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Differential expression of immune factor between patients with chronic prostatitis/chronic pelvic pain syndrome and the healthy volunteers

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Abstract

Purpose Immune mechanisms have been hypothesized to contribute to the development of CP/CPPS. In this study, we investigated the differential expression of immune factors between patients with CP/CPPS and healthy volunteers.

Methods This study was registered in Australian New Zealand Clinical Trials Registry. Healthy volunteers and patients with CP/CPPS were enrolled in this study. The inclusion criteria for patients were below: (1) aged 18–45 years old; (2) prostatitis-related syndrome longer than 3 months; (3) normal routine urine culture and negative bacterial culture in prostatic fluid. Patients were further classified into two groups: types IIIA and IIIB CP/CPPS according to the results of EPS routine test. Serum immune markers include IgA, IgM, IgG, $CD4^+$ and $CD8^+$.

Results There are total 23 CP/CPPS patients, including 12 type IIIB and 11 type IIIA. Relatively, there are 26 healthy volunteers. The serum levels of IgG were higher in CP/CPPS patients compared to healthy volunteers (1141.2 ± 204.3 vs 1031.9 ± 173.7 mg/L, $p = 0.045$), while the serum levels of $CD8^+$ were lower in CP/CPPS patients compared to healthy volunteers (492.8 ± 185.6 vs 640.0 ± 246.8 cells/μL, $p = 0.021$). Furthermore, serum levels of IgG were higher in patients with IIIA CP/CPPS compared to those with IIIB (1244.3 ± 151.6 vs 1054.3 ± 209.3 mg/L, $p = 0.023$).

Conclusions Differential levels of IgG and $CD8^+$ between CPPS patients and healthy volunteers suggest a contributing role of immune mechanisms to the development of CP/CPPS; and IgG may play an important role in inflammatory CPPS.

Clinical Study registration number ACTRN12613000792729.

Keywords Chronic prostatitis · Chronic pelvic pain syndrome · Immune

Abbreviations

CP/CPPS	Chronic prostatitis/chronic pelvic pain syndrome
EPS	Expressed prostatic secretions
NIH-CPSI	NIH-Chronic Prostatitis Symptom Index
IFN-γ	Interferon-γ
IL	Interleukin
IDD3	Insulin-dependent diabetes susceptibility 3
CTLA4	Cytotoxic T lymphocyte antigen 4
$CD4^+$	$CD4^+$ T lymphocyte cell
$CD8^+$	$CD8^+$ T lymphocyte cell

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Introduction

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), accounting for over 90% of all prostatitis diagnosis, affects approximately 2–15% of adult men in different countries [1, 2]. The considerable discomfort or pain of CP/CPPS reduces patients' life quality significantly and lays a huge economic burden (e.g., the direct cost of CP/CPPS care is approximately $4000 a year per patient in USA) [3].

This is mainly ascribed to the complex etiologies and lack of effective treatments for CP/CPPS.

Recently, immune response and autoimmunity activation have been hypothesized to contribute to the development of CP/CPPS [4–6]. Several studies suggested that immune maladjustment led to the damage of self-tolerance to prostatic antigens and development of proinflammatory immune response [7–9]. However, most results are based on animal models; and the results from human studies based on semen and expressed prostatic secretions (EPS) sample are inconsistent. In this study, we investigated the differential expression of immune factors between the patients with CP/CPPS and the healthy volunteers, to gain further insights into the potential roles of immune mechanisms in the development of CP/CPPS.

Patients and methods

Patients and trial design

This study was registered in Australian New Zealand Clinical Trials Registry (ANZCTR) (Trial ID: ACTRN12613000792729) and approved by the Ethics Committee of Changhai hospital. Healthy volunteers and patients with CP/CPPS were enrolled in this study. All participants involved in this study signed the informed consent. Healthy volunteers were recruited from men who had routine health examination in our hospital and who had good physical health, while the patients with CP/CPPS were enrolled via outpatient urology consultation in the same period.

