IL-17 exacerbates experimental autoimmune prostatitis via CXCL1/CXCL2-mediated neutrophil infiltration

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Abstract
Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a poorly understood disease. Accumulating evidence suggests that autoimmune dysfunction is involved in the development of CP/CPPS. Interleukin-17 (IL-17) is associated with the occurrence and development of several chronic autoimmune inflammatory diseases. However, the molecular mechanisms underlying the role of IL-17 in CP/CPPS are not clear. We confirmed that IL-17 was increased in the prostate tissues of experimental autoimmune prostatitis (EAP) mice. Corresponding to the increase of IL-17, neutrophil infiltration and the levels of CXCL1 and CXCL2 (CXC chemokine ligands 1 and 2) were also increased in the prostate of EAP. Treatment of EAP mice with an IL-17-neutralizing monoclonal antibody (mAb) decreased the number of infiltrated neutrophils and CXCL1 and CXCL2 levels. Depletion of neutrophils using anti-Ly6G antibodies ameliorated the inflammatory changes and hyperalgesia caused by EAP. Fucoidan, a could potent inhibitor of neutrophil migration, also ameliorate the manifestations of EAP. Our findings suggested that IL-17 promoted the production of neutrophil infiltration.

Keywords
CXCL1/CXCL2, experimental autoimmune prostatitis, IL-17, neutrophil infiltration

1 | INTRODUCTION

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a frequently occurring disease of urological morbidity in men younger than 50 years old, and it accounts for 90%–95% of all prostatitis diagnoses (Collins et al., 2002; Magistro et al., 2016; Nickel et al., 2017). It is characterized by chronic pelvic pain and symptoms of prostatic inflammation. The incidence of CP/CPPS varied from 2% to 16% in previous reports (Liang et al., 2009; Roberts et al., 1998). In China, the incidence of CP/CPPS was 8.4%, with a mean age of 34.56 ± 13.48 years (Liang et al., 2009). However, the incidence of CP/CPPS in middle-aged and elderly men in China reached 25.3% in a recent report (Zhang et al., 2019). The etiology of CP/CPPS has not been elucidated. Currently, several hypotheses to explain CP/CPPS pathogenesis was proposed, including cryptic infections, abnormal pelvic floor neuromuscular activity, and autoimmune mechanisms (Breser et al., 2017; J. Chen et al., 2019; Liu et al., 2021).

IL-17 is a pro-inflammatory cytokine mainly secreted by T helper 17 (Th17) cell subsets, and it is relevant to the occurrence and development of several autoimmune inflammatory diseases. IL-17 targets...
various inflammatory cell types and induces proinflammatory cytokines (such as IL-1β, G-CSF, GM-CSF) and chemokines (such as CXCL1 and CXCL2) in some inflammatory diseases (Moseley et al., 2003; Y. Zhang et al., 2012). The role of IL-17 in a non-infectious autoimmune-driven mouse model of CP/CPPS experimental autoimmune prostatitis (EAP), is controversial (Motrich et al., 2016; Murphy et al., 2015). Conclusions in these studies were based on C57BL/6 mice, which were proved less susceptible to EAP induction than non-obese diabetic (NOD) mice (Rivero et al., 1998). We have demonstrated that IL-17 exerted a positive effect in EAP-NOD mice (Zhan et al., 2020). This finding suggested that the levels of IL-17 in the prostate tissues from EAP mice were higher than control mice (Zhan et al., 2020). We also found that treatment with a neutralizing antibody against IL-17 decreased the level of IL-17 in prostate tissues and ameliorated the inflammatory changes and pelvic pain in EAP (Zhan et al., 2020). However, the exact mechanisms of IL-17 involvement in the inflammatory manifestations and pelvic pain in the EAP mouse model and CP/CPPS diseases are not certain.

The current study demonstrated the mechanisms of IL-17 modulation of infiltration neutrophils into prostate tissues in an EAP model. Meanwhile, the beneficial effects of fucoidans, a sulphated polysaccharide extracted from algae, on inflammatory diseases and immune dysfunction were demonstrated previously (Aleissa et al., 2020; B. R. Chen et al., 2021). Therefore, we hypothesized that fucoidans would ameliorate EAP-induced inflammation and pain symptoms and explored its possible mechanisms. These findings improve the understanding of CP/CPPS pathogenesis and therapeutic intervention.

