

Original Article

Patients with chronic prostatitis/chronic pelvic pain syndrome show T helper type 1 (Th1) and Th17 self-reactive immune responses specific to prostate and seminal antigens and diminished semen quality

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Objectives

To assess the presence of self-reactive immune responses to seminal and prostate antigens (PAG), biomarkers of inflammation of the male genital tract, and semen quality parameters in patients with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS).

Patients, Subjects and Methods

Peripheral blood and semen samples were collected from patients with CP/CPPS and age-matched healthy control volunteers. We analysed the lymphoproliferative responses of peripheral blood mononuclear cells (PBMC) to different seminal plasma (SP)-derived and purified PAG, serum autoantibodies specific to PAG, leucocyte subpopulations, and inflammatory cytokines in semen, sperm apoptosis/necrosis, and semen quality parameters.

Results

Significantly greater PBMC proliferative responses specific to PAG, with elevated secretion of interferon (IFN) γ and interleukin (IL)-17, were detected in the patients with CP/CPPS vs the controls. Moreover, the patients with CP/CPPS had significantly greater serum immunoglobulin G immune reactivity to SP proteins, such as prostate-specific antigen and prostatic acid phosphatase, than the controls. Inflammation of the male genital tract was exemplified by high levels of IFN γ , IL-17, IL-1 β and IL-8, as well as higher counts of leukocytes, mainly CD4 T lymphocytes and macrophages, in the semen. In addition, this local inflammation was associated with an overall diminished semen quality, i.e., reduced sperm concentration, motility and viability; and higher levels of sperm apoptosis/necrosis in patients with CP/CPPS vs controls.

Conclusion

Patients with CP/CPPS show T helper type 1 (Th1) and Th17 immune responses specific to PAG associated with chronic inflammation of the male genital tract and reduced semen quality. These immune responses may underlie the induction and development of chronic pelvic pain and inflammation of the male genital tract, which in turn could alter normal prostate functioning and impair semen quality.

Keywords

prostatitis, inflammation, autoimmunity, MaleInfertility, semen analysis

Introduction

Prostatitis is a major medical problem, being the most common urological diagnosis in men aged <50 years and the third most common in older men [1]. Prostatitis is currently classified into four categories by the National Institutes of Health (NIH): acute bacterial prostatitis, chronic bacterial prostatitis, chronic non-bacterial prostatitis/chronic pelvic pain syndrome (CP/CPPS), and asymptomatic inflammatory prostatitis [2]. CP/CPPS accounts for >90% of cases of prostatitis and affects 8–14% of men of all ethnic origins [3,4]. CP/CPPS has a considerable negative impact on quality of life, similar to myocardial infarction, angina, or Crohn's disease [4]. It is a poorly understood clinical syndrome characterised by local signs and symptoms of chronic inflammation and genitourinary pain (defined as lasting ≥ 3 of the preceding 6 months) in the absence of identifiable urogenital infections [1]. As its aetiology remains obscure, most therapies are empiric and ineffective [5]. CP/CPPS appears to encompass similar clinical phenotypes resulting from a wide array of heterogeneous conditions. It has been suggested that dysregulated inflammation resulting from autoimmunity against the prostate might be involved in its onset and/or progression [6]. Indeed, no infectious cause has been successfully identified to date and treatments with corticosteroids or immunosuppressive drugs, rather than with antibiotics, have been shown to be effective [5,7–9]. Moreover, the prostate-specific protein transglutaminase-4 (TGM4) has been identified as a target of the autoimmune response in autoimmune polyendocrine syndrome type 1 (APS1), which presents with clinical and histopathological prostatitis and subfertility in males [10]. Regarding the latter, some recent evidence suggested that CP may have andrological implications, as it could have deleterious effects on semen quality compromising male fertility potential [11–13]. Nonetheless, that issue remains controversial highlighting the need for additional and compelling data [14].

In an effort to gain further understanding of the pathophysiology underlying CP/CPPS, in the present study, we analysed the presence of self-reactive immune responses to seminal plasma (SP)-derived and prostate antigens (PAg), inflammatory cytokines and leucocyte subpopulations in the semen, and sperm quality parameters in patients with CP/CPPS.

