two different scenarios: in the first one, the question of how prostate epithelial cells deal with inflammatory stimuli, like microorganisms, is addressed. Whereas the role of infiltrating innate immune cells like macrophages and neutrophils in prostate inflammation has been extensively studied, the particular contribution of prostate epithelial cells to an inflammatory setting has barely been investigated. Prostate epithelial cells can act as early sensors of infection, expressing Toll like receptors, the best well known innate immune receptors. Thus they become sentinels of the prostate gland, like the epithelial cells of other internal organs. The way these cells respond to infection up-regulating pro-inflammatory mediators is discussed. This topic is of particular interest since chronic inflammation has been postulated to be an important driving force to prostate carcinoma. In the second scenario, the situation in which the prostate gland becomes a target of an autoimmune response is analyzed. Chronic inflammatory prostate disease of noninfectious origin seems to be a good example of this situation, since at least 30% of the patients suffering from CP/CPPS exhibited IFN-γ-secreting, proliferating lymphocytes against prostate antigens such as prostatic acid phosphatase (PAP), prostate specific antigen (PSA), and other antigens present in prostate homogenates and seminal plasma. We have dissected the immunological mechanisms that are involved in these patients and in experimental models of autoimmune prostatitis as well as analyzed its consequences on the fertility of these patients.

Keywords: Prostatitis, toll like receptors, inflammation, autoimmunity, cancer.

PROSTATE EPITHELIAL CELLS (PECS) AS EARLY SENSORS OF INFECTION

Prostate: The Neglected Male Sex Gland

The prostate is one of the main male sex accessory glands that constitute the male genitourinary tract. Secretions from this gland help to preserve sperm viability and enhance fertilization capacity of spermatozoa among other several actions ranging from protection of the genital tract against infections to the induction of remodelling of the uterine mucosa in order to facilitate embryo implantation [1]. In spite of the many and important physiological functions of the prostate gland, it has only been the focus of attention of urologists and general practitioners because it is the target of many pathological conditions. Diseases of the prostate gland range from infections, CP / CPPS of a still unknown etiology, to benign hyperplasia (BPH) and prostate cancer (PCa) [2]. Prostate cancer is the second most frequently diagnosed cancer among men and the sixth leading cause of cancer death in men worldwide. Despite a wide array of therapeutic choices and the significant improvement occurred in the outcomes of many treatments and diagnostic approaches of localized PCa during last decade, limited data are available to indicate a clearly defined survival benefit with most current therapies [3]. Currently, neither uniform pathological diagnosis nor uniform tumour grading strategies are being used. This fact constitutes a big pitfall that leads to misdiagnosis and wrong management of patient care [4, 5]. Benign prostatic hyperplasia, and its clinical manifestation as lower urinary tract symptoms, is also a major health concern for aging men. An estimated 42% of men aged 51 to 60 have BPH, compared with over 70% of those aged 61 to 70, and almost 90% of those aged 81 to 90 [6]. There have been significant advances in the diagnosis and treatment of BPH in recent years. Benign prostatic hyperplasia is an enlargement of the prostate gland from the progressive hyperplasia of stromal and glandular prostatic cells [6], which is characterized histologically by the presence of discrete nodules in the periurethral zone of the prostate gland [7]. However, the etiology of BPH leading to an enlarged prostate is still unknown [8]. Several hypotheses have been proposed to describe its etiology but none has been proven to contribute to BPH including endocrine/inflammatory alterations, sexual activity, alcohol, genetic factors and age [9]. Unlike BPH and PCa, 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 [10]. Moreover, prostatitis accounts for more office visits than either BPH or PCa. Infectious agents such as bacteria like Chlamydia or other microorganisms are implicated only in 5-40% of cases of prostatitis [11]. Thus, the remaining majority 60-95% cases of clinical prostatitis (NIH categories IIIA and IIIB or CPPS) have a still unidentified etiology that may involve (i) hormonal imbalance; (ii) autoimmunity; (iii) neurological

*Address correspondence to this author at the Centro de Investigaciones en Bioquímica Clínica e Inmunología, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, 5000 - Córdoba, Argentina; Tel: 54 - 351- 433 4164, Ext. 123; Fax: 54 - 351 - 433 3048; E-mail: criera@bioclin.fcq.unc.edu.ar

1573-3955/10 $55.00+.00 © 2010 Bentham Science Publishers Ltd.

dysfunction; (iv) urinary reflux into the prostate; (v) inappropriate cytokine release and/or (vi) α-adrenergic system abnormalities [11]. Patients typically have repeated episodes of prostatitis, therapy is 'hit or miss' and quality-of-life may be significantly diminished. Furthermore, there is growing awareness that chronic prostate inflammation may play a role in the development of PCa although this is a matter on debate [12].

All these data evidence that prostate gland study has been neglected during last 50 years. Little is still known about prostate physiology in the male genital tract and, although a considerable advance has been shown during last decade, prostate disease diagnosis and management have several pitfalls to be solved.

Hallmarks in Prostate History

In ancient Greek the masculine term "*prostatēs*" meant "president" and was exclusively used in a non-medical sense. The similarly sounding word "*prostatēs*" was used in ancient times to indicate a part of the male reproductive system (the testis or epididymides and/or the spermatic ducts) that played an important "helping" role in procreation [13]. Herophilus, a physician from Alexandria, who made dissection of human corpses, was the first one in mentioning the human prostate. He described the *parastatai adenoides* and *parastatai kirsoeides*, but he confused the prostate with the seminal vesicles and seminal ducts with the bilateral prostate of some animals. No medical text from Byzantine or Islamic period even mentions the unpaired prostate organ because of the research practices of the day. The first to precisely and exactly described the prostate was Nicolo Massa, a Venetian physician, who in 1536 showed the anatomical localization, its relationship with the bladder neck, seminal vesicles and ducts and its macroscopic anatomy. During this Renaissance period, anatomists named the organ "glandulous body". It was the French physician Du Laurens who introduced the metaphoric denomination "prostatae", to designate what he believed was a double organ. Only in the 1800s this anatomical error was corrected [13]. Now we know that a healthy human prostate is slightly larger than a walnut, it surrounds the urethra just below the urinary bladder and its secretion comprises 25-30% of the volume of semen.