2 | MATERIALS AND METHODS

2.1 | Mice and EAP induction

The Committee for Animal Care and Use of the Animal Center of Anhui Medical University approved the animal experiments (No. LLSC20190651). Six- to eight-week-old male NOD mice were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). All mice were maintained under specific pathogen-free conditions at the animal facility of our institution. NOD mice were induced to develop the EAP model using previously reported methods (Rivero et al., 1998; Zhan et al., 2020). Briefly, model mice were administered 0.1 ml mixed emulsion prostate antigens (PAg) and complete Freund’s adjuvant (CFA; Sigma-Aldrich) via subcutaneous injection on days 0 and 14. The control group control group was performed as described previously (Nowacki et al., 2013). Briefly, prostate tissues were fixed in 10% formalin and embedded in paraffin. The details are shown in Supplemental Figure 1.

2.2 | Tactile allodynia assessment

The mice were tested before PAg injection (baseline, day 0) and 14, 28, and 42 days after EAP induction. Referred hyperalgesia and tactile allodynia were tested using von Frey filaments application to the abdominal region near the prostate according to previous studies (Zhan et al., 2020). Three types of behaviors were considered positive responses to von Frey filament stimulation: (1) sharp retraction of the abdomen; (2) immediate licking or scratching of the area of filament stimulation; or (3) jumping. The response frequency was calculated as the percentage of positive responses, and the data were reported as means ± SEM. Tactile allodynia was measured using 50% withdrawal thresholds following stimulation with von Frey filaments, which was described as (50% threshold, g = (10[Xf + kδ])/10,000) (Chaplan et al., 1994; Christensen et al., 2020; Quick et al., 2013).

2.3 | Inflammation scoring

Prostate tissues were fixed in 10% formalin and embedded in paraffin. Five-micron sections were stained with haematoxylin and eosin (HE) and scored blindly using the histopathological classification system for chronic prostatic inflammation (Breser et al., 2013). Briefly, the extent of chronic inflammation was graded from 0 to 3: (a) 0, no inflammation; (b) 1, mild but definite perivascular cuffing with mononuclear cells; (c) 2, moderate perivascular cuffing with mononuclear cells; (d) 3, marked perivascular cuffing, haemorrhage, and numerous mononuclear cells in the parenchyma.

2.4 | Immunohistochemistry and immunofluorescence assays

Immunohistochemistry (IHC) and immunofluorescence (IF) assays were performed as described previously (Nowacki et al., 2019; Zhan et al., 2020). Briefly, prostate tissues were fixed in 10% formalin and embedded in paraffin. Prostate sections were incubated overnight at 4 °C with an anti-IL-17 (Abcam, Ab79056) primary antibodies at a dilution of 1:500. An IHC kit (ZSGB-Bio, SP9000) and 3′,3′-diaminobenzidine (ZSGB-Bio, ZLI-0918) were used for subsequent immunohistochemical analysis according to the manufacturer’s instructions.

Prostate sections were blocked with 5% bovine serum albumin and incubated overnight at 4 °C with rabbit anti-mouse Ly6G primary antibodies (1:200, Elabscience, E-AB-70094). The sections were incubated with Cy3-conjugated goat anti-rabbit IgG (1:400, Beyotime, 0.1 mg/mouse) daily for 1 week after the first and second immunizations (Gao et al., 2010). Fucoidan (Sigma-Aldrich, F5631) or its vehicle was administered via intraperitoneal injection at a dose of 20 mg/kg body weight 1 day before EAP induction and 6, 13, 20, 27, 34 and 41 days after induction (McNamee et al., 2011). The details are shown in Supplemental Figure 1.
A0516) and DAPI (1:1000, Beyotime, C1005) after washing with Tris-buffered saline (TBS). Fluorescence images were acquired using a Zeiss LSM510 confocal microscope.

### 2.5 RNA isolation and quantitative real-time PCR

Total RNA from prostate tissues was extracted using TRizol reagent according to the manufacturer's instructions. Total RNA was synthesized into cDNA using the Fast Quant RT kit (Tiangen, KR106-02). Quantitative real-time PCR was performed using Super Real Premix Plus (SYBR green) (Tiangen, FP205-02). The gene-specific primer sets used to amplify each gene were synthesized by Sangon Biotech Co., Ltd. (Shanghai) (Supplemental Table 1). Gene expression was assessed using the comparative C_T method. The results were normalized to Gapdh.

### 2.6 Analysis of chemokine and cytokine expression in prostate tissue

The concentrations of cytokines in prostate tissue homogenate samples were determined using enzyme-linked immunosorbent assays (ELISAs). The levels of IL-17 (Elabscience, E-EL-M0047c), CXCL1 (Elabscience, E-EL-M0018c), and CXCL2 (Elabscience, E-EL-M0019c) in the prostate tissues were measured using ELISA kits according to the manufacturer’s instructions.