Patients, Subjects and Methods

Patients and Subjects

CP/CPPS was defined as pain or discomfort in the perineum and the suprapubic region with LUTS or sexual/ejaculatory dysfunction, without infection [2]. Diagnosis was based on a detailed history, physical examination, ultrasonography, and laboratory evaluations. Inclusion criteria were men aged 18–55 years with history of pain or discomfort in the prostate

region, painful DRE, negative Meares–Stamey test, no other lower urinary tract pathology, and for ≥ 3 of the preceding 6 months a CP/CPPS history >1 year, and a NIH Chronic Prostatitis Symptoms Index (NIH-CPSI) total score >15. Control subjects had no history of any genitourinary symptoms, instrumentation or surgery. Exclusion criteria included specific disease-associated pelvic pain/discomfort caused by non-CP/CPPS diseases (e.g. acute prostatitis, bacterial prostatitis, BPH, prostate cancer, urogenital infection), serious or acute disease of the heart, liver, kidney or blood, vasectomy, toxic/pollutant exposure, any drug, alcohol or marijuana consumption, antibiotic, steroids or NSAID treatment during the preceding 12 weeks. Patients and controls were prospectively included in the study and they donated blood and semen samples. This study was carried out in accordance with the Declaration of Helsinki and the Argentinean legislation for protection of personal data (Law 25326). The experimental protocol was approved by the Institutional Ethics Committee from the Hospital Privado de Cordoba (Ref. #HP-4-132). All participants provided a signed written informed consent prior to enrolment.

Lymphoproliferation Assays

Peripheral blood samples were obtained and collected in sterile heparinised tubes. PBMC were purified by density gradients (Ficoll-Paque PLUS, GE Healthcare Life Sciences, Pittsburgh, PA, USA) and lymphoproliferation assays specific to SP (SP0, human prostate homogenate (PHg), PSA and prostatic acid phosphatase (PAP) were performed as previously described [15]. In brief, PBMC were divided as responder or antigen-presenting cells (APC). APC were loaded with different PAg sources: dilutions (1:25) of pooled SP from normal donors, PHg (100 $\mu\text{g}/\text{mL}$), PSA (10 $\mu\text{g}/\text{mL}$, Sigma-Aldrich, St. Louis, MO, USA), PAP (10 $\mu\text{g}/\text{mL}$, Sigma-Aldrich), or medium alone, and then irradiated at 3000 cGy. Pulsed APC were added to plates containing 1.5×10^5 responder PBMC in quadruplicate and incubated for 96 h at 37 °C/5% CO₂. Concanavalin A (5 $\mu\text{g}/\text{mL}$, Sigma-Aldrich) was used as a positive control of proliferation. Supernatants were collected and frozen at –80 °C for cytokine quantification. DNA synthesis was measured by adding 37000 Bq of [methyl-³H] thymidine (Perkin Elmer, Waltham, MA, USA) in fresh medium per well 18 h before harvesting cells onto glass fibre filters. Labelled material was counted and results expressed as a Proliferation Index (PI) calculated from counts/min (cpm) incorporated in antigen-pulsed cultures/cpm incorporated in cultures with medium. A PI of ≥ 2.00 was considered positive.

PAg-specific Antibodies in Serum

Immunoglobulin G (IgG) antibodies specific to SP, PSA or PAP were assayed by conventional ELISA as previously

described [15]. Serum reactivity was expressed as optical density at 450 nm.

Semen analysis

Semen samples were obtained by masturbation after 4–7 days of sexual abstinence and semen analysis performed according to the WHO Semen Analysis Manual [16]. Semen analysis was performed at least twice in each patient and control. The sperm concentration and motility were evaluated soon after liquefaction in a Makler chamber (Sefi-Medical Instrument, Haifa, Israel) on a phase-contrast microscope. Sperm viability and morphology were analysed using eosin Y (Sigma-Aldrich) staining and the Papanicolaou technique, respectively. The concentration of round cells was evaluated using the Makler chamber and peroxidase-positive cells were quantified among round cells using a cytochemical assay [17]. Semen citrate and fructose concentrations were measured as biomarkers of prostate and seminal vesicles function, respectively, by colorimetric assays. Anti-sperm IgG antibodies were tested using the direct SpermMAR test (FertiPro, N.V., Beernem, Belgium). Routine sperm parameters were assessed in at least 200 spermatozoa/sample by two operators, rendering a total of 400 scored spermatozoa.

Semen Leucocyte Analysis by Flow Cytometry

Leucocyte subpopulation analysis was performed in total semen single-cell suspensions obtained after washing semen aliquots with 10% fetal bovine serum, 2 mM EDTA, and 50 mM 2-mercaptoethanol supplemented Roswell Park Memorial Institute (RPMI)-1640 medium. Cell suspensions were stained with fluorescent-labelled antibodies to cluster of differentiation (CD)45, CD3, CD4 and CD19 (BioLegend, San Diego, CA, USA), and to CD14 (eBioscience, San Diego, CA, USA). Cells were acquired and analysed by fluorescence-activated cell sorting/flow cytometry (FACS) on FACSCanto II cytometer (BD Biosciences). A total of 100 000 cells/sample were analysed.

