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Immune mediators of chronic pelvic pain syndrome

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Abstract

The cause of chronic pelvic pain syndrome (CPPS) has yet to be established. Since the late 1980s, cytokine, chemokine, and immunological classification studies using human samples have focused on identifying biomarkers for CPPS, but no diagnostically beneficial biomarkers have been identified, and these studies have done little to deepen our understanding of the mechanisms underlying chronic prostatic pain. Given the large number of men thought to be affected by this condition and the ineffective nature of current treatments, there is a pressing need to elucidate these mechanisms. Prostatitis types IIIa and IIIb are classified according to the presence of pain without concurrent presence of bacteria; however, it is becoming more evident that, although levels of bacteria are not directly associated with levels of pain, the presence of bacteria might act as the initiating factor that drives primary activation of mast-cell-mediated inflammation in the prostate. Mast cell activation is also known to suppress regulatory T cell (T_{reg}) control of self-tolerance and also activate neural sensitization. This combination of established autoimmunity coupled with peripheral and central neural sensitization can result in the development of multiple symptoms, including pelvic pain and bladder irritation. Identifying these mechanisms as central mediators in CPPS offers new insight into the prospective treatment of the disease.

Introduction

Prostatitis (inflammation of the prostate) can be categorized into five different subsets. Of these groups, chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is the most common, accounting for 90–95% of all prostatitis diagnoses. This syndrome is most easily distinguished from the other types of prostatitis by the lack of bacterial association with pain symptoms, which is the case for acute prostatitis (type I) and chronic bacterial prostatitis (type II).¹ CPPS is further divided by the presence or absence of inflammatory markers in the expressed prostatic secretion (EPS) of affected patients into inflammatory CPPS (type IIIa) and noninflammatory CPPS (type IIIb),¹ respectively. Finally, the last subset of prostatic disease, asymptomatic prostatitis (type IV),^{2,3} is associated with inflammation of the prostate without the emergence of pain symptoms, and is usually diagnosed coincidentally (Box 1).

Competing interests

The authors declare no competing interests.

Author contributions

S.F.M and P.T researched the literature, wrote, edited, and reviewed the article. A.J.S. made substantial contributions towards discussions of contents and reviewed the manuscript prior to submission.

Box 1**Prostatitis classification****Type I (acute bacterial prostatitis)**

- Presence of a severe and acute, possibly septic, bacterial infection that is detectable in the urine of patients
- Leukocytes and white blood cells (WBCs) are detectable in expressed prostatic secretion (EPS) >10/hpf
- Requires immediate medical attention and can be life-threatening, with patients commonly presenting with high levels of pain

Type II (chronic bacterial prostatitis)

- Nonseptic bacterial infection is detectable in the urine of presenting patients and WBCs are detectable in EPS samples
- Patients might not present with high-level pain but might suffer from intermittent UTI and prostate infection

Type III (chronic pelvic pain syndrome)

- The most common classification of prostatitis, comprising 90–95% of diagnoses
- Classified by the absence of detectable bacterial infection at diagnoses with high levels of chronic pain
- Further subcategorized by the presence (type IIIa) or absence (type IIIb) of WBCs in EPS samples

Type IV (asymptomatic inflammatory prostatitis)

- Patients have no pain and no history of UTI
- WBCs present in EPS

CPPS is a condition with an estimated prevalence rate of up to 15% of the male population, with most diagnoses occurring between the ages of 35–45 years.^{2, 4} However, incidence varies widely between studies, ranging from 2–16% of adult men.^{4, 5} Studies of large male cohorts of varying ages from Canada,⁶ Australia,⁷ and China⁸ have revealed incidences of 3–8%, 2%, and 8.4%, respectively, with average pain scores of 8 and total index scores of approximately 16.⁹ Incidence and symptom severity might vary based on geographic location and ethnicity, but limited data currently support this theory.¹⁰

Diagnosed by the presence of pain in the absence of bacterial infection for >3 months, CPPS has unknown, probably complex, aetiology, which has thus far hampered efforts to determine effective treatment strategies for ameliorating pain.^{11–13} The heterogeneous nature of the symptoms and the length of the disease course prior to detection, presentation, and diagnosis further exacerbate these issues. The complex nature of the disease is reflected

by the vast array of studies that have been published, ranging from genome-wide association studies (GWAS) to immunological, cell biological, and microbiological investigations. In this article, we collate the disparate published data, enabling a better understanding of the mechanisms behind CPPS and providing insight into ways to enhance the efficacy of treatment.

CPPS symptoms and diagnosis

CPPS is a complex disease with symptoms that are difficult to both quantify and effectively treat.^{12–15} To overcome these challenges, systems have been developed—such as The National Institute of Health Chronic Prostatitis Symptoms Index (NIH-CPSI) and the Urinary, Psychosocial, Organ Specificity, Infection, Neurologic, and Tenderness (UPOINT) system that help physicians to assess the current and ongoing needs of the patient.^{2, 16, 17} These systems demonstrate succinctly to researchers the complex nature of the disease and the high degree of variance between patient symptoms.¹⁸ In doing so, they provide direct avenues of research into the causative effects of CPPS.