### 2.7 Analysis of prostate-infiltrating neutrophils

Freshly harvested prostate tissues were mechanically disrupted and enzymatically digested in RPMI 1640 medium containing 1 mg/ml collagenase D (Sigma-Aldrich, C9891) and 0.05% DNase I (Sigma–Aldrich, D5025) for 45 min at 37°C. After digestion, suspensions were filtered through 75-μm cell strainers and single-cell suspensions were washed with PBS. The cells were stained with APC-conjugated anti-Ly6G (BD Bioscience, 560,599) for FACS analysis. A FACSCalibur flow cytometer (BD Bioscience) was used to analyse the stained cells, and the data were analysed by FlowJo Software X (Tree Star).

### 2.8 Analysis of MPO activity

Myeloperoxidase (MPO) is produced by activated neutrophils, and it is an established marker of neutrophil migration. MPO activity in prostate tissues was analysed by an MPO activity measurement kit (Elabscience, E-BC-K074) according to the manufacturer's instructions. MPO activity was determined by a spectrophotometer at 450 nm and expressed as U/g prostate tissues.
2.9 | Neutrophil depletion assays

For neutrophil depletion studies, we used an anti-Ly6G antibody (clone 1A8; Bio-X-Cell) (Daley et al., 2008; Sercundes et al., 2016). Briefly, EAP mice were treated with an intraperitoneal injection of 0.25 mg/dose of anti-Ly6G or control rat IgG (clone GL117, Bio-X-Cell) 1 day before EAP induction and then once weekly. Peripheral blood from mice was collected via cardiac puncture for leukocyte quantification using a HEMAVET 950 multispecies haematology cell counter.

2.10 | Statistical analysis

Statistical analysis were performed using two-tailed Student’s t test and two-way ANOVA followed by Bonferroni post hoc testing. Data are representative of three independent experiments with four mice per group. The data are expressed as means ± SEM. *p < 0.05 indicated a significant difference in the analyses. Statistical analyses were performed using SPSS software version 21.0 and GraphPad Prism 6 software.

3 | RESULTS

3.1 | IL-17 was increased in the prostate tissues of EAP mice

We first analysed the changes between control and EAP mice 42 days after the EAP first immunization. As shown in Figure 1a, pathological changes were observed in EAP mice, including a large amount of

**FIGURE 2** IL-17 mRNA and protein expression paralleled EAP-induced prostate inflammation and pain symptoms. (a) IL-17 mRNA expression in prostate tissues was measured using real-time PCR at the indicated times after the first EAP immunization. (b) IL-17 levels in prostate homogenates were determined using ELISA at the indicated times after the first EAP immunization. (c,d) The mice were sacrificed at the indicated times after the first EAP immunization and formalin-fixed prostate sections were stained with HE. Representative images of HE staining (C) and inflammation scores (D) of prostate tissue sections from the control and EAP mice at different times after EAP induction. Original magnification: ×100. Scale bars = 100 μm. (E) Chronic pelvic pain was assessed by tactile allodynia using von Frey filament stimulation at different times after the first EAP immunization. *p < 0.05, **p < 0.01. Data represent the mean ± SEM for four mice per treatment group of three independent experiments.
inflammatory cell infiltration and tissue disorder. The inflammation scores of the control and EAP groups were 0.40 ± 0.16 versus 2.50 ± 0.17, respectively (Figure 1b, *p < 0.01). Behavioural tests showed significant increases in the response frequencies to von Frey filament stimulation of EAP mice 42 days after the first immunization with forces of 0.4, 1, 2, or 4 g compared to control mice (Figure 1c, *p < 0.05). As shown in Figure 2, the IL-17 mRNA and protein expression levels, and inflammation scores, response frequency to von Frey filament stimulation in EAP mice were consistently increased during days 0–42. According to the results shown in Figure 2, we found a positive correlation between the increased expression of IL-17 in prostate tissues and the development of prostatitis in EAP mice. We tested the IL-17 expression in the prostate tissues of control and EAP mice 42 after the first immunization. The mRNA and protein levels of IL-17 in the prostate tissues were significantly increased in the EAP group compared to the control group (Figure 1d and Figure 1e, *p < 0.05). The IHC results also verified that IL-17-positive cells were increased in the prostate tissues of EAP mice compared to control mice (Figure 1f).