Immunobiology of the Prostate

Neglected by most and underestimated in its significance, the immunology of the prostate has recently developed into a new field of research in urology. Twenty years ago, the prostate gland was considered to be an immunologically privileged organ based on the lack of afferent lymphatic vessels [14] the high incidence of occult carcinoma [15], and the immunosuppressive properties of seminal plasma [16, 17]. However, progress made in the field during the last two decades has completely changed this concept. It is nowadays accepted that the prostate is capable of initiating an innate immune responses which in turn will activate specific lymphocytes in order to keep the reproductive tract sterile [18]. The immune system of the prostate develops gradually during gestation. The presence of T lymphocytes in the fetal human prostate can be found as early as the week 12 of gestation, with a subsequent peak between the weeks 21 and 30 [19, 20]. They are predominantly distributed intraglandularly between epithelial cells (ECs). As early as in this stage of gestation, the immune system of fetal prostate mirrors some of the immunologic features observed in the normal adult prostate: the prevalence of CD8+ over CD4+ T lymphocytes, the co-expression of CD3 in association with the TCR α/β, but rarely with the TCR γ/δ and the presence of HLA-DR+/CD11c+ macrophage like cells [19].

The adult human prostate, defined as the prostate in 18-25-yr-old individuals and with a volume around 20 cc, contains only small numbers of T and B lymphocytes, macrophages and mast cells [21]. Lymphocytes are more numerous in the stroma than within the epithelium. T lymphocytes account for the 90% of total lymphocytes from both stromal and intraepithelial compartments. It has been shown that CD8+ suppressor/cytotoxic T lymphocytes prevail over CD4+ helper T cells and are evenly distributed throughout the interstitium and in between the ECs [19]. However, other reports contradicts these data showing that, although lymphocytes are in fact more numerous in the stroma than within the epithelium and that they are chiefly T cells in both compartments, stromal T cells are mainly helper/inducer cells with a clear prevail of CD4+ T cells over CD8+ T cells [22]. Nevertheless, these authors reported, in agreement with previous data, that intraepithelial T cells are mainly cytotoxic/suppressor CD8+ T cells [22]. The reason of the prevalence of CD8+ T cells in the intraepithelial compartment of the prostate is still unknown, but this finding was also shown in the epithelium of the epididymis [23]. Most probably the CD8+ T-cell subset forms a major immunologic barrier that under normal circumstances prevents the development of cellular autoimmune responses to sperm and other prostatic antigens [24]. B lymphocytes are also present and their distribution is similar to that of the T lymphocytes but in much less proportion. Macrophages are much less abundant than B lymphocytes, especially in the stromal compartment. In most cases, macrophages occurred in aggregates in the periglandular infiltrates [25]. IFN-γ, IL-1β, IL-8, IL-15 and IL-17, among other cytokines and pro-inflammatory mediators, were shown to be produced in normal human prostate homogenate [21], a fact that reflects the immunocompetence of the prostate gland. Although the knowledge of the immunobiology of the prostate gland seems to have experienced an improvement, we are far away from the current understanding of mucosal immune systems as the gastrointestinal mucosal immune system. Intense research is promptly required to better understand the immunobiology of the normal human prostate.

Toll Like Receptors (TLRs) and Prostate Epithelial Cells

Toll like receptors conform a family of receptors that mediates the recognition of pathogen-associated molecular patterns (PAMPs), which have been evolutionarily conserved in specific classes of microbes [26]. Each TLR recognizes a different PAMP and the binding of these PAMPs to TLRs triggers a complicated series of biochemical events involving adapter proteins like MyD88, MAL, TRIF and TRAM that ultimately will lead to the activation of transcription factors such as nuclear factor-κB (NF-κB), AP-

1 and IRF3 [27]. The consequence of this signaling cascade is an augmented expression of pro-inflammatory cytokines, chemokines and effector molecules. The response to PAMPs is not unique to immune cells, but it is also elicited by a variety of hematopoietic and nonhematopoietic cells [28, 29]. Best characterized in this respect is the response to lipopolysaccharide (LPS), an endotoxin from the outer envelope of Gram-negative bacteria, which is recognized by TLR4 [30]. Lipopolysaccharide can induce pro-inflammatory cytokine production in a large variety of cell types including epithelial cells, adipocytes, keratinocytes and smooth muscle cells [28, 29]. Interestingly, inflammation can also be triggered in sterile conditions arguing for the existence of PAMP-independent pathways of the immune response. Aberrant exposure of normal cell constituents, like hyaluronan fragments, fibronectin, high-mobility group box 1 and heat shock proteins can signal through TLR4, promoting tissue repair but also representing a potentially hazardous situation if they are leaved uncontrolled [31-35].

Epithelial cells have classically been considered important players in innate immune defence mechanisms, merely acting as physical barriers capable of secreting some antimicrobial substances when disrupted. At present, there are a compelling number of reports showing that ECs can recognize bacterial products through the family of TLRs, a fact that confers ECs a more protagonic role [36]. Epithelial cells may, therefore, contribute to the initiation of the immune response. Indeed, a study done by Nolte *et al*. [29], using a chimeric system in irradiated mice, which allows to eliminate hematopoietic cells, showed that recognition of LPS by the radioresistant cell compartment (among which are the ECs) was sufficient to induce local and systemic inflammation, with production of inflammatory cytokines and chemokines, which can recruit and activate immune cells.