The NIH-CPSI was designed to assess the symptoms and quality of life of patients with CPPS,¹⁹ and has been shown to be an effective tool for gauging the effect of chronic pain on morbidity.^{16, 20} Using this system, patients are diagnosed with CPPS when they present with perineal or ejaculatory pain and an index score >4. However, it is stymied by the fact that, for the most part, it is defined based on a subjective questionnaire and overall scores are determined by a cumulative scoring of symptoms that might or might not be related to one another, or indeed to the underlying causes of pathology.² More recently, the UPOINT system of classification was developed to guide treatment decision-making (Box 2).²¹ Rather than grouping patients based on an aggregate score of symptoms, this system considers each group of symptoms, including urinary²², psychosocial²³, organ-specific, infection, neurological, and tenderness²⁴ symptoms, separately (Box 2).²⁰ The number of symptom groups involved has been shown to correlate with NIH-CPSI²⁵ scores, and multimodal treatment of combinations of symptom groups has been shown to result in decreased scores after 50 weeks of treatment (mean reduction of 12 points),²⁶ suggesting that a more individually tailored approach to CPPS might be more effective than a standardized approach.^{15, 18, 27–30}

Box 2

UPOINT system

- Urinary problems (specifically irritative or obstructed voiding and urinary tract issues, which might be indicative of interactions between the prostate and the bladder)
- Psychosocial problems (such as clinical depression)
- Organ specificity issues (encompassing a range of problems, such as prostate tenderness and presence of leukocytes in expressed prostatic secretions)

- Infection (even in the absence of an ongoing UTI and, despite the ‘abacterial’ nature of type IIIa and IIIb CPPS, microbial cultures can be grown from prostatic fluid and used to determine the initiating factors of disease)
- Neurological pain outside the pelvic region (sometimes associated with systemic chronic pain syndromes, such as fibromyalgia)
- Tenderness (generally localized to pelvic floor muscles; data suggest that sexual dysfunction might be an emerging symptom of this disease)

Abbreviations

CPPS	chronic pelvic pain syndrome
UPOINT	Urinary, Psychosocial, Organ Specificity, Infection, Neurologic, and Tenderness

Another issue that has hampered research into the factors causing CPPS is the comorbidity of symptoms.^{30, 31} Chronic pain syndromes such as fibromyalgia and chronic fatigue syndrome have been shown to be associated with CPPS, as has irritable bowel syndrome (IBS).³² Epidemiological studies have also linked cardiovascular problems to CPPS; in one study, patients with CPPS were six times more likely than controls to have cardiovascular disease, and were also independently shown to have an increased prevalence of arterial stiffness.^{33, 34} Assessment of pelvic floor tension, by monitoring the motility of muscles using ultrasonography, linked increased tension to CPPS.³⁵ Although there has been some interest in investigating whether pelvic floor tension acts as a causative factor or direct mediator of CPPS, its role in determining patient outcomes has yet to be established.²⁰

Infection as an initiating factor

Distinguishing CPPS from the other types of prostatitis requires evidence of bacterial infection.^{36–38} Although effective for determining patient diagnoses, pinpointing causes of disease using such definitions might not be so clear cut. Studies of the role of bacteria in disease initiation are emerging from our, and other, laboratories, which might be informative for future therapeutic intervention.^{39–41} We isolated a strain of *Escherichia coli*, designated CP1, from the EPS of a patient with CPPS and demonstrated that it induced symptoms of pain when introduced into the prostate and urethra of nonobese diabetic (NOD) mice.⁴¹ NOD mice are genetically distinct from C57BL/6 mice and have been shown to develop spontaneous chronic pain and autoimmune prostatitis over time. Upon prostatic instillation with CP1 bacteria, NOD mice developed bacterial colonization in both the bladder and the prostate,⁴¹ resulting in chronic pain in the NOD mice that was not detectable (by behavioral tactile allodynia scoring) in C57BL/6 mice. Although the pain symptoms persisted, no bacteria were detectable 35 days after instillation with the microbe, suggesting that infection with CP1 might initiate disease in genetically susceptible hosts, but that fundamental changes in the immune responses of the prostate are responsible for chronic pain.⁴¹ In agreement with this, it is interesting to note that the incidence of chronic inflammatory prostatic disease of noninfectious origin is shown to be eightfold higher than that of bacterial

prostatitis.⁴¹ Whether similar models are applicable to humans, such that early infection with a pathogen might cause disease but microbes are undetectable at diagnosis of CPPS, resolving the 'abacterial' definition of CPPS IIIa and IIIb categories of prostatitis, remains to be elucidated.