3.2 Neutrophil infiltration and the expression levels of CXCL1 and CXCL2 were increased in the prostate of EAP mice

Previous studies suggested that IL-17 promoted the recruitment of neutrophils to inflammatory sites (Catar et al., 2020; Eskan et al., 2012). We were interested in whether the high level of IL-17 in the prostate tissues of EAP could increased neutrophil infiltration. Therefore, we analysed neutrophil infiltration into the prostate tissues using various methods. As shown in Figure 3a, the percentage of Ly6G+ cells in prostate tissues from EAP mice was significantly higher than control mice (*p < 0.05). The IF staining results for the neutrophil marker Ly6G indicated that EAP prostate tissue sections showed significantly elevated levels of Ly6G-positive neutrophils around the prostatic glandular cavity (Figure 3b). MPO is produced by activated neutrophils, and it is an established marker of neutrophil migration. To quantitatively assess neutrophil migration, we measured MPO activity in prostate tissues from control and EAP mice. The results showed that MPO activity in prostate tissues of the EAP mice was higher than
In summary, the results shown in Figure 3a–c suggested increased neutrophil infiltration in EAP mice. Neutrophils are not direct targets of IL-17 because these cells do not express the IL-17 receptor subunit (Gelderblom et al., 2012). Neutrophils express chemokine receptors, such as CXC chemokine receptor 2 (CXCR2) and readily react to the chemokines, CXCL1 and CXCL2 (Williams et al., 2017). Neutrophil recruitment from peripheral blood is a consequence of the local production of chemokines, primarily CXCL1 and CXCL2 (Scalerandi et al., 2018), which may be elicited by IL-17 (Moseley et al., 2003; Y. Zhang et al., 2012). Therefore, we measured the mRNA and protein levels of CXCL1 and CXCL2, which are downstream of the IL-17 signalling pathway, in prostate tissues from control and EAP mice. The mRNA and protein levels of CXCL1 and CXCL2 in the prostate tissues of EAP mice were higher than control mice (Figure 3d,e, p < 0.05). Therefore, we hypothesized that IL-17 promoted neutrophil infiltration of the prostate tissues via the high levels of CXCL1 and CXCL2 in EAP mice rather than control mice.

3.3 | IL-17 neutralization reduced CXCL1/CXCL2 production and neutrophil recruitment

To verify our hypothesis that IL-17 induced neutrophil infiltration via CXCL1 and CXCL2, we treated EAP mice with an IL-17-neutralizing mAb. As a result, IL-17 neutralization decreased IL-17 levels in prostate tissues and ameliorated the inflammatory changes and pelvic pain associated with EAP (Supplemental Figure 2, p < 0.05).

We measured neutrophil infiltration into the prostate in the EAP + IgG and EAP + anti-IL-17 groups by flow cytometry and IF staining. As shown in Figure 4a, the percentage of Ly6G+ cells in prostate tissues from the EAP + anti-IL-17 group was significantly lower
than the EAP + IgG group (p < 0.05). IF staining for Ly6G in prostate tissues showed that IL-17 neutralization decreased Ly6G-positive neutrophils in the prostate sections of the EAP + anti-IL-17 group (Figure 4b). We also found that MPO activity in the prostate tissues from the EAP + anti-IL-17 group was decreased compared to the EAP + IgG group (Figure 4c, p < 0.01). These results confirmed that blockade of IL-17 reduced neutrophil infiltration in the prostate tissues of EAP mice.

Furthermore, we analysed the effect of IL-17 neutralization on the expression of the chemokines CXCL1 and CXCL2. The qRT-PCR results showed that blockade of IL-17 decreased the mRNA levels of CXCL1 and CXCL2 (Figure 4d, p < 0.01). Consistent with the qRT-PCR results, anti-IL-17 treatment also decreased the protein levels of CXCL1 and CXCL2 (Figure 4e, p < 0.01). Taken together, these results suggested that IL-17 promoted the recruitment of neutrophils through the inflammatory chemokines CXCL1 and CXCL2. Additionally, we hypothesized that the amelioration of inflammatory changes and pelvic pain in EAP + anti-IL17 mice was because of the decrease in neutrophil infiltration in prostate tissues.

3.4 | Depletion of neutrophils ameliorated inflammatory changes and EAP-induced hyperalgesia

Recent studies demonstrated that neutrophil infiltration played a vital role in inflammatory disease (Prado et al., 2020). We hypothesized that the depletion of neutrophils would alleviate the severity of EAP-induced inflammation. To verify this hypothesis, we treated EAP mice with anti-Ly6G or IgG isotype control. As shown in Figure 5a, anti-Ly6G treatment decreased neutrophils without affecting lymphocytes or monocytes in circulating blood. We examined the effect of neutrophil depletion treatment on the manifestation of EAP. HE staining showed that the pathological alterations were significantly alleviated in the EAP + anti-Ly6G mice compared to the EAP + IgG mice (Figure 5b,c). The response frequencies to individual filament stimulation of the pelvic area also exhibited a significant decrease compared to EAP + IgG mice (Figure 5d, p < 0.05). Taken together, these data suggested that neutrophils were fundamental to the development of EAP and decreasing neutrophils in prostate tissues might be a promising therapeutic strategy for EAP or CPPS.
Fucoidan ameliorated EAP-induced inflammatory changes and pain symptoms by decreasing the infiltration of neutrophils