Toll like receptors have been shown to be expressed in mucosal epithelia, such as intestinal [37-41], tracheo-bronchial [42], renal [43, 44], bladder [45], oral [46], and ocular ECs [47]. TLR 1-10 as well as many essential TLR-related genes products are expressed throughout the male reproductive tract, in the testis, epididymis, vas deferens and accessory glands of the human and/or laboratory rodents [48-51]. For instance, Sertoli cells express TLR2, 4, 5, and 6. Stimulation of Sertoli cells with macrophage-activating lipopeptide-2, agonist of TLR2/TLR6, and with flagellin, agonist of TLR5, induces augmented secretion of the chemokine MCP-1 [49]. The abundant expression of the majority of TLR family members together with expression of TLR adaptors and activation targets provides strong evidence that TLRs play important roles in innate immunity of the male reproductive tract.

Although the prostatic gland normally preserves a sterile environment, bacterial infection of the prostate gland may occur as a result of ascending urethral infection or by reflux of infected urine into prostatic ducts emptying into the posterior urethra [52]. Other possible routes of infection include invasion of rectal bacteria through direct extension or by lymphogenous or hematogenous spread. In the prostate, as in other organs of the genitourinary system, the ECs are the first cells that contact with pathogens. Therefore, efforts in improving the current knowledge about how PECs respond to inflammatory stimuli such as microorganisms or their products are necessary. Several studies have shown that *Escherichia coli* [53] and non-*E. coli* strains like *Chlamydia trachomatis, Pseudomonas aeruginosa, Serratia spp., Klebsiella spp., Proteus spp., Enterococcus spp., Neisseria gonorrhoeae, Mycobacterium tuberculosis, Salmonella spp., Clostridium spp* and parasitic or mycotic organisms are able to induce acute and chronic bacterial infection/inflammation within the prostate [54,55]. Although the vast majority of men with prostatitis have the nonbacterial variant, a small number of men have acute or chronic bacterial prostatitis [52]. Two causative organisms frequently associated to both conditions are enterobacteria, particularly *E. coli* and *C. trachomatis* [52].

The expression of TLR4 in the prostate has been addressed in several animal models. Studies revealed a constitutive expression of TLR4 in the ECs of rat ventral prostate [56] as well as in a rat adenocarcinoma cell line and in prostate primary cell cultures [57, 58] (Table **1**). In addition, an up-regulation of TLR4 expression in both stromal and ECs was found in castrated rats or in rat cell lines stimulated with TLR4 agonists [56, 57].

Prostate primary cell cultures have been shown to be a useful tool to address the role of ECs against inflammatory stimulus. *In vitro C. trachomatis* infection of these cultures induces the secretion of cytokines and chemokines. Although these cultures contain a small percentage of contaminating CD45+ cells (which comprise total leukocyte population), when they are eliminated, the secretion of cytokines and chemokines was still observed indicating that ECs and other non-leukocyte cells can respond to this pathogen. Furthermore, when primary prostate cultures were performed using prostate from MyD88 deficient mice, results showed that the absence of this adaptor molecule on CD45+ and CD45- cells eliminate the ability of these cells to respond to *C. trachomatis* (manuscript under submission).

Prostate epithelial cells also up-regulate the levels of TLR4, CD14 and the adaptor molecule MyD88 in response to *Chlamydia muridarum* infection, recruiting these molecules to the vicinity of the bacterial inclusion membrane [58]. Ligation of TLR4 to LPS on PECs activates NF-κB, induces the expression of iNOS, and the consequent secretion of nitric oxide. Even more, the expression of numerous chemokine and cytokine genes is up-regulated or induced in prostate cell lines as well as in normal PECs, showing that PECs are competent to recognize the presence of *E. coli* in the prostate gland and to contribute to the initiation of the immune response [57]. The role of TLR4 in the control of the prostate infection has also been addressed by *in vivo* experiments using *Tlr4* knockout mice. Indeed, wild type mice have non significant prostate infection 5 days after intraurethral inoculation with uropathegenic *E. coli*, while *Tlr4* deficient mice have severe symptoms of infection [59]. Whereas this study highlights the role of TLR4 in the defence mechanisms of the prostate, the particular contribution provided by TLR4 present on resident innate immune cells or on PECs was not considered.

The expression of TLR4 in the human prostate gland has been reported, and the differential expression of TLR4 at the stromal and epithelial cell compartments in different prostatic human samples has been recently analyzed by our

Table 1. Expression of TLRs in the Prostate

	TLRs	TLR Stimulus	Refs. as in Text
Human			
Prostate cancer cell line			
	TLR2 [a]	Mycoplasma	[63]
	TLR4 [a]	LPS	[53, 62]
	TLR9 [a]	CpG	[64]
Normal prostate tissue			
	TLR1-10 [b]		[48]
Benign prostatic hyperplasia tissue	TLR4, 5, 7, 9 [b]		[60]
Rat			
Primary prostate epithelial cells	TLR2, 4[a]	Chlamydia, LPS	[56-58]
Prostate adenocarcinoma cell line	TLR2, 4[a]	LPS	[57]

[a] Protein expression, [b] mRNA expression.