Cytokine and PSA Quantification

Interferon (IFN) γ , interleukin (IL)-10, IL-8, IL-17A and IL-1 β concentrations in SP or culture supernatants were analysed using ELISA specific kits. IFN γ , IL-10 and IL-8 were respectively quantified by the BD OptEIA™ Human IFN γ , IL-8 and IL-10 ELISA sets (BD Biosciences, cat. # 555142, 5126542 and 555157). IL-17A and IL-1 β were respectively assayed by the Human IL-17A ELISA Ready-SET-Go and Human IL-1 β ELISA Ready-SET-Go kits (eBioscience, cat. # 88-7010 and 88-7176). Serum PSA concentrations were determined using chemiluminescence immunometric assay kits (Immulite, Malvern, PA, USA).

Sperm apoptosis/necrosis

Sperm apoptosis/necrosis was assessed immediately after semen liquefaction by annexin V/propidium iodide staining and analysed by flow cytometry as previously described [18].

Statistics

Statistical analysis was performed using the Mann–Whitney test. A $P < 0.05$ was considered statistically significant. Compromise power analyses were calculated using G*Power3 data analysis software. Considering ρ : 0.5 and α : 0.05, the statistical power of the study ($1-\beta$) was 0.68.

Results

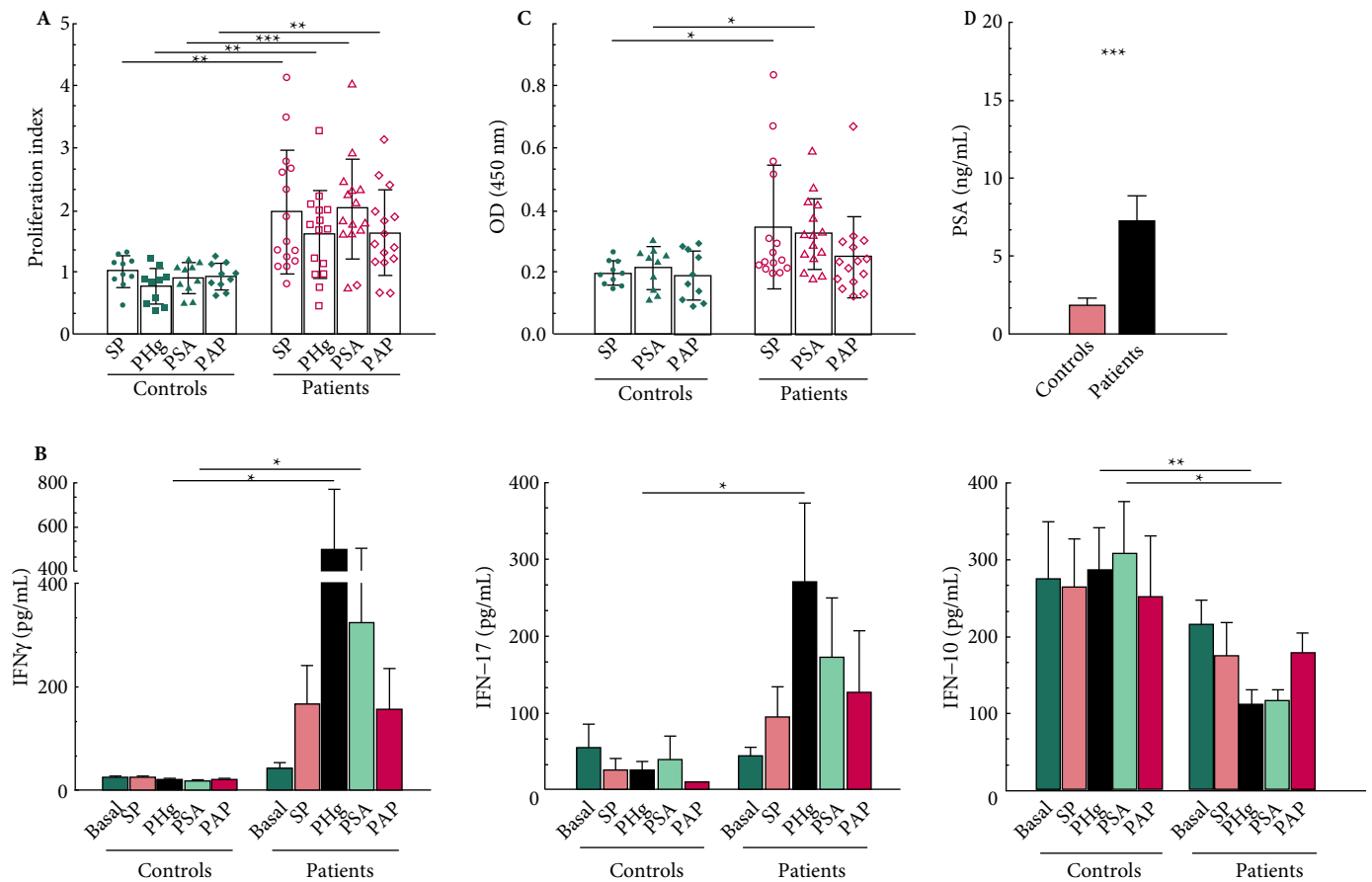
Detection of PAg-specific T helper type 1 (Th1) and Th17 immune responses in patients with CP/CPPS