Fucoidan has been proved to ameliorate inflammation and hyperalgesia in various inflammatory diseases (McNamee et al., 2011; Park et al., 2017; Yu et al., 2018). Its most important therapeutic effects is potent inhibition of neutrophil recruitment to inflammatory sites (McNamee et al., 2011). Fucoidan is a competitive inhibitor of selectins, which are necessary for neutrophil migration. We were curious whether fucoidan was effective for the treatment of CPPS. Therefore, we treated EAP mice with rIL-17 (to enhance neutrophil recruitment to prostate tissues) or fucoidan previously described (Gao et al., 2010; McNamee et al., 2011). The percentages of Ly6G+ cells in the prostate tissues in the four groups were 5.92% ± 0.31% for EAP + vehicle, 7.45% ± 0.44% for EAP + rIL-17, 4.50% ± 0.14% for EAP + fucoidan, and 4.33% ± 0.67% for EAP + rIL-17 + fucoidan (Figure 6a,b). These results suggested that fucoidan treatment decreased the severity of prostate inflammation and chronic pain behaviour. (a,b) Ly6G+ neutrophils in the prostate tissues from each group were analysed using flow cytometry 42 days after the first EAP immunization. (c) the MPO activity of the prostate tissues from each group analysed using the MPO colorimetric assay kit 42 days after the first EAP immunization. (d,e) representative HE staining images (d) and inflammation scores (e) of prostate tissue from each group 42 days after the first EAP immunization: 1, EAP + vehicle; 2, EAP + rIL-17; 3, EAP + fucoidan; 4, EAP + rIL-17 + fucoidan. (f) The response frequencies to individual filament stimulation of the pelvic area 42 days after the first EAP immunization. (EAP + fucoidan vs. EAP + vehicle, *p < 0.05, **p < 0.01 and EAP + rIL-17 + fucoidan vs. EAP + rIL-17, #p < 0.05, ##p < 0.01). Data represent the means ± SEM for four mice per treatment group of three independent experiments.
decreased the percentage of Ly6G+ neutrophil infiltration in prostate tissues (Figure 6a,b, *p < 0.05). Similarly, fucoidan treatment also decreased the MPO activity in prostate tissues (Figure 6c, *p < 0.01). These results confirmed the efficiency of fucoidan-mediated neutrophil inhibition.

Finally, we investigated the effect of fucoidan treatment on the severity of prostate inflammation and chronic pain behaviour. HE staining showed showed that the histological appearance of prostate tissues from fucoidan-treated-EAP mice was alleviated, which was evidenced by a reduction in stromal mononuclear cell infiltration (Figure 6d,e, *p < 0.01). Moreover, chronic pain development analysis suggested that the response frequency was ameliorated in the EAP + fucoidan group compared to the EAP + vehicle group (Figure 6f, ***p < 0.05, **p < 0.01). Compared to the EAP + rIL-17 group, the inflammation changes and pain symptoms were significantly decreased in the EAP + rIL-17 + fucoidan group. (Figure 6d, f, *p < 0.05). These results supported fucoidan as a potential drug for EAP treatment by inhibiting neutrophil infiltration into the prostate tissues.

4 | DISCUSSION

CP/CPPS is a complex syndrome with an unclear aetiology, but the involvement of immune dysfunction, especially autoimmunity, has received considerable support from a variety of human and mouse studies (Breser et al., 2017; Murphy et al., 2014; Pontari & Ruggieri, 2008). We recently demonstrated that the characteristic features of CPPS, namely, pelvic pain and infiltration of inflammatory cells into the prostate, were observed in the EAP mouse model (Zhan et al., 2020). The pelvic pain in this model was represented by referred visceral hyperalgesia of the somatic area. Our previous study demonstrated that IL-17 exerted a positive effect in an EAP mouse model (Zhan et al., 2020). In this study, we examined the molecular mechanism of IL-17, which is the main effector cytokine of Th17 cells, in the development of pelvic pain and prostate inflammation in EAP model mice. That was, IL-17 secreted by Th17 cells recruited neutrophils by increasing the expression of CXCL1/CXCL2, which promoted the occurrence of EAP.