In addition, the virulence of bacterial isolates was fundamental to the initiation of disease in this mouse study.⁴¹ Phylogenetic analyses of the CP1 strain identified a group B1 strain of uropathogenic *E. coli* (UPEC), distinct in both virulence gene and fimbriae expression from the typical group B2 bacteria, which are commonly associated with acute bacterial infection of the urogenital tract. The virulence and invasiveness of CP1 was compared with the typical cystitis-related UPEC group B2 strain NU14.⁴² The authors demonstrated in both *in vitro* and *in vivo* systems that, although both bacteria have the same adherence capabilities, CP1 was dramatically more invasive than NU14.⁴¹ Interestingly, when NU14 is instilled into the prostate of NOD mice, chronic pain does not develop. We are now investigating the possibility that instillation of nonpathogenic strains of atypical urogenital bacteria can reverse pain symptoms in murine models of CPPS. These studies are, at present, only preliminary but seem to indicate that prostatic instillation of certain species of microbiota can reverse inflammation in the prostate and, over time, can significantly reduce pain.^{43, 44} This finding is hoped to lead to the development of probiotic treatments for CPPS in humans.

The immune responses mediating these effects, and leading to the development of pain in the CP1 model, are an area of current investigation. Another bacteria-focused study recently associated *Helicobacter pylori* infection with CPPS, when patients with CPPS were compared with controls based on antibody seropositivity.⁴⁵ These determinants were not correlated with severity of symptoms and only a relatively small cohort of patients were recruited, so it remains to be seen whether these preliminary findings will affect the development of future therapies.^{34, 45, 46} The hypothesis that bacterial infection acts as the initiating factor in CPPS is further strengthened by studies demonstrating mixed therapeutic efficacy of antibiotics.^{38, 47}

Immunological basis of autoimmune activation

T-cell activation and CP1 infection

Numerous lines of evidence and ongoing investigations underline the role of the adaptive immune response and activation of autoimmunity in the development of CPPS (Figure 1a and 1b). Inflammation has been shown to have an important role in the development of both prostate cancer and benign prostate hyperplasia (BPH),^{48–51} with studies to show increased levels of the cytokines IL6, IL8, IL15, and IL17 compared with controls.⁴⁹ Generally, the prostate contains only a small number of CD3⁺ T cells (approximately seven per mm² of tissue), of which over two-thirds are also CD8⁺. These numbers greatly increase with age (to about 55/mm² in men aged 50 years, correlating with increased rates of benign prostatic disease in ageing men.⁴⁹ The role of inflammation in CPPS pathogenesis is being uncovered using data from both human studies and murine models. Analysis of the immunology behind the CP1 model of chronic pain has demonstrated that T cells can mediate pelvic pain in the absence of a persistent bacterial infection.⁵² In a recently published article, our lab

demonstrated CP1-mediated activation of Th1/Th17 CD4⁺ T-cell response and showed that these lymphocytes were sufficient to cause pain upon adoptive transfer to naive uninfected NOD mice.⁵² IL17A, a secreted effector of Th17 cells, was fundamental to the development of pain in the absence of IFN γ , the main effector of Th1 cells, and pain was increased in IFN γ knockout mice upon CP1 instillation.⁵² These results implicate IL17A as an essential mediator in the development of pain,^{53, 54} without the need for intact Th1 responses. Transfer of serum from naive or infected mice did not cause increased pain responses in these mice, indicating that, although there is a strong autoimmune component to this model of CPPS (NOD background only),⁵⁵ no current evidence supports the existence of a systemic circulating antigen or activating factor in this model.⁵²

Murine models of prostatitis

Given the diagnostic value of separating bacterial prostatitis from conditions that are not associated with persistent infection, murine models can offer insight into the alternative mechanisms that might mediate disease progression. Experimental autoimmune prostatitis (EAP) has consistently been linked with underlying immune responses associated with the development of pain in mice. Indeed, this model has been used for the past 20 years to delineate possible mechanisms underlying pathogenesis in humans.⁵⁶ The EAP model of CPPS was first investigated in Wistar rats, but our lab and others use similar models in mice.⁵⁷ Using one such murine model, investigators demonstrated that a particular antigen, prostatic steroid binding protein (PSBP) (also known as prostatein), is capable of inducing EAP when injected with an adjuvant alone, and data suggest that additional antigens might be present in rat prostate homogenates that augment these effects.^{58–60} In our laboratory, we use a 1:1 ratio of rat prostate homogenate to adjuvant that is injected subcutaneously, and pain is assessed by tactile allodynia after 7 days (for up to 30 days).⁵⁷ Studies of this model in multiple mouse strains have revealed that the development of pain occurs at the highest level, without the need for a second injection of antigen and adjuvant in the NOD background.⁶¹ NOD mice demonstrate unchecked T-cell immune responses against B-islet cells of the pancreas, resulting in the development of diabetes mellitus.^{62, 63} These mice have also been shown to develop autoimmune sialitis, thyroiditis, peripheral neuropathy, systemic lupus erythematosus, and spontaneous prostatitis, and have multiple genetic abnormalities that directly affect their immune systems. Polymorphisms have been identified in *IDD3*, a gene that controls expression of IL2, an essential cytokine in T-cell development; alterations in the expression pattern of this gene increase the risk of autoimmunity.⁶³ A second mutation—in *CTLA4*, a negative regulator of T-effector cell activation—results in incorrect splicing and leads to loss of autoreactive T-cell suppression and development of immune responses against insulin-producing cells.⁶⁴ These data, combined with the high incidence of spontaneous prostatitis in NOD mice, highlight loss of T_{reg} suppression as a possible key factor in the development of EAP.⁶³