To search for the presence of immune reactivity against PAg in patients with CP/CPPS, we analysed peripheral blood immune responses in the patients and controls. A total of 15 patients [mean (SD, range) age 40.3 (7.4, 30–55) years] and 10 controls [mean (SD, range) age: 37.7 (6.4, 24–45) years] were included. As shown in **Figure 1A**, significantly greater proliferative responses were detected when PBMC from patients with CP/CPPS were *in vitro* stimulated with different sources of PAg (SP or PHg) or purified PAg (PSA and PAP) vs controls. Indeed, 53.3% (eight out of 15) of the patients had positive lymphoproliferative responses to one or more of the PAg assayed. Moreover, when assaying PAg-specific cytokine secretion in culture supernatants, higher levels of specific IFN γ and IL-17 secretion to one or more of the PAg assayed were detected in cell cultures from the patients with CP/CPPS compared with the controls (**Figure 1B**). Conversely, lower levels of PSA- and PAP-specific IL-10 secretion were observed (**Figure 1B**). On the other hand, patients with CP/CPPS had higher serum levels of SP-, PSA-, and PAP-specific IgG autoantibodies than the controls (**Figure 1C**). Also, significantly higher levels of serum PSA were found in patients with CP/CPPS than in the controls, suggesting a state of inflammation of the prostate (**Figure 1D**). These results indicate that patients with CP/CPPS have Th1 and Th17 self-reactive immune responses and also autoantibodies specific to PAg.

Patients With CP/CPPS Show Local Inflammation in the Male Genital Tract

As shown in **Figure 2A**, flow cytometry analysis of semen samples revealed that the patients with CP/CPPS had significantly more leucocytes in their semen when compared with the controls. When analysing leucocyte subpopulations, macrophages and CD4⁺ T helper cells were the most

Fig. 1 Self-reactive Th1 and Th17 immune responses specific to PAg in patients with CP/CPPS. (A) Lymphoproliferative responses of PBMC from patients with CP/CPPS and age-matched control subjects to different SP-derived and PAg; dilutions (1:25) of SP obtained from pooled donor samples having a normal semen analysis, PHg, PSA, PAP, or medium alone. Lymphoproliferative responses were expressed as PI calculated from counts/min (cpm) incorporated in antigen-pulsed cultures/cpm incorporated in cultures with medium. (B) $\text{INF}\gamma$, IL-17 and -10 secretion of PBMC from patients with CP/CPPS and age-matched control subjects cultured with the same PAg sources assayed by sandwich ELISA. (C) Serum levels of IgG antibodies specific to SP, PSA and PAP in patients with CP/CPPS and age-matched control subjects examined by indirect ELISA in 1:50 serum dilutions. Serum reactivity was expressed as optical density (OD) at 450 nm. (D) Serum levels of PSA in patients with CP/CPPS and age-matched control subjects assayed by chemiluminescence immunoassays and results expressed as ng/mL. Experiments were performed at least in triplicate and repeated twice with similar results. Data are shown as mean \pm SD; patients with CP/CPPS, $n = 15$; age-matched control subjects, $n = 10$. Mann-Whitney test; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.005$.