In fact, the pathogenesis of TH17 cells in autoimmune diseases, such CP/CPPS, has attracted the attention of many scholars (Murphy et al., 2015; Zhan et al., 2020). The role of neutrophils in immune diseases was reported in many studies. As early as 1993, A.H. et al. reported the role of neutrophil cytoplastic antibodies in autoimmune liver diseases (Mulder et al., 1993). Other scholars subsequently studied the association between neutrophils and various autoimmune diseases (Kwon et al., 2018; Lande et al., 2011). Simmons et al. showed that IL-17 promoted ELR(+) chemokine-mediated neutrophil recruitment to the brain (Simmons et al., 2014).

In this study, our group examined the pathogenesis of CPPS using Th17 cells and neutrophils together. We demonstrated that IL-17 secreted by Th17 cells upregulated the expression of CXCL1/CXCL2, which are neutrophil chemokines that recruit neutrophils by binding with CXCR1 and CXCR2 receptors. We also confirmed that fucoidan ameliorated the severity of prostate inflammation and chronic pain behaviour in EAP by inhibiting neutrophil recruitment to prostate tissues.

In conclusion, the current study revealed that IL-17 exacerbated the manifestation of EAP via neutrophil infiltration in prostate tissues. Neutrophil recruitment was necessary for the development of EAP. The depletion of neutrophils by anti-Ly6G antibodies and the decrease in neutrophil infiltration by fucoidan effectively ameliorated prostate inflammation and chronic pain in EAP mice. The results also suggest the use of fucoidan as a potential therapeutic drug for the treatment of CP/CPPS in the near future.

AUTHORS’ CONTRIBUTIONS

Chang-Sheng Zhan, Chao-Zhao Liang, and Li Zhang designed and conceived the project. Chang-Sheng Zhan, Cheng Zhang and Jia Chen acquired and analysed the data and wrote the manuscript. Mei-Juan Zheng and Xian-Guo Chen provided technical support.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest in the data of this article.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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REFERENCES

Aleissa, M. S., Alkahtani, S., Abd Eldaim, M. A., Ahmed, A. M., Bungäu, S. G., Almutairi, B., Bin-Jumah, M., Alkahtane, A. A., Alyousif, M. S., & Abdel-Daim, M. M. (2020). Fucoidan ameliorates oxidative stress, inflammation, DNA damage, and hepatorenal injuries in diabetic rats intoxicated with aflatoxin b1. *Oxidative Medicine and Cellular Longevity*, 2020, 9316751.

Breser, M. L., Motrich, R. D., Sanchez, L. R., Mackern-Oberti, J. P., & Rivero, V. E. (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. *The Journal of Immunology*, 190(7), 3121–3133.

Breser, M. L., Salazar, F. C., Rivero, V. E., & Motrich, R. D. (2017). Immunological mechanisms underlying chronic pelvic pain and prostate inflammation in chronic pelvic pain syndrome. *Frontiers in Immunology*, 8, 898.

Catar, R. A., Chen, L., Cuff, S. M., Kift-Morgan, A., Eberl, M., Kettritz, R., Kamhieh-Milz, J., Moll, G., Li, Q., Zhao, H., Kawka, E., Zickler, D., Parekh, G., Davis, P., Fraser, D. J., Dragun, D., Eckardt, K. U., Jörres, A., & Witowski, J. (2020). Control of neutrophil influx during peritonitis...
by transcriptional cross-regulation of chemokine cxcl1 by il-17 and ifn-y. *Journal of Pathology,* 251(2), 175–186.

Chaplan, S. R., Bach, F. W., Pogrel, J., Chung, J., Yaksh, T. J. J., & o. n. m. (1994). Quantitative assessment of tactile allodynia in the rat paw. *Journal of Neuroscience Methods,* 53(1), 55–63.

Chen, B. R., Hsu, K. T., Hsu, W. H., Lee, B. H., Li, T. L., Chan, Y. L., & Wu, C. J. (2021). Immunomodulation and mechanisms of fucoidan from cladosiphon okamuranus ameliorates atopic dermatitis symptoms. *International Journal of Biological Macromolecules,* 189, 537–543.

Chen, J., Zhan, C., Zhang, L., Zhang, L., & Chen, X. (2019). The hypermethylation of foxp3 promoter impairs the function of treg cells in eap. *Inflammation,* 42(4).

Christensen, S. L., Hansen, R. B., Storm, M. A., Olesen, J., Hansen, T. F., Ossipov, M., Iarzugaza, J. M., Porreca, F., Kristensen, D. M. J. E. J., & o. P. (2020). Von frey testing revisited: Provision of an online algorithm for improved accuracy of 50% thresholds. *European Journal of Pain,* 24 (4), 783–790.