Development of autoimmunity

D3tx mice have their thymus removed 3 days after birth and develop spontaneous inflammation as a result,⁶⁵ owing, at least in part, to the absence of self-tolerized T_{reg} cells (known as natural T_{reg} cells),⁶⁶ which form the backbone of central tolerance.^{67, 68} Thymic epithelial cells surrounding the thymus control induction of T-cell maturation—and,

therefore, the development of tolerance—by upregulating expression of the autoimmune regulator (AIRE)^{69, 70} transcription factor. AIRE has been shown to regulate expression of, and therefore increase tolerance to, prostate antigens.^{71, 72} All AIRE-deficient NOD mice develop inflammation of the prostate by 20 weeks of age, further supporting the loss of tolerance in mouse models of chronic prostatitis.^{73, 74} Notably, the spontaneous inflammation observed in D3tx is commonly localized to the prostate,^{65, 75} indicating that loss of tolerance to self-prostate antigens has a central role in driving CPPS development.⁷⁶ Peripheral tolerance is also regulated by inducible T_{reg} (iT_{reg}) cells that mature in a tissue-specific manner in the periphery.⁷⁷ Presentation of antigen from immature dendritic cells in the periphery can induce T-effector cell activation and proliferation in the absence of an iT_{reg} response, prompting autoreactive T cells to drive inflammation in an unchecked manner.⁷⁸ This activation status could explain the immune activation that is commonly seen in murine models of CPPS, with increased number of CD4⁺ T cells leading to increased secretion and expression of IL17 and IFN γ . Furthermore, a broad analysis of polymorphisms in a variety of immune-regulatory genes, including TGF β , TNF α , IL10, and IL6, revealed an association between low levels of IL10 production and CPPS, implicating loss of T_{reg} function and tolerance as key mediators of inflammation.⁷⁹ TNF α was also shown to be closely associated with type IIIa CPPS, suggesting that this subtype might have a more proinflammatory phenotype. Failure of treatment with quercetin,⁸⁰ a mast cell stabilizer, in men with CPPS with a more regulatory phenotype is associated with high IL10 and low TNF α levels,⁷⁹ an interesting observation given that IL10 can regulate and dampen mast cell activity. This finding suggests that mast cells in these patients are already under the regulatory control of IL10, such that further stabilization of degranulation will not ameliorate symptoms.

However, no evidence exists to support spontaneous prostate inflammation in IL10-knockout mice, which suggests that, although activation of autoreactivity might drive CPPS development, mechanisms that are distinct from iT_{reg} suppression via IL10 must be responsible for maintaining tolerance in healthy individuals. Furthermore, preliminary data from our laboratory have demonstrated that IL10-knockout mice develop pelvic pain in response to EAP, but instillation with the probiotic bacteria that decreases pain in NOD mice is ineffective in the absence of IL10. These data demonstrate the fundamental role of IL10, not in restraining onset of autoreactivity, but rather controlling it once it has occurred (P. Thumbikat, unpublished work).

Cytokine and chemokine association

Human studies

Since the late 1980s, numerous studies have aimed to establish an association between expression of certain immunomodulatory proteins and numbers of particular cell types in samples from men with CPPS. Analysis of these samples, which have been taken from various urogenital and systemic sources including EPS, seminal plasma, postprostatic massage urine, and semen, has demonstrated increased levels of numerous proinflammatory cytokines, including, IL1b, TNF α , IL6, IL8, and IgA,^{81–87} as well as decreased levels of IL2R (involved in T-cell development) in patients with CPPS compared with controls.

Studies involving multiple sample types (including EPS, postprostatic massage urine, and semen) have shown increased numbers of granulocytes, macrophages, and both activated T cells and B lymphocytes in the cellular infiltrate of patients with CPPS.⁸⁸ Prostate inflammation has also been examined histologically,³ with data to show increased leukocyte infiltration and increased numbers of T cells (more specifically, CD8⁺ T cells) compared with controls.⁸⁹

The T-cell immune response to prostate antigens has also been investigated and CD4 T cells have been shown to respond to seminal plasma from patients in two independent studies.^{90, 91} Moreover, IFN- γ -producing Th1 cells have been identified in the peripheral blood of men with CPPS that are specific to PSA.^{92,93} High PSA levels in blood are commonly associated with prostate tissue damage and carcinogenesis.⁹⁴ A similar study identified IgA antibodies that are specific to two additional prostate antigens, MAD-PRO-34 and Ny-Co-7, in patients with CPPS.⁹⁵ These studies, and others, point to autoreactivity to prostate antigens as a potential driver of CPPS and support the existence of organ-resident autoreactive T cells, which become activated following an initiation event.⁸⁴