For CP/CPPS patients, the inclusion criteria were below: (a) aged 18–45 years old; (b) prostatitis-related syndrome longer than 3 months; (c) normal routine urine culture and negative bacterial culture in prostatic fluid. The exclusion criteria included: (a) CP/CPPS types I, II, or IV; and (b) the presence of other pelvic diseases that may explain the prostatitis-like symptoms; (c) history of bacterial prostatitis; (d) recent history of vaccination; (e) autoimmune disease; and (f) recent infections. For CP/CPPS patients, the symptom duration, treatment history, NIH-Chronic Prostatitis Symptom Index (NIH-CPSI) scores and EPS routine test were evaluated. Patients were further classified into two groups: types IIIA and IIIB CP/CPPS according to the results of EPS routine test. More than or equal to 10 leukocytes per high-power field in EPS were classified as type IIIA, and < 10 were classified as type IIIB. Before EPS collection, the patients were required to abstinence for more than 5 days. We also cleaned and disinfected patients' penis and meatus before EPS collection.

Serum immune markers including IgA, IgM, IgG, $CD4^+$ and $CD8^+$ were assayed in both patients and healthy volunteers. We assayed $CD4^+$ and $CD8^+$ through flow cytometry using BD FACS CatoII with six-color TBNK kit (BD biosciences, USA). Immunoglobulin panel (IgA, IgM, IgG) was detected using Modular P (Roche, Switzerland). All the tests followed the manufacturers' protocols.

Statistical analysis

SPSS Statistics 19 software was used for data processing. Statistical analysis was performed using Student *t* test and Mann–Whitney. Statistical significance was defined as p value < 0.05.

Results

A total of 23 CP/CPPS patients and 26 healthy volunteers were enrolled into the study. The age of patients and volunteers was 26.6 ± 3.4 and 26.8 ± 6.0 years, respectively ($p > 0.05$). The symptom duration time was 26 ± 9.4 months of CP/CPPS patients, and the NIH-CPSI score was 21.3 ± 4.4. None of the patients were taking a-receptor blockers for the treatment of CP/CPPS.

Of these 23 CP/CPPS patients, 12 patients were classified as having type IIIB CP/CPPS, while the other 11 patients had IIIA CP/CPPS. The serum levels of IgA, IgM, IgG, $CD4^+$ and $CD8^+$ in healthy volunteers and the patients are shown in Table 1. The serum levels of IgG were higher in CP/CPPS patients compared to healthy volunteers (1141.2 ± 204.3 vs 1031.9 ± 173.7 mg/L, $p = 0.045$), while the serum levels of $CD8^+$ were lower in CP/CPPS patients compared to healthy volunteers (492.8 ± 185.6 vs 640.0 ± 246.8 cells/μL, $p = 0.021$).

Furthermore, the serum levels of IgA, IgM, IgG, $CD4^+$ and $CD8^+$ were compared between patients with IIIA and IIIB CP/CPPS, and the results are shown in Table 2.

Table 1 Differential level of serum markers in both CP/CPPS patients and healthy volunteers

	IgA (mg/L)	IgM (mg/L)	IgG (mg/L)	$CD4^+$ (cells/μL)	$CD8^+$ (cells/μL)
CPPS ($n = 23$)	237.1 ± 72.1	104.8 ± 48.1	1141.2 ± 204.3	619.4 ± 142.9	492.8 ± 185.6
Volunteers ($n = 26$)	202.2 ± 68.4	107.6 ± 41.9	1031.9 ± 173.7	727.1 ± 227.8	640.0 ± 246.8
p	0.071	0.711	0.045*	0.105	0.021*

Values are expressed as mean ± SD. Mann–Whitney *U* was used for statistical analysis

*$p < 0.05$

Table 2 Differential level of serum markers between baseline patients with IIIA and IIIB CP/CPPS

	IgA (mg/L)	IgM (mg/L)	IgG (mg/L)	$CD4^+$ (cells/μL)	$CD8^+$ (cells/μL)
IIIA ($n = 11$)	252.8 ± 69.1	103.6 ± 41.8	1244.3 ± 151.6	630.0 ± 130.5	522.5 ± 169.4
IIIB ($n = 12$)	222.6 ± 74.8	106.0 ± 55.1	1054.3 ± 209.3	612.4 ± 159.0	465.5 ± 202.8
p	0.242	0.806	0.023*	0.902	0.325

Values are expressed as mean ± SD. Mann–Whitney U was used for statistical analysis

*$p < 0.05$

Serum levels of IgG were higher in patients with IIIA CP/CPPS compared to those with IIIB (1244.3 ± 151.6 vs 1054.3 ± 209.3 mg/L, $p = 0.023$). However, there was no significant difference in IgA, IgM, $CD4^+$ and $CD8^+$ between the two groups.