group [60]. König *et al.* [61] demonstrated the presence of TLRs 4, 5, 7, and 9 mRNA in BPH tissue. Also, many associated genes, including MyD88, TRIF, TIRAP/Mal and CD14 (a key molecule involved in the recognition of LPS), are expressed in human prostate [48, 60, 61]. Whereas the expression of TLR4 mRNA in tissue samples was demonstrated, no discrimination between its particular expression in stromal or ECs was performed. Our results show that the main site of expression of TLR4 in the normal prostate gland is the stroma [60]. However, the epithelial layer also exhibits TLR4 expression at lesser extent and is capable of responding to TLR4 agonists [60]. At protein level, TLR4, TLR2 and TLR9 have been shown to be expressed by human PCa cell lines (Table **1**) [60-64]. Our results from the human prostate adenocarcinoma cell line, DU-145, reveal that stimulation of these cells with TLR4 agonists activates NF-κB and induces the expression of pro-inflammatory cytokines [60]. This activation is specific and the MyD88-dependent pathway is involved, indicating once again that PECs are able to respond to TLR4 agonists. The expression of TLR2 has also been detected in a human prostate adenocarcinoma cell line. Stimulation with *Mycoplasma hominis* induced activation of NF-κB and IL8 secretion through TLR2-dependent mechanism, suggesting that PECs can also promote inflammation in response to *Mycoplasma* in the prostate [63].

In summary, PECs recognize TLR2 and 4 ligands inducing an inflammatory immune response. Activation of an early immune response by PECs could be a key mechanism to ensure effective elimination of the organism, preventing the progression of infection to restricted sites of the male genital tract, including epididymis and testis. Prostate is fully competent to react upon the invasion of environmental antigens. This is not surprising since, as other organs exposed to regular contacts with foreign antigens (e.g. lungs), it has developed specific defence mechanisms in order to preserve the gland integrity and functionality.

Toll Like Receptors, Inflammation and Prostate Cancer

As the percentage of elder population rises in Western countries, the number of males with PCa experiences an alarming increase. PCa is one of the major causes of death in western population, being the most common non-skin malignant neoplasm in men in industrialized countries [65]. The pathogenesis of PCa, as well as of other cancer types, involves heritable and enviromental factors [66]. Among these environmental factors, chronic inflammation has been postulated to be an important driving force to PCa [67]. Causally evidence implicates chronic inflammation in the development of a diverse range of human cancers, like esophagus, lung, pancreas and stomach cancer [67]. Inflammation increases cell proliferation and therefore mutation and cell and genome damage. Recently, direct evidence of malignant transformation of BPH human cells by *Mycoplasma* has been reported [68]. Causes of prostate inflammation that have been proposed as etiological agents of PCa are direct infection by sexually transmitted organisms (like *C. trachomatis* or Gram negative bacteria like *E. coli*, *Mycoplasma spp.*), chemical irritation from urine reflux, autoimmunity and trauma [68, 69]. Epidemiological studies show significant association between infection and inflammation and PCa. Moreover, men with a previous history of certain sexually transmitted infections have a higher incidence of PCa than age matched controls [70].

In this inflammatory setting, TLRs represent a central piece. Recently, sequence variants in several TLR genes have been linked to PCa risk, including *Tlr4* and the *Tlr1_6_10* cluster [71-74]. Although the biological consequences of these risk variants have not been investigated, the association of PCa risk and polymorphisms in this family of genes, which is so intimately related to innate immunity, indicates that an inflammation related process could be important in PCa development.

Much has been said about the role of innate immune cells in inflammation, but little is known about the particular contribution from epithelial cells in an inflammatory setting. As key players in innate immunity to pathogens, TLRs have been under the focus of recent research directed to understand the role that these receptors play in cancer. Whereas the importance of TLR ligands as adjuvants to promote effective immune response against tumors is unquestionable [75], controversy exists regarding the

consequences of activating TLRs present on tumor cells themselves [76]. Several reports suggest that TLR agonists directly stimulate tumor cell proliferation and resistance to apoptosis as well as an increment in tumor cell invasion and metastasis [77, 78]. These findings support the hypothesis that TLR signaling on tumor cells promotes tumor growth. However, there are also reports that claim that under certain conditions TLR-signaling on tumor cells could be beneficial to the anti-tumoral immune response [79, 80].

Our group has recently demonstrated that in the normal human gland, TLR4 expression was detectable mainly on stromal cells, composed of muscular fibers and fibroblasts. A consistent expression in the basal epithelial cell compartment in a lower percentage of samples was also detected. Interestingly, a decay of TLR4 expression in the gland as long as the histopathological grade of the tumor increases was also observed. As the tumor becomes less differentiated the amount of stroma is reduced, therefore it is reasonable to think that TLR4 expression in the stroma could virtually disappear. Nevertheless, the same pattern of decreased staining was also observed in the epithelial cell compartment [60].

Interactions between the epithelium and stroma in the adult prostate appear to regulate normal growth homeostasis. Alterations in the stromal-epithelial dialogue that recapitulate features of the mesenchymal-epithelial interactions during development may play a critical role in the development and progression of PCa [81, 82]. It could be hypothesized that in a situation of chronic inflammation, a sustained stimulus could continuously activate the prostate stroma (and at a lesser extent the epithelium) through TLRs, promoting the secretion of pro-inflammatory mediators with the consequences already discussed elsewhere. The fact that TLR4 expression gradually diminishes as the tumor becomes more aggressive could be a consequence of the loss of differentiation that accompanies the process. Other receptors, like the androgen-receptors, which are key molecules in the functioning of the prostate, are also lost in this process. It may also be supposed that TLR4 expression could be somehow regulated by unknown factors that are also involved in PCa progression.

Taken together, our data allow us to hypothesize that TLR4 in prostate cells could synergize with innate immune cells contributing to an eventual inflammatory process, which in genetically prone individuals could promote carcinogenesis.