Collins, M. M., Meigs, J. B., Barry, M. J., Corkery, E. W., Giovannucci, E., & Lande, R., Gangule, D., Fachinetti, V., Frasci, L. , Corti, R ., Bassett, R., Amuro, H., Fukuhara, S., Ito, T., Liu, Y. J., & Gilliet, M. (2011). Neutrophils in mice. The *Journal of Leukocyte Biology,* 83(1), 64–70.

Eskandari, M. V., Hashim, A., Curtis, M. A., Chavakis, T., & Hajishengallis, G. (2012). The leukocyte integrin antagonist del-1 inhibits the IL-17-mediated inflammatory bone loss. *Nature Immunology,* 13(5), 465–473.

Gao, X., Ding, G., Wang, Z., Fu, H., Ni, Z., Ma, J., Song, S., Liu, F., & Fu, Z. (2010). Adjuvant treatment suppresses il-17 production by t cell-independent myeloid sources in nonobese diabetic mice. *Molecular Immunology,* 47(14), 2397–2404.

Gelderblom, M., Weyman, A., Bernreuther, C., Velden, J., Arunachalam, P., Steinbach, K., Orthey, E., Arumugam, T. V., Leyboldt, F., Simova, O., Thomas, V., Friese, M. A., Prinz, I., Hälscher, C., Glatzel, M., Korn, T., Gerloff, C., Tolosa, E., & Magnus, T. (2012). Neutralization of the IL-17 axis diminishes neutrophil invasion and protects from ischemic stroke. *Blood,* 120(18), 3793–3802.

Kwon, O. C., Lee, E. J., Chang, T. J., Youn, J., Ghang, B., Hong, S., Lee, C. K., Yoo, B., & Kim, Y. G. (2018). IL-17A (+)/Gm-csf(-) neutrophils are the major infiltrating cells in intestinal lung disease in an autoimmune arthritis model. *Frontiers in Immunology,* 9, 1544.

Lande, R., Ganguly, D., Facchinetti, V., Frasca, L., Conrad, C., Gregorio, J., Meller, S., Chamilos, G., Sebagdiari, R., Ricceri, V., Bassett, R., Amuro, H., Fukuhara, S., Ito, T., Liu, Y. J., & Gilliet, M. (2011). Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Science Translational Medicine,* 3(73), 73ra19.

Li, C., Li, H., Wang, Z., Xing, J., Hu, W., Zhang, T., Ge, W., Hao, Z., Zhang, X., & Zhou, J. (2009). The prevalence of prostatitis-like symptoms in China. The *Journal of Urology,* 182(2), 558–563.

Liu, Y., Tang, M., Zhang, Q., Li, C., Lv, R., Min, H., & Zhou, X. (2021). T2 peptide represents a major autoantigen epitope in experimental autoimmune prostatitis. *Inflammation,* 44(1), 243–248.

Magistro, G., Wagenlehner, F. M., Grabe, M., Weidner, W., Stief, C. G., & Nickel, J. C. (2016). Contemporary management of chronic prostatitis/chronic pelvic pain syndrome. *European Urology,* 69(2), 286–297.

McNamee, K. E., Alzabin, S., Hughes, J. P., Anand, P., Feldmann, M., Williams, R. O., & Inglis, J. J. (2011). IL-17 induces hyperalgesia via tnf-dependent neutrophil infiltration. *Pain,* 152(8), 1838–1845.

Moseley, T. A., Haudenschild, D. R., Rose, L., & Reddi, A. H. (2003). Interleukin-17 family and IL-17 receptors. *Cytokine and Growth Factor Reviews,* 14(2), 155–174.

Motrich, R. D., Brerer, M. L., Sánchez, L. R., Godoy, G. J., Prinz, I., & Rivero, V. E. (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(3), 585–597.

Mulder, A. L., Horst, G., Haagsma, E. B., Limburg, P. C., Kleibeuker, J. H., & Kallenberg, C. G. H. (1993). Prevalence and characterization of neutrophil cytoplasmic antibodies in autoimmune liver diseases. *Hepatology,* 17(3), 411–417.

Murphy, S. F., Schaeffer, A. J., & Thubrikat, P. (2014). Immune mediators of chronic pelvic pain syndrome. *Nature Reviews. Urology,* 11(5), 259–269.

Murphy, S. F., Schaeffer, A. J., Done, J., Wong, L., Bell-Cohn, A., Roman, K., & Thubrikat, P. (2015). IL17 mediates pelvic pain in experimental autoimmune prostatitis (eap). *PLoS One,* 10(5), e0125623.

Nickel, J. C., Freedland, S. J., Castro-Santamaria, R., & Moreira, D. M. (2017). Chronic prostate inflammation predicts symptom progression in patients with chronic prostatitis/chronic pelvic pain. *The Journal of Urology,* 198(1), 122–128.