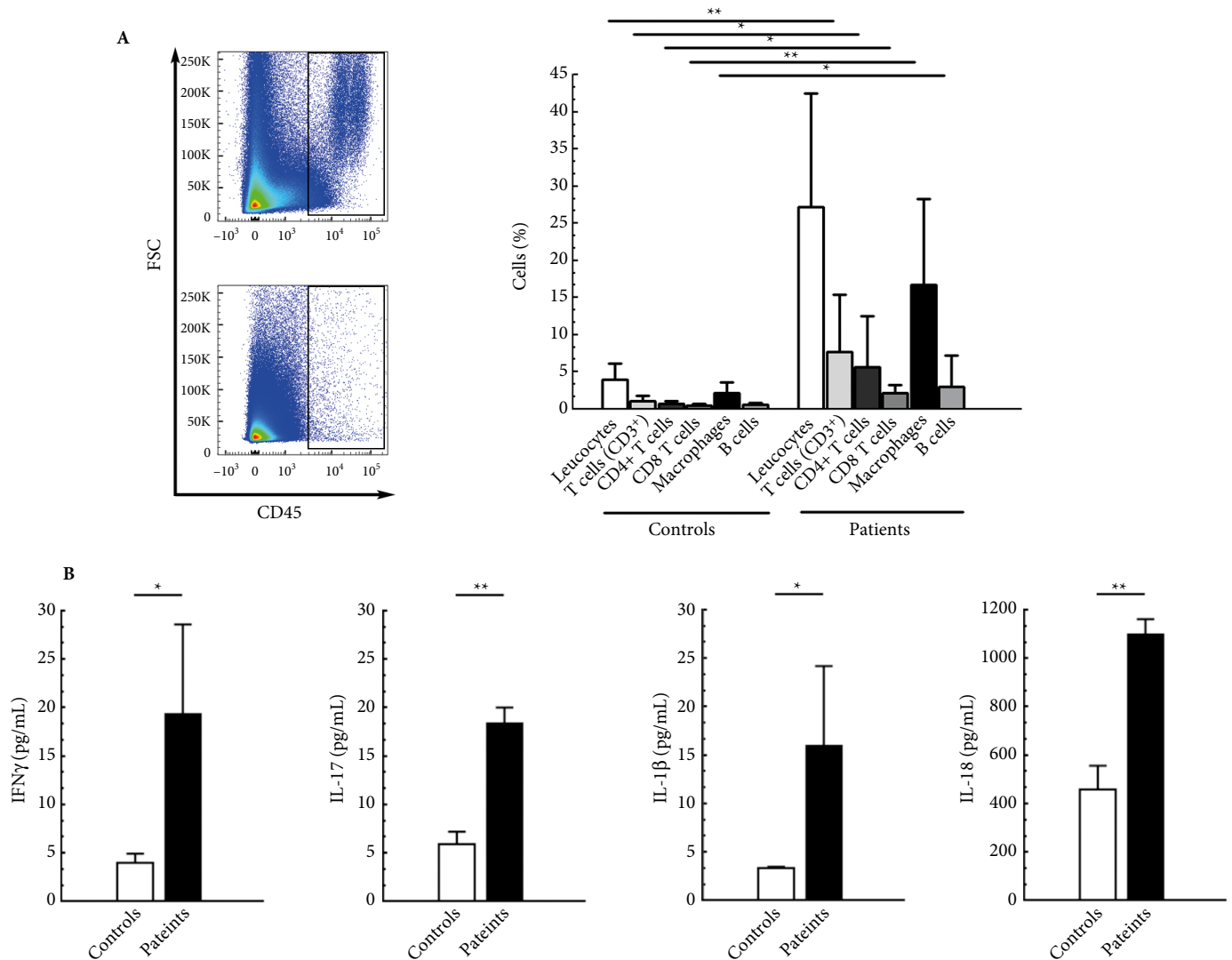
prominent cell subsets. Moreover, significantly higher levels of inflammatory cytokines, such as $\text{INF}\gamma$, IL-17, IL-1 β and IL-8, were detected in the SP from patients with CP/CPPS compared with the controls (Figure 2B). These results indicate a state of chronic inflammation in the male genital tract of patients with CP/CPPS, probably due to PAg-reactive immune responses.

Patients With CP/CPPS Have Decreased Semen Quality

To analyse if local male genital tract inflammation was associated with diminished semen quality, we performed semen analysis in the patients and controls. No significant

alterations in semen volume, pH values, fructose levels and peroxidase-positive cell concentrations were found in the patients with CP/CPPS compared with the controls. Moreover, no presence of anti-sperm antibodies was observed in either the patients or controls (Table 1). Conversely, significantly lower seminal citrate levels were detected in the patients than in the controls (Table 1). These data indicate that the inflammatory milieu in the urogenital tract in patients with CP/CPPS might impair prostate physiology. Sperm quality parameters analysis showed significantly lower sperm concentration, motility and viability, and higher counts of round cells in the patients with CP/CPPS vs controls (Table 1). Indeed, when sperm apoptosis/necrosis was assessed, significantly lower levels of live spermatozoa and