PROSTATE AS A TARGET OF THE AUTOIMMUNE RESPONSE

Non-Infectious Chronic Prostatitis

Prostatitis is a highly prevalent disease in men [10, 83]. Prostatitis is often described as an infection of the prostate, but it can also be an inflammation with no sign of infection. Just 5% to 40% of cases are caused by bacterial infection [11]. While acute prostatitis is commonly caused by bacterial infection, most patients with CP/CPPS have no evidence of urinary tract infection. Chronic prostatitis is a common disease of unclear etiology. The diagnosis of this syndrome still relies mainly on reported pain in the pelvic zone, perineum, rectum, and/or prostate by affected men. Chronic pelvic pain syndrome often relapses and remits without clear triggers.

The CPPS has no defined treatment and the therapy used for this disease is nonspecific and usually involves empiric treatment with antibiotics and α-adrenergic blockers whose efficacy is not clear. Accumulating evidence suggests that CPPS could be an autoimmune disease of the prostate [1, 84]. In some patients with noninfectious CPPS, elevated levels of inflammatory cytokines in seminal plasma or expressed prostate secretion have been detected [85, 86].

Central and Peripheral Tolerance to Prostate Antigens

It has been extensively demonstrated that prostate antigens together with a wide spectrum of tissue-specific autoantigens are presented in the thymus and drive central tolerance. The presence of specific mRNA for numerous tissue-specific proteins has been described in medullary thymic epithelial cells [87]. This promiscuous expression plays an important role in the establishment of central tolerance to peripheral antigens. Lack of tolerance to prostate antigens as well as other tissue-specific antigens, including testis, thyroid, pancreas and stomach antigens, has been reported in animals deficient for the transcription factor called AutoImmune REgulator (AIRE) [88]. AIRE shows high expression in medullary thymic epithelial cells and its deficiency generates autoimmunity against different organs, including parathyroid and / or adrenal glands, ovaries, stomach and also prostate. Indeed, it has been shown by Anderson *et al.* [88] that prostate-specific antigens, such as spermine-binding protein, are expressed in the thymus, its expression being regulated by AIRE [88]. In a further work, the same authors determined the role of central tolerance in the development of spontaneous prostatitis [89]. They identified a prostate-specific protein, seminal vesicle secretory protein 2 (SVS2), against which reactive $CD4^+$ T cells and antibodies develop spontaneously in *Aire*-KO mice. They also demonstrated that thymic expression of this autoantigen is AIRE dependent, and also showed that immunization with SVS2 breaks tolerance to this antigen and induces autoimmune prostatitis in *Aire*-sufficient wild type mice, demonstrating that central tolerance to this antigen was incomplete in wt-mice. Furthermore, they showed that patients with CPPS show immune responses to semenogelin, the human counterpart to SVS2, speculating that these results may provide new approaches to the diagnosis and treatment of CPPS [89].

An alternative mechanism of inducing self tolerance in the thymus might be the signals for generation of T regulatory cells (Tregs) in response to localized antigen presentation, resulting in a more robust regulation of autoreactivity [90]. These Tregs are generated in a burst of activity during early stages of fetal and neonatal T-cell development. The existence of these natural Tregs is clearly demonstrated in murine models of neonatal thymectomy [90]. Studies on CD4+ CD25+ Tregs with transgenic T-cell receptors indicate that Tregs may receive continuous antigen stimulation in the periphery. However, the consequence of this antigen encounter and its relevance to physiologic polyclonal Tregs function are not established. During the past 10 years, CD4(+)CD25(+)Foxp3(+) Tregs have been

extensively studied for their function in autoimmune disease. Suri-Payer *et al.* [91] presented evidence for a role of Tregs in suppression of innate and adaptive immune responses in experimental models of autoimmunity including arthritis, colitis, diabetes, autoimmune encephalomyelitis, lupus, gastritis, oophoritis, prostatitis, and thyroiditis. Regulatory T cells suppress conventional T cells by direct cell contact *in vitro*. However, downmodulation of dendritic cell function and secretion of inhibitory cytokines such as IL-10 and TGF-β might underlie Tregs function *in vivo*. The final outcome of autoimmunity *vs* tolerance depends on the balance between stimulatory (TLR engagement, costimulation, and antigen dose) and inhibitory signals.

In the day-3 thymectomized (d3tx) mice occurs organ-specific autoimmune diseases, which affects the stomach, thyroid, ovaries, testes and prostate. This lack of tolerance to prostate antigens is due to the loss of a thymus-derived regulatory T-cell population that becomes activated in the periphery [92]. In experimental autoimmune prostatitis (EAP) in the d3tx mice, Setiady *et al.* [93] demonstrated that the superior EAP-suppressing function was acquired after a 6-day exposure to prostate antigens in the periphery, unaffected by sex hormones. Thus, a brief exposure of physiologic prostate antigens capacitates peripheral polyclonal Tregs to suppress EAP.