Discussion

The pathogenesis of CP/CPPS is unknown. Infection, trauma and stress have been reported to be involved in the development of CP/CPPS [4]. Although CP/CPPS has been considered as a bacterial chronic prostatitis, evidences showed that *E. coli* and helicobacter pylori antibodies can be detected in EPS and serum of those patients, respectively [4]. Furthermore, it has been reported that a strain of *E. coli* (strain CP1), isolated from the EPS of a CP/CPPS patient, could induce bacterial prostate colonization in non-obese diabetic (NOD) mice [10]. The pathogenesis CP/CPPS may be multifactorial, including infection, cell immune response, involvement of mast cells and activation of peripheral neurons. Thus, bacterial infection might play an important role in disease initiation, followed by cell immunity, activation of mast cells and peripheral neurons.

Recently, more studies have reported the roles of immune response and autoimmunity activation in the progression of CP/CPPS. In the murine models, $CD4^+$ T cells, such as IFN-γ-producing Th1 cells and IL-17A-producing Th17 cells, could contribute to the development of pain even in the absence of a persistent bacterial infection [11]. Moreover, investigators further identified that mutations of insulin-dependent diabetes susceptibility 3 (IDD3) and cytotoxic T lymphocyte antigen 4 (CTLA4), both genes associated with the T-cell development and regulation, increase the risk of autoimmunity in experimental autoimmune prostatitis murine models [12, 13]. In addition, the loss of self-tolerized T_{reg} cells and autoimmune regulator (AIRE) transcription factor might drive inflammation in CP/CPPS [14]. As a fact, TGF-β, TNF-α, IL-10, IL-6, MCP1 and MIP1a have been reported to be closely related to the development and progression of CP/CPPS [15].

Moreover, mast cells identified as the main mediator of T_{reg} and Th17 cells have been reported to play an important role in regulation of cellular immunity with progression of prostatitis [16]. Activation of peripheral neurons responsible for chronic pelvic pain is also closely associated with mast cells [17, 18]. Despite the complex multifactorial etiology of CP/CPPS (infection, cell immune response, activation of mast cells and peripheral neurons), the cell immune response appears to be the core of multiple factors contributing to CP/CPPS. Nevertheless, the potential mechanism of cell immune involved in progression of CP/CPPS is still unknown.

In our study we investigated the differential serum levels of IgA, IgG, IgM, $CD4^+$ and $CD8^+$ in healthy volunteers and CPPS patients. These immune factors were chosen for analysis primarily because they are closely associated with autoimmunity and can be easily tested. Compared to healthy volunteers, we found that the CP/CPPS patients had lower serum levels of $CD8^+$ and higher serum levels of IgG. It seems that both cell immunity and humoral immunity are involved in the development of CP/CPPS. These results suggested that immune-related mechanisms may be involved in CP/CPPS and immunomodulatory drugs may be tested as a potential treatment for CP/CPPS.

IgG is the most common type of serum antibodies in humans. It is widely distributed in respiratory, gastrointestinal and urogenital mucosa and protects the body from infection. However, the abnormal increase in IgG is associated with autoimmune diseases, such as autoimmune hepatitis and rheumatoid arthritis [19, 20]. Furthermore, previous articles also suggest that IgG can activate or enhance activation of mast cell and further induce proinflammatory cytokines expression and secretion, which is closely associated with inflammatory response [21, 22]. For CP/CPPS, distinguishing type IIIA from IIIB often confused us. Although we usually distinguish type IIIA and IIIB based on the amount of leukocytes in EPS, it may be artificial since both types show the increase of multiple differential proinflammation cytokines [23]. In this study, our results not only showed that the serum level of IgG was higher in CP/CPPS patients compared to healthy volunteer, but also is higher in IIIA compared to IIIB. So, maybe increased IgG plays important roles as an upstream inflammatory trigger in CP/CPPS. Although mast cell also could be activated through other signaling pathways [24], we think that the level of IgG could help us better distinguish between types IIIA and IIIB in clinical work.