Nowacki, T. M., Lenz, P., Bettenworth, D., Brückner, M., Bokemeyer, A., Tepasse, P. R., Helfen, A., Wildgruber, M., & Eisenblatter, M. (2019). Target-specific fluorescence-mediated tomography for non-invasive and dynamic assessment of early neutrophil infiltration in murine experimental colitis. *Cell,* 8(11), 1328.

Park, J., Cha, J. D., Choi, K. M., Lee, K. Y., Han, K. M., & Jang, Y. S. (2017). Fucoidan inhibits lps-induced inflammation in vitro and during the acute response in vivo. *International Immunopharmacology,* 43, 91–98.

Pontari, M. A., & Ruggieri, M. R. (2008). Mechanisms in prostatitis/chronic pelvic pain syndrome. *The Journal of Urology,* 179(5 Suppl), S561–S567.

Prado, D. S., Veras, F. P., Ferreira, R. G., Damasceno, L. E. A., Melo, P. H., Zamboni, D. S., Cunha, T. M., Cunha, F. Q., & Alves-Filho, J. C. (2020). Nlrp12 controls arthritis severity by acting as a checkpoint inhibitor of th17 cell differentiation. *The FASEB Journal,* 34, 10907–10919.

Quick, M. L., Done, J. D., & Thubrikat, P. J. J. (2013). Measurement of tactile allodynia in a murine model of bacterial prostatitis. *JoVE,* 16(71), e50158.

Rivero, V. E., Cailleau, C., Deplante-Depaoli, M., Riera, C. M., & Carnaud, C. (1998). Non-obese diabetic (nd) mice are genetically susceptible to experimental autoimmune prostatitis (eap). *Journal of Autoimmunity,* 11(6), 603–610.

Roberts, R. O., Lieber, M. M., Rhodes, T., Girman, C. J., Bostwick, D. G., & Jacobsen, S. J. (1998). Prevalence of a physician-assigned diagnosis of prostatitis: The omsted county study of urinary symptoms and health status among men. *Urology,* 51(4), 578–584.

Scalerandi, M. V., Peinetti, N., Leimgruber, C., Cuello Rubio, M. M., Sercundes, M. K., Ortolan, L. S., Debone, D., Soeiro-Pereira, P. V., Gomes, E., Atikken, E. H., Condino-Neto, A., Russo, M., MR. D. I. L., Alvarez, J. M., Portugal, S., Marinho, C. R., & Epiphaniou, S. (2016). Targeting neutrophils to prevent malaria-associated acute lung injury/acute respiratory distress syndrome in mice. *PLoS Pathogens,* 12(12), e1006054.

Simmons, S. B., Liggitt, D., & Goverman, J. M. (2014). Cytokine-regulated neutrophil recruitment is required for brain but not spinal cord inflammation during experimental autoimmune encephalomyelitis. *The Journal of Immunology,* 193(2), 555–563.
Williams, A. E., José, R. J., Mercer, P. F., Brealey, D., Parekh, D., Thickett, D. R., O’Kane, C., McAuley, D. F., & Chambers, R. C. (2017). Evidence for chemokine synergy during neutrophil migration in ARDS. Thorax, 72(1), 66–73.

Yu, H. H., Chengchuan Ko, E., Chang, C. L., Yuan, K. S., Wu, A. T. H., Shan, Y. S., & Wu, S. Y. (2018). Fucoidan inhibits radiation-induced pneumonitis and lung fibrosis by reducing inflammatory cytokine expression in lung tissues. Marine Drugs, 16(10), 392.

Zhan, C. S., Chen, J., Chen, J., Zhang, L. G., Liu, Y., Du, H. X., Wang, H., Zheng, M. J., Yu, Z. Q., Chen, X. G., Zhang, L., & Liang, C. Z. (2020). Camk4-dependent phosphorylation of akt/mTOR underlies Th17 excessive activation in experimental autoimmune prostatitis. The FASEB Journal, 34(10), 14006–14023.

Zhang, J., Zhang, X., Cai, Z., Li, N., & Li, H. J. A. (2019). The lifetime risk and prognosis of chronic prostatitis/chronic pelvic pain syndrome in the middle-aged Chinese males. American Journal of Men’s Health, 13(4), 155798319865380.

Zhang, Y., Chen, L., Gao, W., Hou, X., Gu, Y., Gui, L., Huang, D., Liu, M., Ren, C., Wang, S., & Shen, J. (2012). IL-17 neutralization significantly ameliorates hepatic granulomatous inflammation and liver damage in Schistosoma japonicum infected mice. European Journal of Immunology, 42(6), 1523–1535.

SUPPORTING INFORMATION
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