Experimental Autoimmune Prostatitis

Animal model(s) might be useful in elucidating mechanisms involved in the molecular pathogenesis of chronic nonbacterial prostatitis and CPPS syndrome. Given that prostatitis might have a multifactorial etiology, several animal models with unique features may prove helpful [2]. In the last years, the group of Gupta [94] examined in a review a number of experimental rodent models of prostatitis and evaluated their advantages and limitations. Also, Zhou *et al.* [95] immunized male rats with prostate tissue homogenate supernatants with complete Freund's adjuvant (CFA) and observed that there is a remarkable up-regulation of TNF-α expression. The results of histopathology and iNOS expression analysis also revealed the same tendency. In a recent work, Kim *et al.* [96] induced non-bacterial prostatitis using 17β-estradiol and dihydrotestosterona demonstrating that oligomerized polyphenol has a beneficial effect on prevention and treatment of the disease. Over the past decades, our laboratory has developed rat and mouse models of EAP that are characterized by organ specific mononuclear infiltration and cellular and humoral autoimmune response against prostate antigens [2]. In the rat model, infiltrating mononuclear cells appear approximately at day 7 after priming. The main isotypes involved in this autoimmune phenomenon are IgG2b and IgG2c. A strong association between the cellular autoimmune response measured by the DTH response and the IgG2b and IgG2c isotype is found at early stages of the disease. Since the DTH response and the IgG2b isotype have been previously associated with Th1-like activity in rats, our results suggested that Th1-like cells could be playing an active role in early stages of this disease in rats [97]. Further studies were performed using non-obese diabetic mice (NOD). NOD mice are a well characterized model of type-1 diabetes, and also develop spontaneous manifestations of organ-specific diseases affecting salivary and lachrymal glands, thyroid, parathyroids and the adrenal cortex in the course of their lives. Interestingly, NOD mice develop spontaneous autoimmune prostatitis with advanced age [2]. Immunization of young NOD males with prostate extracts in CFA, accelerates the disease, causing a severe inflammatory reaction in the prostate. Florid infiltrates circumscribed to lateral and dorsal prostatic lobes are observed in these animals, accompanied by a humoral and T cell-mediated response. Immunized mice develop a T cell-mediated response to prostate antigens assessed by *in vitro* proliferation and accompanied by the release of IFN-γ, whereas IL-4 is not detectable in the same culture supernatants [98]. The experimental disease is characterized by prostate-specific IFN-γ-secreting cells in periphery and Th1-related cytokines in the target organ. Increased IFN-γ and IL-12 levels are observed in prostates from autoimmune animals, while IL-10 and IL-4 levels are decreased or unaltered, respectively. Mice deficient in factors involved in IFN-γ signalling cascade (such as IRF-1 and STAT-1) are resistant to the development of EAP. These mice show no infiltration or alterations in the prostate [99]. In addition, Penna *et al.* [100] showed that the EAP in NOD mice is markedly delayed in IFN-γ-deficient NOD mice. They also showed that Th1-type CD4 (+) T cells are able to transfer autoimmune prostatitis into NOD.SCID recipients.

The identification of the target antigens in autoimmune diseases is always an important step towards understanding the etiology of this group of pathologies. We purified one of the main prostatic autoantigens recognized by the humoral autoimmune response. Amino acid sequence analysis identified the purified protein as prostatein or rat prostatic steroid binding protein (PSBP), a member of the uteroglobin superfamily. We also examined its ability to induce autoimmune prostatitis in male Wistar rats and in NOD mice, and concluded that, when incorporated into an adequate adjuvant, PSBP elicits cellular and humoral autoimmune responses and lesion in the prostate gland [101, 102]. Liu *et al.* [103] also showed the same results and proposed PSBP as a target antigen for vaccine and T cell therapy against PCa.

All together these results argue for a putative role of autoimmunity in the pathogenesis of the disease and encourage to intense research in order to better understand the etio-pathogenic mechanisms underlying CP/CPPS.

Chronic Prostatitis/Chronic Pelvic Pain Syndrome and Autoimmunity

In the last decade, evidence indicating an autoimmune component in the pathogenesis of CP/CPPS has begun to emerge [104-107]. Autoreactive $CD4^+$ and $CD8^+$ T cells specific for prostate antigens exist in normal individuals [108]. Moreover, T cells from CP/CPPS patients proliferate in response to seminal plasma [109-111] and to specific prostate antigens indicating expression of the autoreactive T cell repertoire in disease pathogenesis [112, 113]. High titer IgG autoantibodies to prostate-disease-associated proteins are found in patients with CP/CPPS [114]. In addition, patients with a clinical diagnosis of CP/CPPS show higher levels of pro-inflammatory cytokines, like IL-1β, IL-6, and TNF-α, compared to controls, in seminal plasma [115-118].

Recently, it has also been documented significantly increased levels of several chemokines in seminal plasma form these patients [85].

A group of patients with infectious and non-infectious chronic prostatitis was analyzed by our lab, in order to search for the presence of a possible autoimmune response to prostate antigens [112]. The presence of lymphocytes able to proliferate in response to known human prostate antigens such as PSA and PAP was demonstrated in a group of patients with non-infectious chronic prostatitis. We observed that, as in other autoimmune diseases, a proliferative response against two or more autoantigens was a common feature. Moreover, when IFN-γ and IL-10 levels were measured in culture supernatants, significantly elevated levels of IFN-γ were detected only in samples from patients with positive proliferative response to prostate antigens. Interestingly, only these patients showed significantly elevated levels of inflammatory cytokines (IL-1 and TNF-α) in seminal plasma, arguing for a local inflammation of non-infectious cause. Our results show that IFN-γ secreting lymphocytes specific to prostate antigens are in fact detected in 34% of the patients with chronic non-infectious prostatitis. We speculate that these cells could be involved in the inflammatory process taking place in the prostate gland and therefore could alter its biological function [112]. Recent data by Kouiavskaia *et al.* [113] support these findings. These authors described specific PAP and PSA antigenic epitopes that are preferentially recognized by the immune system from CPPS patients.

To better understand CP/CPPS, Rudick *et al.* [119] used a murine experimental autoimmune prostatitis model to examine the development, localization, and modulation of pelvic pain. Pelvic pain is detected 5 days after antigen instillation and is sustained beyond 30 days. The pain is attenuated by lidocaine treatment into the prostate, but not into the bladder or the colon, suggesting that pain actually originates in the prostate. Experimental autoimmune prostatitis histopathology is confined to the prostate with focal periglandular inflammatory infiltrates in the ventral, dorsolateral, and anterior lobes of the mouse prostate. Inflammation and pelvic pain are positively correlated and increase with time. Morphologically, only the dorsolateral prostate shows significantly increased neuronal fiber distribution. Pelvic pain is attenuated by treatment with the neuromodulator gabapentin, suggesting spinal and/or supraspinal contribution to chronic pain. These results provide the basis for identifying mechanisms that regulate pelvic pain and the testing of therapeutic agents that block pain development in CP/CPPS [119].