A subset of $CD8^+$ cells with the innate non-antigen-specific capabilities can lead to autoimmunity [25]. Moreover, mast cells could be activated to secrete chemokines, which could recruit effector $CD8^+$ cells [26]. Thus, $CD8^+$ are usually recruited to infected tissues via chemokines release [27]. Accordingly, histological examination of prostate inflammation shows increased numbers of $CD8^+$ compared with normal controls [11, 28]. In this study, we further found decreased serum levels of CD8 + in CP/CPPS patients but no significant difference of serum levels of $CD8^+$ between IIIA and IIIB. Obviously, the decreased level of $CD8^+$ cells related to autoimmunity seems to contribute the progression of CP/CPPS. However, no further differential level of $CD8^+$ cells between IIIA and IIIB suggests the possible involvement of cell immune based on $CD8^+$ in both groups. Furthermore, a lack of the $CD8^+$ level of prostate tissue hindered us from understanding the role of $CD8^+$ in CP/CPPS. Anyhow, the detail mechanism needs further investigation.

There are several limitations of the study. First, the sample size is not large. Second, the normal reference levels of these immune factors in Chinese population were not yet established, even though we have demonstrated differences between CP/CPPS patients and healthy volunteers. Finally, we find immune factors involved in CP/CPPS but still need to further investigate the detail mechanism.

Conclusions

Differential levels of IgG and $CD8^+$ between CP/CPPS patients and healthy volunteers suggest the contributing role of immune mechanisms to the development of CP/CPPS. The differential levels of IgG between patients with IIIA CP/CPPS and those with IIIB further indicate that humoral immune response based on IgG maybe plays a more important role in the inflammatory changes of CP/CPPS than cell immune response.

Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Liang CZ, Li HJ, Wang ZP, Xing JP, Hu WL, Zhang TF et al (2009) The prevalence of prostatitis-like symptoms in China. J Urol 182:558–563
2. Ferris JA, Pitts MK, Richters J, Simpson JM, Shelley JM, Smith AM (2010) National prevalence of urogenital pain and prostatitis-like symptoms in Australian men using the National Institutes of Health Chronic Prostatitis Symptoms Index. BJU Int 105:373–379
3. Schaeffer AJ (2008) Epidemiology and evaluation of chronic pelvic pain syndrome in men. Int J Antimicrob Agents 31(suppl 1):S108–S111
4. Pontari MA (2013) Etiology of chronic prostatitis/chronic pelvic pain syndrome: psychoimmunoneurendocrine dysfunction (PINE syndrome) or just a really bad infection. World J Urol 31:725–732
5. Murphy SF, Schaeffer AJ, Thumbikat P (2014) Immune mediators of chronic pelvic pain syndrome. Nat Rev Urol 11:259–269
6. Thakkinstian A, Attia J, Anothaisintawee T, Nickel JC (2012) α-blockers, antibiotics and anti-inflammatories have a role in the management of chronic prostatitis/chronic pelvic pain syndrome. BJU Int 110:1014–1022
7. Krieger JN, Thumbikat P (2016) Bacterial prostatitis: bacterial virulence, clinical outcomes, and new directions. Microbiol Spectr 4(1). http://doi.org/10.1128/microbiolspec.UTI-0004-2012
8. Motrich RD, Breser ML, Sánchez LR, Godoy GJ, Prinz I, Rivero VE (2016) IL-17 is not essential for inflammation and chronic pelvic pain development in an experimental model of chronic prostatitis/chronic pelvic pain syndrome. Pain 157:585–597
9. Jiang Y, Cui D, Du Y, Lu J, Yang L, Li J et al (2016) Association of anti-sperm antibodies with chronic prostatitis: a systematic review and meta-analysis. J Reprod Immunol 118:85–91
10. Rudick CN, Berry RE, Johnson JR, Johnston B, Klumpp DJ, Schaeffer AJ et al (2011) Uropathogenic *Escherichia coli* induces chronic pelvic pain. Infect Immun 79:628–635
11. Quick ML, Wong L, Mukherjee S, Done JD, Schaeffer AJ, Thumbikat P (2013) Th1–Th17 cells contribute to the development of uropathogenic *Escherichia coli*-induced chronic pelvic pain. PLoS ONE 8:e60987
12. Gallegos AM, Bevan MJ (2004) Driven to autoimmunity: the nod mouse. Cell 117:149–151
13. Kim JM, Rudensky A (2006) The role of the transcription factor Foxp3 in the development of regulatory T cells. Immunol Rev 212:86–98
14. de Vries VC, Wasiuk A, Bennett KA, Benson MJ, Elgueta R, Waldschmidt TJ, Noelle RJ (2009) Mast cell degranulation breaks peripheral tolerance. Am J Transpl 9:2270–2280
15. Shoskes DA, Albakri Q, Thomas K, Cook D (2002) Cytokine polymorphisms in men with chronic prostatitis/chronic pelvic pain syndrome: association with diagnosis and treatment response. J Urol 168:331–335
16. Brown MA, Sayed BA, Christy A (2008) Mast cells and the adaptive immune response. J Clin Immunol 28:671–676
17. Woolf CJ, Salter MW (2000) Neuronal plasticity: increasing the gain in pain. Science 288:1765–1769
18. Bali KK, Venkataramani V, Satagopam VP, Gupta P, Schneider R, Kuner R (2013) Transcriptional mechanisms underlying sensitization of peripheral sensory neurons by granulocyte-/granulocyte-macrophage colony stimulating factors. Mol Pain 9:48
19. Lakos G, Soós L, Fekete A, SzabóZ Zeher M, Horváth IF et al (2008) Anti-cyclic citrullinated peptide antibody isotypes in rheumatoid arthritis: association with disease duration, rheumatoid factor production and the presence of shared epitope. Clin Exp Rheumatol 26:253–260
20. Lin S, Sun Q, Mao W, Chen Y (2016) Serum Immunoglobulin A (IgA) Level is a potential biomarker indicating cirrhosis

during chronic hepatitis B infection. Gastroenterol Res Pract 2016:2495073
21. Lechowski S, Bischoff SC, Lorentz A (2010) Role of IgG-subclasses in activation of human intestinal mast cells. Aktuelle Ernährungsmedizin 35(06):295–300
22. Lappalainen J, Lindstedt KA, Oksjoki R, Kovanen PT (2011) OxLDL-IgG immune complexes induce expression and secretion of proatherogenic cytokines by cultured human mast cells. Atherosclerosis 214(2):357–363
23. Pontari MA, Ruggieri MR (2008) Mechanisms in prostatitis/chronic pelvic pain syndrome. J Urol 179(5 suppl):S61–S67
24. Riccardo Sibilano, Barbara Frossi, Pucillo Carlo E (2014) Mast cell activation: a complex interplay of positive and negative signaling pathways. Eur J Immunol 44(9):2558–2566
25. Berg RE, Forman J (2006) The role of $CD8^+$ T cells in innate immunity and in antigen non-specific protection. Curr Opin Immunol 18:338–343
26. Orinska Z, Bulanova E, Budagian V, Metz M, Maurer M, Bulfone-Paus S (2005) TLR3-induced activation of mast cells modulates CD8 + T-cell recruitment. Blood 106:978–987
27. Ebert S, Becker M, Lemmermann NA, Büttner JK, Michel A, Taube C et al (2014) Mast cells expedite control of pulmonary murine cytomegalovirus infection by enhancing the recruitment of protective CD8 T cells to the lungs. PLoS Pathog 10(4):e1004100.10.1371
28. Breser ML, Motrich RD, Sanchez LR, Mackern-Oberti JP, Rivero VE (2013) Expression of CXCR3 on specific T cells is essential for homing to the prostate gland in an experimental model of chronic prostatitis/chronic pelvic pain syndrome. J Immunol 190:3121–3133