MCP1 and MIP1 α

Two chemokines have been associated with the development and progression of pain in multiple studies of CPPS.⁹⁶ Monocyte chemoattractant protein 1 (MCP1),⁹⁷ also known as chemokine C-C motif ligand 2 (CCL2),⁹⁸ is a chemotactic factor that attracts monocytes and basophils to sites of inflammation and is implicated in the pathogenesis of numerous autoimmune diseases, including rheumatoid arthritis,⁹⁹ aortic aneurysms,¹⁰⁰ and coronary artery disease.¹⁰¹ Macrophage inflammation protein 1 α (MIP1 α),^{99, 102} also known as chemokine C-C motif ligand 3 (CCL3),^{103, 104} is a potent inducer of proinflammatory responses and has been shown to be upregulated in patients with rheumatoid arthritis, systemic lupus erythematosus, and ulcerative colitis. Levels of both chemokines are elevated in EPS samples from men with type IIIa and type IIIb CPPS compared with those with BPH and healthy controls.¹⁰⁵ It was hypothesized that, although type IIIb CPPS (noninflammatory) is categorized as a distinct subset of disease from type IIIa, it might, in fact, represent a distinct snapshot in the natural course of the disease when inflammation is ongoing but neutrophilic infiltration has yet to start. In the same study, levels of MIP1 α , but not MCP1, positively correlated with National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) pain score in patients. MCP1 was, however, positively correlated with the number of white blood cells in EPS samples,¹⁰⁵ indicating that, although levels of both chemokines are increased in CPPS samples, each chemokine might have distinct effects in terms of mediating different symptoms of CPPS.¹⁰⁶

Another, more-recent, study from our laboratory also investigated CCL2 and CCL3 as potential biomarkers for CPPS using the murine EAP model. This study assessed the source, nature, and kinetics of chemokine expression and the phenotype of the associated immune infiltrate.¹⁰⁷ The results highlighted important roles for CCL2 and CCL3 in the pathogenesis of pelvic pain in EAP. Increases in CCL2 in prostate tissue were shown at the mRNA level at day 5 and day 20 after EAP; CCL3 increases were observed at day 20. Prostatic epithelial cells expressed the highest levels of CCL2 and CCL3, and were identified as the main source

of both chemokines in EAP-treated prostates.¹⁰⁷ Expression levels of these cytokines were significantly elevated in the stroma of EAP-treated prostate tissue compared with control tissue. These differences were confirmed using immunohistochemical analysis, which demonstrated increased expression of CCL2 and CCL3 in the epithelial compartment and increased expression of another chemokine, CCL12, in the stroma.¹⁰⁷ The effect of these expression changes on cellular infiltrate was also examined and numbers of various cell types; CD4 T cells were decreased at days 5 and 10, but increased at day 20, CD8 T cells were decreased at each time point after day 0, mast cells were increased at days 5 and 10, macrophage and neutrophils increased at day 10 and B cells at days 5 and 10.¹⁰⁷ Although a plethora of studies have attempted to define a biomarker for CPPS, few have used the resulting data to delineate a functional consequence for altered levels of certain cytokines or chemokines. However, the investigators in this study were able to demonstrate the functional consequences of changing the cellular milieu of mice undergoing EAP. Currently, studies are underway to identify the role of these chemokines in mediating neuroinflammation and neuropathic pain in murine models of CPPS.

Numerous studies using high-throughput arrays to measure cytokine and chemokine levels in EPS samples from patients with CPPS are underway. One such preliminary study from our laboratory has revealed positive correlations between levels of a known mediator of T-cell immunity with each of the three metrics used to categorize symptoms in patients, including the pain subscale, urinary subscale, and quality of life. Expression levels of this cytokine are currently being investigated in various mouse models of prostatitis (P. Thumbikat, unpublished work),¹⁰⁸ hopefully leading to a greater understanding of how the cytokine and chemokine milieu leads to progression of disease.