Fig. 2 Evidence of local inflammation in the male genital tract of patients with CP/CPPS. (A) Frequencies of leucocyte and different leucocyte cell subsets in semen samples from patients with CP/CPPS and age-matched control subjects. Total leucocytes (CD45+), T lymphocytes (CD3+), helper T lymphocytes (CD3+ CD4+), cytotoxic T lymphocytes (CD3+ CD4-), macrophages (CD14+) and B lymphocytes (CD19+) were assayed in semen samples by flow cytometry. Analyses were performed in gates on the total leucocyte cell population (CD45+) using forward side scatter (FSC) vs CD45 dot plots. A total of 100 000 cells/events per specimen were analysed. Results were expressed as % of cells. (B) INF γ , IL-17, IL-1 β and IL-8 levels in SP samples from patients with CP/CPPS and age-matched control subjects assayed by sandwich ELISA. Experiments were performed in triplicate and repeated three-times with similar results. Data are shown as mean \pm SD; patients with CP/CPPS, $n = 15$; age-matched control subjects, $n = 10$. Mann-Whitney test; * $P < 0.05$ and ** $P < 0.01$.



higher levels of early apoptotic and late apoptotic/necrotic spermatozoa were found in the patients vs controls (Figure 3).

Overall, these results indicate that patients with CP/CPPS show Th1- and Th17-associated self-immune responses specific to PAG and higher levels of inflammatory cytokines and leucocytes in their semen. Thus, the patients had local inflammation with lower levels of biomarkers of prostate physiology and reduced semen quality.

Discussion

Although CP/CPPS is the most common type of prostatitis and genitourinary problem in adult males aged <50 years, it is still an enigmatic disease mostly due to its uncertain aetiology. That makes its diagnosis of exclusion, inadequate and cumbersome. Moreover, therapies are empirical and results ineffective for most patients [5]. Over the past decade, chronic inflammation has been explored as underlying

Table 1 Semen analysis in patients with CP/CPPS

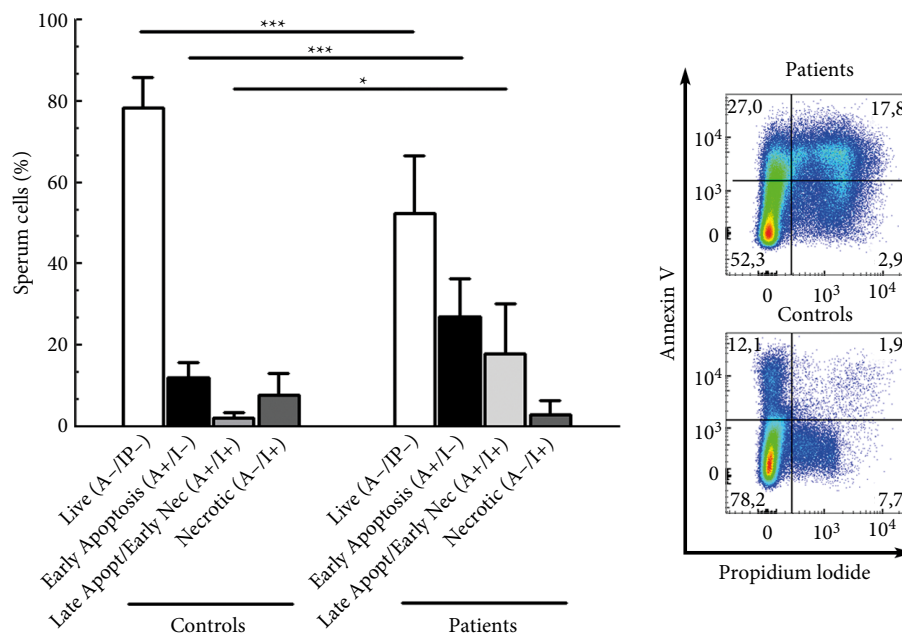
Variable, mean (SD)	Controls	Patients	P
Demographics			
Age, years	37.7 (6.4)	40.3 (7.4)	0.540
Abstinence, days	4.6 (3.2)	5.4 (2.4)	0.411
Semen analysis			
Volume, mL	3.2 (1.2)	4.1 (2.0)	0.438
pH	7.7 (0.1)	7.6 (0.2)	0.302
Concentration, $\times 10^6$ /mL	136.3 (84.6)	57.0 (58.0)	0.040
Total motility, %	65.2 (12.2)	43.1 (19.4)	0.043
Normal morphology, %	10.3 (6.4)	7.1 (3.5)	0.303
Viability, %	89.5 (5.6)	82.1 (5.9)	0.034
Round cells, $\times 10^6$ /mL	0.48 (0.28)	1.58 (0.91)	0.012
Peroxidase (+) cells, $\times 10^6$ /mL	0.19 (0.23)	0.81 (1.16)	0.386
Citrate, mg/dL	402.9 (54.1)	319.4 (98.1)	0.026
Fructose, mg/dL	349.9 (109.5)	334.2 (134.3)	0.971
Anti-sperm antibodies (ASA)*			
IgG, % sperm agglutination	0.67 (0.81)	1.64 (5.10)	0.260