Consequences of Prostate Autoimmunity on Semen Quality

The relationship between chronic prostatitis and fertility has been controversial for many years. Batstone *et al.* found that there are T cell responses to seminal plasma in CP/CPPS patients [110]. Severe abnormalities are seen in patients with no evident infection and with an autoimmune response against prostate antigens. Moreover, significantly increased levels of pro-inflammatory cytokines are detected in seminal plasma from these patients. Besides, chronic prostatitis patients with cellular autoimmune response to prostate antigens present important alterations in their semen quality parameters. Therefore, it was hypothesized that an autoimmune response against prostate antigens and the consequent inflammatory process, may affect male fertility [120]. To investigate when, where, and how sperm cells from autoimmune prostatitis individuals are being damaged, we took advantage of the experimental model of EAP in Wistar rats. A marked reduction in sperm concentration, almost null sperm motility and viability, and an increased percentage of apoptotic spermatozoa are detected in samples from animals with the disease. Prostate-specific autoantibodies as well as elevated levels of NO, TNF-α, and IFN-γ are also detected in their seminal plasma. In contrast, epididymal spermatozoa remain intact, indicating that sperm damage occurs at the moment of joining of prostate secretion to sperm cells during ejaculation. These results were further supported by experiments in which a mixture of normal sperm cells with autoimmune seminal plasma was performed [121]. Further on, the putative direct consequences of autoimmunity to the prostate on male semen quality and fertility potential was analyzed. A significant decrease in the fertility index and augmented percentages of embryo loss are observed in the autoimmune group. These results allow us to conclude that an autoimmune response to the prostate impairs its functionality and induces important alterations in seminal oxidative balance, producing higher levels of lipid peroxidation and sperm DNA damage [122].

Searching for New Therapeutic Alternatives: Taking Advantage of the Vitamin D Endocrine System

The prostatitis syndromes are among the most common and frustrating clinical challenges for urologists in outpatient practice. Available therapy, especially for the chronic prostatitis syndromes, is poor, with no standard treatment producing significant cure rates. Currently corticosteroids are not a standard treatment option in CP/CPPS. Theoretically, experimental and scarce clinical evidence suggest that treatment with low dose corticosteroids could be beneficial in some patients with chronic prostatitis [123]. In the last years, with the advances in the knowledge of mechanisms of the immune response, the treatments of diseases with immune compromise became more rationale. The vitamin D endocrine system is involved in a variety of biological processes able to modulate immune responses, and the tolerogenic properties of vitamin D receptor (VDR) agonists render this class of compounds particularly suitable for the treatment of autoimmune diseases [124-127]. Treatment of EAP by a VDR agonist represents a novel application for these hormone-like agents. Work done by Penna *et al.* [128] shows that treatment with normocalcemic doses of the VDR agonist Elocalcitol for 2 weeks in already established EAP is able to significantly inhibit the intraprostatic cell infiltrate, leading to a profound reduction in the number of infiltrating leukocytes, whereas dexamethasone administration is much less effective. Interestingly, in NOD mice with established EAP, numerous leukocyte infiltrates are observed adjacent to the ganglia of the prostate peripheral nervous system, whereas ganglia are mostly free of infiltrating cells in mice treated with Elocalcitol, suggesting a possible effect on the pain component of autoimmune prostatitis.