Mast cells

Tryptase and cellular function

Mast cells have become a major focus of current research, with proposed roles as the main mediator and effector cells in disease progression from initiation to breaking of tolerance, neuronal activation, and, eventually, sensitization.^{109, 110} Mast cells are derived from CD34⁺ haematopoietic precursor cells in bone marrow; they circulate as immature cells, only fully maturing once they are resident at a specific tissue or site, where they become activated. This process suggests a tissue-specific or organ-specific cell phenotype.¹¹¹ Mast cells are also thought to be the first line of defense against infection by microbes and are usually located in (or near to) blood or lymphatic vessels and nerves.^{110, 111} They demonstrate both protective and pathological effects in a context-dependent manner, with roles in wound healing, angiogenesis, and inflammation.^{110, 111} Crosslinking of their IgE receptor leads to cell sensitization (as seen in conditions such as asthma). Upon activation, mast cells release several molecules stored in granules, including histamine, serotonin, tryptase, chymase, prostaglandin D2 (PGD2), and leukotriene B4, triggering early-stage allergic responses.^{112–114} Aside from the effect of IgE crosslinking, activation of mast cells is classified as nonallergic and can originate from a diverse range of sources including pathogen-associated molecular patterns, complement, cytokines, drugs, hormones, and physical stressors such as temperature and pressure.^{110, 111} Mast cells can also directly

interact with T cells and B cells via antigen presentation and can also be involved in reverse signalling, particularly through CD40 and CD40L ligands.¹¹⁵ Finally, mast cells have also been shown to mediate signals related to TNF α stimulation in order to control neutrophilic recruitment.¹¹⁶

Mast cells and inflammatory conditions

In order to assess the role of mast cells in CPPS, it is useful to draw parallels between their functions in other chronic inflammatory pain disorders and establish whether these functions can also be attributed to the development of CPPS. Experimental autoimmune encephalopathy results from a breach of the blood brain barrier, which allows infiltration of immune cells into the central nervous system.^{117, 118} Encephalogenic CD4⁺ IFN γ -producing Th1 cells are the major contributor to the pathogenic autoimmune response.^{119, 120} IL17a-producing Th17 cells and IL9-producing Th9 cells have been shown to have major roles in the development of experimental autoimmune encephalopathy.^{121, 119} Patients with multiple sclerosis display increases in mast cell numbers at sites of inflammatory demyelination,¹²² and increased levels of tryptase in their cerebrospinal fluid.¹²³ *In vitro* analyses have revealed that mast cell proteases are also capable of degrading myelin, further implicating mast cells and mast cell degranulation in the progression of this debilitating disease.^{124–126} Mast cells are also associated with rheumatoid arthritis, a chronic systemic autoimmune disease that results in loss of mobility and flexibility of synovial joints.^{112, 127} Increased numbers of mast cells and secreted tryptase have been found at affected synovial joints and in synovial fluid, respectively.^{128, 129} Increased levels of proinflammatory cytokines, such as IL6, IL17, and TNF α , have also been associated with rheumatoid arthritis and mast cells have been identified as the main source of IL17 expression.^{130, 131}

Mast cells have been shown to mediate both T_{reg} and Th17 cells, and are consistently found in close proximity to secondary lymphoid organs.¹³² The best-defined mast: T-reg cellular interaction is between the OX40 receptor expressed by T_{reg} and OX40L ligand expressed by mast cells.^{133, 134} Activation of T_{reg} cells have been shown to result in decreased levels of Fc ϵ R1 receptors on mast cells, thus preventing IgE crosslinking and degranulation in allergic responses. Mast cells, on the other hand, can decrease suppression of T-effector cells by T_{reg} cells and can also reduce the susceptibility of these cells to suppression.¹³⁵ Mast cells express a variety of cytokines at high levels including IL6, IL21, IL23, and TGF β ,¹³⁶ all of which have been shown, in various combinations, to control naive Th17/T_{reg} cell differentiation and plasticity.¹³⁶

EPS samples from men with CPPS have been shown to have increased levels of both mast cell number and mast cell tryptase content compared with controls.¹³⁷ Furthermore, mast-cell-deficient Kit^{W-sh}/Kit^{W-sh} Sash mice have attenuated pain responses (tactile allodynia) upon induction of EAP, but exhibit no difference in levels of inflammatory infiltrate,^{126, 138} suggesting that, although mast cells are important for the development of CPPS, additional mediators of pain might be present, in both humans and mice, that account for the increased numbers of cytokines and lymphocytes in tissues of affected individuals. Current studies in our laboratory focus on the role of mast cell tryptase in mediating pain in murine EAP, specifically through the protease-activated receptor 2 (PAR2) receptor, a potent mediator of

innate and adaptive immune responses.¹³⁹ These data implicate the PAR2 signalling network as being fundamental for the development of chronic inflammation and pain.

Neuronal mechanisms

Mast cells contribute to rapidly occurring neuronal peripheral sensitization,¹⁴⁰ which is mediated by the neurotrophin nerve growth factor (NGF).^{141–143} Mast cells are known to express TrkA receptors on their cell membrane and, therefore, NGF binding might cause degranulation, establishing a feedback mechanism that would promote sensitization mechanisms. As peripheral tissue insults are often associated with some degree of nerve damage, increased levels of NGF might be produced as the tissue regenerates. In men with CPPS, NGF levels are elevated in seminal plasma and directly correlated with pain severity.¹⁴⁴ This intriguing result suggests the existence of underlying peripheral sensitization mechanisms in CPPS, with NGF and mast cell proteases as potential mediators. Increased NGF expression is known to increase neuronal excitability and enhance neuronal density in peripheral tissues. In this context, previous work has demonstrated increased neuronal density in prostate tissue sections from animal models of prostatitis.⁵⁷ These results suggest that peripheral sensitization mediated through NGF (and potentially involving mast cell activation) might underlie chronic pelvic pain.