*Mann-Whitney test; bolded values indicate statistical significance (P < 0.05).
Assayed by direct SpermMAR test.

pathogenic mechanism in CP/CPPS [6]. Prostate biopsies revealed chronic inflammation in a considerable proportion of patients and histological prostatitis was associated with a shorter time to symptom progression [19,20]. Schwartz *et al.* [21] reported that prostate inflammation significantly increased urinary voiding frequency, induced hypersensitivity

to bladder distention, and sensitised bladder nerve afferents, indicating cross-organ sensitisation and explaining the urinary symptoms features of CP/CPPS. Although cryptic or difficult-to-culture micro-organisms have been suggested as putative causative factors, studies have systematically failed to identify infectious agents as causative of this syndrome [22]. Moreover, most patients' symptoms are refractory to antibiotic monotherapy [9]. Nonetheless, if not pathogenic, infections could be the initiating factor of dysregulated inflammation in the form of autoimmunity [23]. An autoimmune basis for CP/CPPS is a prominent theory based on evidence obtained from studies in patients and animal models [6,24]. Such animal models mirror most features of human CP/CPPS and have shown that prostate inflammation induction is mediated by T-cell responses specific to PAg. In addition, they have shown that prostate inflammation induces chronic pelvic pain development and has deleterious effects on semen quality compromising male fertility [6,25]. However, compelling available data from studies in patients are limited [6,25,26]. In the present study, we provide novel evidence indicating that Th1 and Th17 self-reactive immune responses specific to PAg are detected in a considerable proportion of patients with CP/CPPS, which may be responsible for the observed inflammation of the male genital tract. Significantly increased lymphoproliferative responses

Fig. 3 Patients with CP/CPPS show increased levels of sperm apoptosis/necrosis. Sperm apoptosis/necrosis was evaluated by annexin V (AV)/propidium iodide (PI) staining and FACS. Frequencies of live (AV−, PI−), early apoptotic (AV+/PI−), late apoptotic/early necrotic (AV+/PI+) and late necrotic (AV−/PI+) spermatozoa in semen from patients with CP/CPPS and age-matched control subjects. Left, representative dot plots showing the different labelling patterns in the bivariate PI-AV analysis that identified the different cell populations designated as viable, early apoptotic cells, late apoptotic cells/early necrotic, and necrotic cells. Results are expressed as % of cells/100 000 events. Experiments were performed at least in triplicate and repeated three-times with similar results. Data are shown as mean \pm SD; patients with CP/CPPS, n = 15; age-matched control subjects, n = 10. Mann-Whitney test; *P < 0.05 and ***P < 0.005.



were seen in patients when their PBMC were *in vitro* stimulated with different SP-derived or purified PAg. Moreover, increased levels of PAg-specific IFN γ and/or IL-17 secretion were associated with these responses indicating a Th1/Th17 mixed immune phenotype. In addition, these PAg-reactive immune responses were concomitant with the presence of local inflammation of the urogenital tract evidenced by more macrophages/monocytes and CD4⁺ T lymphocytes, high levels of IFN γ , IL-17, and other pro-inflammatory cytokines, such as IL-1 β and IL-8, in the semen. Moreover, these patients had low seminal levels of citric acid, indicative of prostate dysfunction, and alterations in sperm quality such as low sperm concentration, motility, and viability, and higher levels of sperm apoptosis/necrosis.

Our present results are in agreement with the scarce reported data [27,28]. Indeed, Kouivaskaia *et al.* [29] showed that patients with CP/CPPS have increased frequencies of CD4⁺ T cells specific to PAP. Moreover, it has been recently reported that 30% of patients with IgG4-related disease have symptoms of CP/CPPS and histopathological and immunohistochemical evidence of prostatitis, which improve after treatment with corticosteroids or immunosuppressive drugs [7]. Noteworthy, these patients typically develop autoimmune pancreatitis [7]. Also, Lu *et al.* [30] reported that a significant proportion of patients with CP/CPPS have autoantibodies against different immunodominant prostate proteins, which were able to induce autoimmune prostatitis in mice upon immunisation. Besides, and as mentioned above, most patients with APS1 present elevated serum levels of autoantibodies to TGM4, indicating that prostate autoimmunity is a feature of APS1 that could also be involved in male subfertility [10]. Chronic inflammation in the form of autoimmunity develops when there is continuous and uncontrolled induction and activation of the immune response to self-antigens, which then causes persistent injury and tissue damage. As the inflammatory process is not interrupted, the production of inflammatory mediators such as cytokines, chemokines and reactive oxygen species persists. Autoimmune inflammation is sustained due to persistent expression of self-antigens and by the lack of efficient control by regulatory immune cell populations (e.g. regulatory T cells), which usually bias the immune response to a destructive Th1/Th17 phenotype. In particular, Th1 cells secrete IFN γ , a cytokine that causes activation of macrophages, which have a dominant role in chronic inflammation, as they contribute by secreting cytokines and growth factors, such as IL-1 β , IL-8, IL-23 and IL-12, further enhancing inflammation by activating other cells, in particular T cells [31]. In fact, Th1 and Th17 cells are known to drive the pathogenesis of many autoimmune diseases [32]. In addition, IL-17 has been shown to be involved in inflammatory and neuropathic pain induction [33]. Neurogenic processes, immune injury and inflammatory