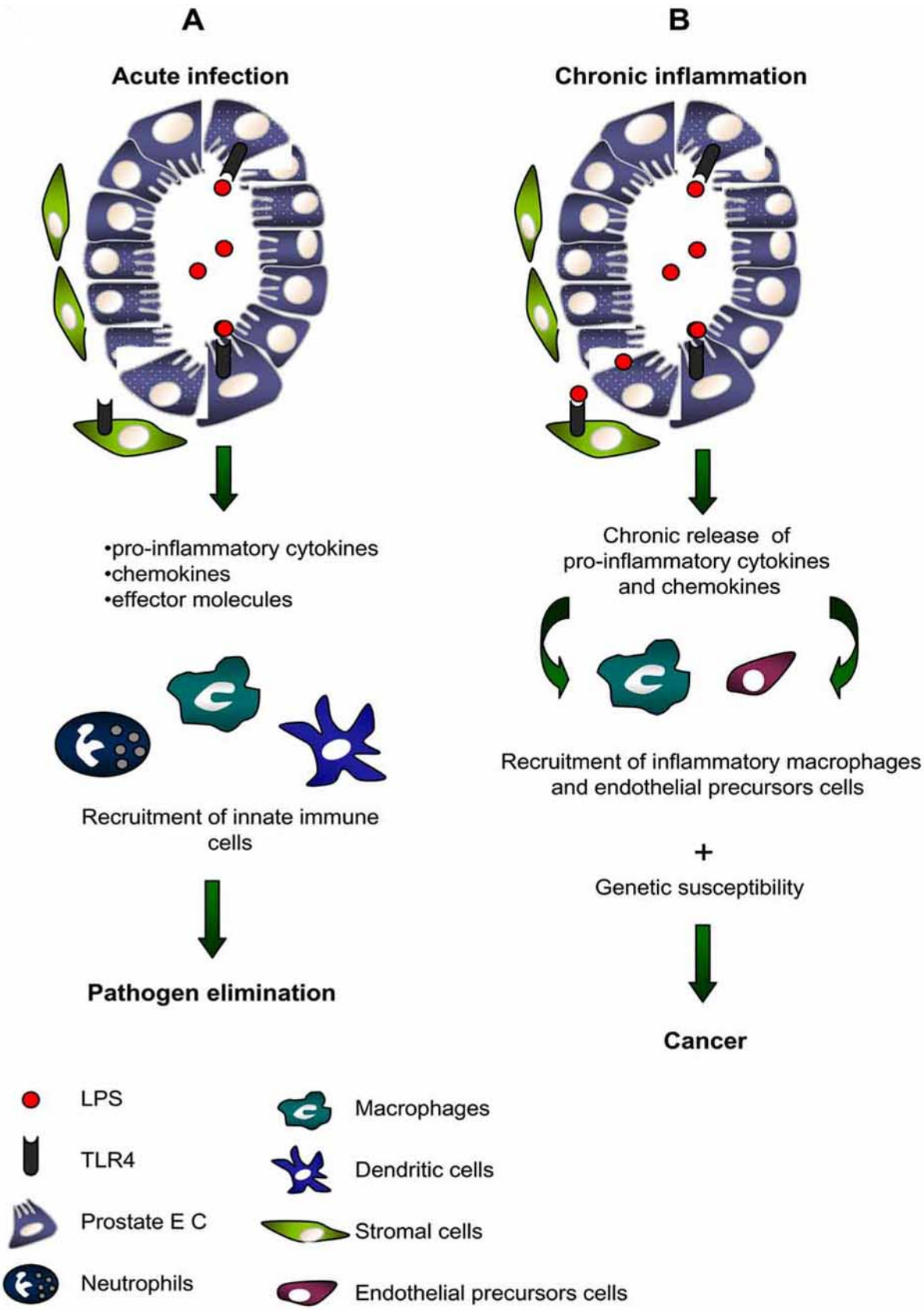

Fig. (1). Proposed role of Toll like receptors (TLRs) in the prostate gland. **A**) TLR4 is expressed on PECs and on prostate stromal cells. PECs can recognize LPS (TLR4 ligand) at early stages of infection, triggering an early inflammatory response that could ensure effective elimination of microorganism and prevent the progression of infection. **B**) If it turns into a situation of chronic inflammation, it becomes a potentially hazardous situation, increasing the risk of malignant transformation and cancer.

In a later work, we investigated the impact of vitamin D receptor silencing by using VDR-deficient NOD mice and the immune-modulating effect of the vitamin D3 analog TX527 on the development of EAP. VDR-deficient NOD mice develop a more aggressive form of autoimmune prostatitis characterized by a greater lymphoproliferative response against prostate antigen *in vitro* and higher levels of specific IFN-γ secretion. This is accompanied *in vivo* by more severe lesions and augmented mononuclear cell infiltration in the prostate gland. On the other hand, although analog-treated mice show a significant reduction in the spleen T-cell specific proliferative response against prostate antigen *in vitro*, no effect on disease development is observed. Therefore, vitamin D receptor modulation holds the promise of interfering with autoimmune prostatitis. Introduction of more powerful analogs or combinations with anti-T-cell reagents may represent therapeutic solutions for this group of patients [129].

CONCLUSIONS

Although the knowledge of the immunobiology of the prostate gland seems to have experienced an improvement, we are far away from the current understanding of mucosal immune systems as the gastrointestinal mucosal immune system.

The prostate gland normally is preserved in a sterile environment; however, bacterial infection of the prostate gland may occur as a result of ascending urethral infection or by reflux of infected urine into prostatic ducts emptying into the posterior urethra. As in other organs of the genitourinary system, PECs are the first cells that contact with pathogens. TLRs, important innate immune receptors, are expressed on PECs and on prostate stromal cells. PECs can recognize TLR ligands at early stages of infection, triggering an early inflammatory response that could be a key mechanism to ensure effective elimination of the organism and to prevent the progression of infection to restricted sites of the male genital tract, like epididymis and testis. The prostate is therefore fully competent to react upon the invasion of environmental antigens (Fig. **1A**). However, if this process gets uncontrolled and it turns into a situation of chronic inflammation, it becomes a potentially hazardous situation, increasing the risk of malignant transformation and cancer (Fig. **1B**).

Chronic pelvic pain syndrome also represents a situation in which, in a large proportion of patients, inflammation of the prostate gland is due to the presence of an autoimmune response against prostate antigens. Probably, as it has been suggested in other autoimmune diseases, microorganisms could be also involved in triggering this autoimmunity. Also, a link between CP/CPPS and PCa has been hypothesized. Whereas the past decade has witnessed an advance in the knowledge of the etiopathogenic mechanisms involved in CP/CPPS, intense research is promptly required to better understand the immunobiology of the normal human prostate and this prevalent disease.

ACKNOWLEDGEMENTS

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT) PICT 2007 00974, PICT 2006 2369, Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET) PIP 2005 Nº 6437. R.M, G.G, J.P.M. and V.A. are fellows of CONICET. M.M. V.R. and C.M.R. are members of the Researcher Career of CONICET.

ABBREVIATIONS

BPH	=	Benign Prostatic Hyperplasia
CFA	=	Complete Freund´s Adjuvant
CP/CPPS	=	Chronic Prostatitis/Chronic Pelvic Pain Syndrome
EAP	=	Experimental Autoimmune Prostatitis
ECs	=	Epithelial Cells
IFN γ	=	Interferon Gamma
LPS	=	Lipopolysaccharide
PAP	=	Prostatic Acid Phosphatase
PCa	=	Prostate Cancer
PECs	=	Prostate Epithelial Cells
PSA	=	Prostate Specific Antigen
TLRs	=	Toll Like Receptors
Tregs	=	Regulatory T Cells

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Received: September 29, 2009 Revised: October 6, 2009 Accepted: October 13, 2009