In addition to, or as a consequence of, persistent peripheral neuronal activation, it is thought that changes in the spinal cord might occur in places where persistent activation of spinal cord neurons enhances pain responses. Persistent activation causes alteration in the dorsal horn of the spinal cord, where nociceptor paths enter the central nervous system. Changes include enhanced expression of neurotransmitter receptors (such as N-methyl-D-aspartate receptor), increased neurotransmitter biosynthesis and release, and the sprouting of nociceptor neurons.¹⁴⁵ Sustained nociceptor firing results in progressive amplification of dorsal horn neuron activity, which can either remain restricted to the activated synapse or spread to adjacent synapses. This hypersensitivity of dorsal horn neurons can mediate pain after the peripheral damage has healed. Thus, central sensitization within the spinal cord of patients with CPPS could mediate pain long after the resolution of any prostate inflammation or other pelvic insult that might initiate symptoms.

Conclusions

CPPS is a complex syndrome of unknown aetiology; thus, uncovering and implementing effective treatments has so far proven difficult. It is our hypothesis, however, that an initiating event, such as a bacterial infection, activates the prostatic immune response in an unchecked manner. A large amount of evidence suggests that loss of regulation of autoreactive T cells has a fundamental role in driving pathogenesis. This loss of regulation amounts to a breaking of tolerance to normal prostatic antigens (self, bacterial, or otherwise), further exacerbation of an adaptive proinflammatory immune response, and development of neural sensitization. We propose that these events are mediated through mast cells, by a variety of cytokines, chemokines, and cellular effectors such as tryptase. Activation of these responses can prevent normal regulation of activated T cells, resulting in autoimmunity and unchecked inflammation, especially in a genetically susceptible host. An

open question that remains, however, relates to the role of neural sensitization. Although we, and others, think this effect is also likely to be driven by mast cells, given the chronic nature of patient pain, it is not yet known whether removal of the inflammatory stimulus can reverse neural changes. Resolving these issues would improve our understanding of therapies for men with CPPS, which we believe should focus, at least in part, on restoration of immune balance and tolerance within the prostate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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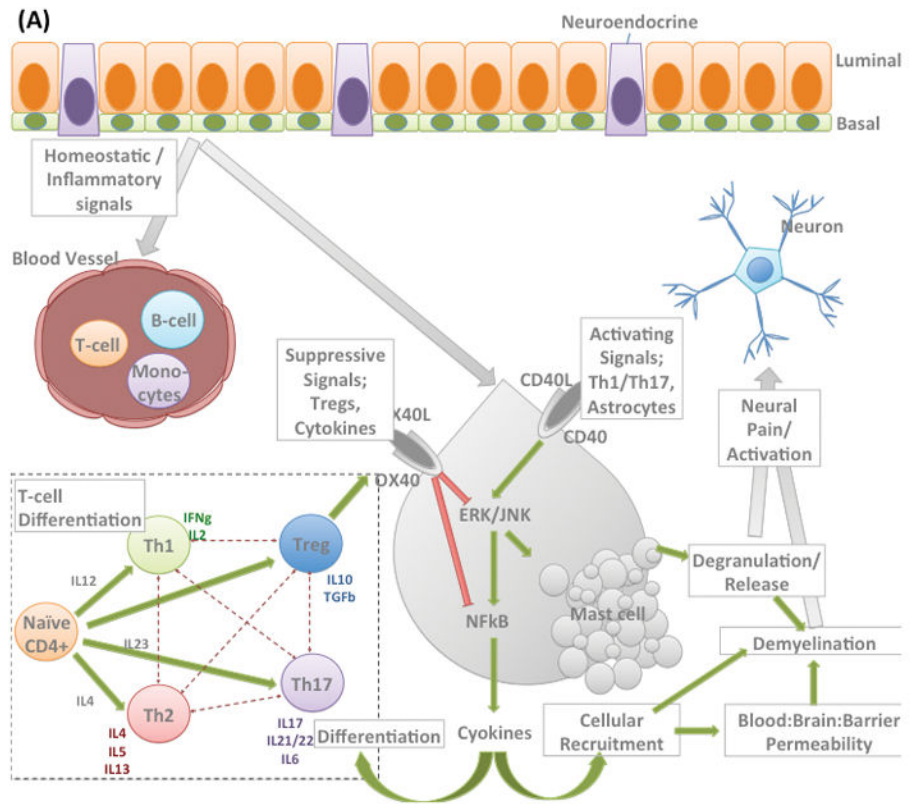
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Key points