mediators have been proposed to trigger pain development in CP/CPPS [6,25]. In that regard, Th1 cells driving prostate inflammation have been shown to mediate prostate inflammation induction and chronic pelvic pain development in an animal model of CP/CPPS [18]. In addition, Roman *et al.* [34] recently showed that prostate inflammation induces neuropathic changes and chronic pelvic pain through the non-selective cation channel transient receptor potential vanilloid 1 (TRPV1). Remarkably, IL-17 has also been postulated to play a key role in the induction of chronic pelvic pain in another animal model of CP/CPPS [35]. Our present results revealed the presence of Th1 and Th17 immune responses specific to PAg and increased SP levels of IFN γ and IL-17 in patients with CP/CPPS. Altogether, this evidence further supports the notion that IFN γ and IL-17 may be involved in the onset and progression of inflammation and chronic pelvic pain development in CP/CPPS.

The prostate is the major male accessory gland and secretes several factors that exert crucial functions for human reproduction [25,36]. Therefore, it is reasonable to hypothesise that inflammation of the prostate could alter male fertility potential [36]. In fact, prostatitis has been postulated as a potential risk factor for male infertility [11,25]. However, the impact of prostatitis on semen quality remains controversial, as conflicting results have been reported [36]. Although some reports have shown a negative impact of CP/PPS on sperm quality [37–39], other studies did not reveal any difference in sperm quality parameters between patients with CP/CPPS and controls [40,41]. Our present results showed that patients with CP/CPPS had increased levels of macrophages, T lymphocytes and inflammatory cytokines in their semen, and alterations in semen quality parameters. Flow cytometry analysis revealed increased levels of leucocytes, mostly macrophages and T lymphocytes, in the semen from patients with CP/CPPS. However, cytochemical analysis of those semen samples revealed no increases in peroxidase-positive cells. These results highlight the need to properly interpret negative results when assaying leucocytes in semen by the peroxidase assay, as it mainly identifies neutrophils but no other leucocyte subsets. In addition, patients with CP/CPPS had sperm quality alterations such as lower sperm concentration, motility and viability associated with elevated levels of IFN γ , IL-17, IL-1 β and IL-8, and more sperm apoptosis/necrosis. Cytokines such as tumour necrosis factor α , IL-1 β , IL-6, and IL-8 have already been reported to be elevated in patients with CP/CPPS [42–44]. Moreover, IL-6 and IL-8 have been proposed as biomarkers of urogenital inflammation [45–47]. Nonetheless, and to the best of our knowledge, we report for the first time elevated levels of IL-17 and IFN γ in urogenital secretions from patients with CP/CPPS [42].

Although our present results are consistent with cumulative reported evidence, they should be interpreted with caution, as the statistical power of the study is limited. Additional studies including larger patient populations are needed to further support our present data.

In conclusion, our present results provide novel evidence indicating that patients with CP/CPPS show Th1 and Th17 self-reactive immune responses to PAg, associated with inflammation of the male genital tract and reduced semen quality. Our present results suggest that dysregulated inflammation in the form of autoimmunity may underlie the induction and development of chronic pelvic pain and inflammation of the male genital tract in CP/CPPS. Moreover, the local inflammatory milieu could alter normal prostate functioning and impair semen quality, thus compromising male fertility potential.

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Conflict of interest

None declared.

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Abbreviations: APC, antigen-presenting cells; APS1, autoimmune polyendocrine syndrome type 1; CP/CPPS, chronic prostatitis/chronic pelvic pain syndrome; FACS, fluorescence-activated cell sorting/flow cytometry; IFN, interferon; IL, interleukin; NIH-(CSPI), National Institutes of Health (Chronic Prostatitis Symptoms Index); PAg, seminal plasma-derived and prostate antigens; PAP, prostate acid phosphatase; PBMC, peripheral blood mononuclear cells; PHg, prostate homogenate; PI, proliferation index; SP, seminal plasma; Th(1)(17), T helper type (1)(17).