- Chronic pelvic pain syndrome (CPPS) is the most common classification of prostatitis and affects up to 16% of men at some point in their lives
- CPPS is classified as an ‘abacterial’ disease at time of diagnoses but acute bacterial infection might initiate inflammation and development of pain
- Loss of regulation and suppression of CD4⁺ T-cell activity might result in constitutively active mast cells and loss of tolerance in CPPS
- Mast-cell-released factors, such as tryptase and nerve growth factor (NGF), might mediate both inflammation and neural sensitization
- CPPS has a complex aetiology, with evidence suggesting interplay between T cells, mast cells and neurons in the development of autoimmunity



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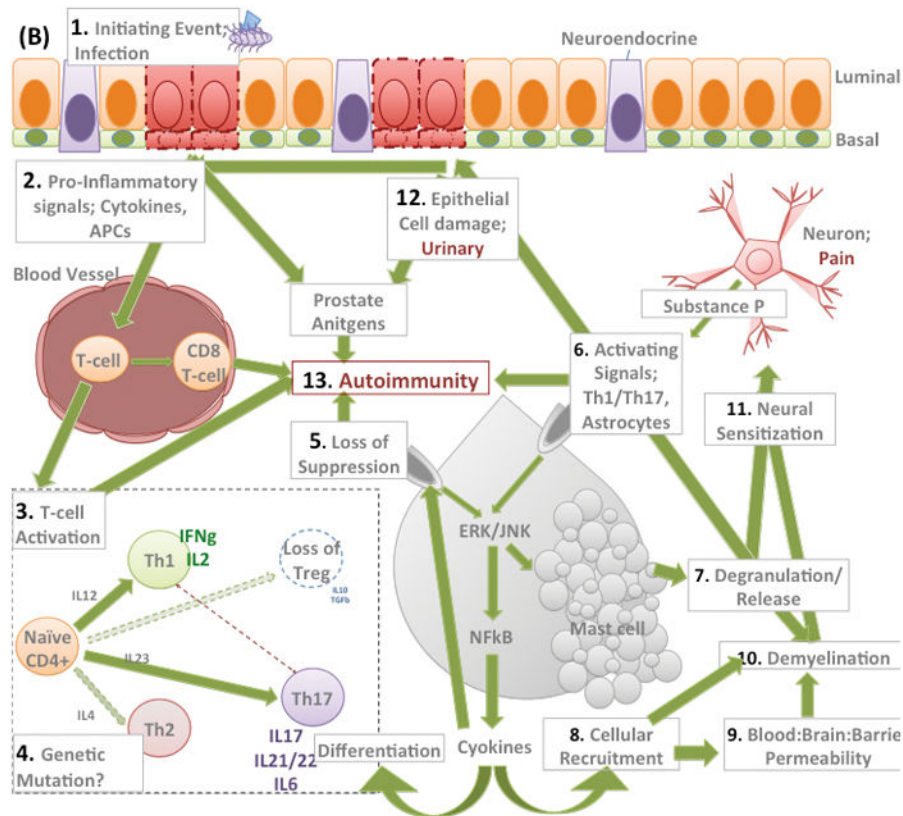


Figure 1. Hypothetical model of CPPS

(A) Intercellular homeostatic signaling demonstrating; differentiation of CD4 T-cells in a context dependent manner; Regulation of mast cell activation by direct interaction e.g. through OX40 receptor; Activation of Mast cells and subsequent deactivation. And how these signaling cascades interact to stimulate neurons. The system is tightly controlled and regulated with numerous checkpoints.

(B) Schematic representation of modulated immune system in CPPS. **1.** We hypothesize that an initiating event, such as bacterial infection, drives prostatic epithelial cell damage and **2.** Promotes secretion and activation of pro-inflammatory cytokines, chemokines and presentation of antigen via APCs **3.** These signaling cascades result in CD4 T-cell activation which is initially of Th1-type (IFN γ) but Th17 activation is also implicated in the pathway possibly at later stages of chronic pain development. **4.** Data suggests a loss of IL-10 secreting suppressive T-regs and a skewing towards Th1/17 responses, this may be attributable to genetic polymorphisms regulating expression of these factors. **5.** The loss of suppression of mast cells as a result of unchecked T-cell activation results in a positive feedback loop in the mast cell resulting in; **7.** degranulation and releases of proteases such as tryptase, chymase and allergy mediators such as histamine., and **8.** cytokine secretion (IL6, IL17, TNF α , IL6) resulting in recruitment of inflammatory cells. Together **7.** and **8.** can disrupt the Blood Brain Barrier, **9.** and/or directly **10.** demyelinate neurons. Taken together these processes result in **11.** neuronal activation and sensitization. The mast cell mediates these events and positive feedback loops increase these processes through **6.** Further epithelial cell damage, **12.,** is one consequence of such increased mast cell activity. Prostate

antigens generated from damage to the epithelium in the presence of an activated CD4 T-cell response, with unchecked mast cell degranulation and increased numbers of CD8 Tcells can result in development of **13.** autoimmunity, which only further exacerbates these mechanisms.

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