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Pentraxin 3 promotes the expression of pro-inflammatory cytokines and the migration of macrophages in myocarditis

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Abstract

Background This study aims to investigate the expression of Pentraxin 3 (PTX3) and Nod-like receptor family pyrin domain-containing 3 (NLRP3) in myocarditis and to elucidate their roles and potential interplay in the pathogenesis of myocarditis.

Methods Immunofluorescence staining was performed on myocardial autopsy specimens from deceased patients with severe myocarditis or severe trauma. H9C2 cardiomyocytes were divided into five groups: Control, Lipopolysaccharide (LPS), LPS + PTX3 overexpression, LPS + small interfering RNA targeting PTX3 (si-PTX3), and LPS + PTX3 overexpression + si-NLRP3. The expression levels of PTX3 and NLRP3 at the RNA level were quantified using quantitative real-time polymerase chain reaction (qPCR), while protein expression was assessed via western blot. The concentrations of interleukin-1β (IL-1β) and IL-18 were determined by enzyme-linked immunosorbent assay (ELISA). Macrophages migration was evaluated using Transwell assays.

Results Immunofluorescence staining revealed co-localization and increased expression of PTX3 and NLRP3 in the myocardium of patients with severe myocarditis. In vitro experiments demonstrated that PTX3 enhanced the expression of NLRP3, IL-1β, and IL-18 in LPS-stimulated cardiomyocytes. Furthermore, PTX3 was shown to promote macrophage migration by regulating NLRP3 expression, as assessed by Transwell assays.

Conclusion Our findings suggest that PTX3-mediated NLRP3 activation contributes to inflammatory responses and promotes macrophage migration in myocarditis. This study provides a foundation for future investigations into PTX3-targeted therapies for myocarditis.

Keywords Myocarditis, PTX3, NLRP3, Transwell, Macrophage migration

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Background

Myocarditis, defined as the inflammation of the myocardium, is a common cause of dilated cardiomyopathy (DCM) [1]. Once tissue damage and systolic dysfunction develop, the transition from myocarditis to DCM becomes irreversible [2]. Currently, there is no effective treatment for these patients aside from supportive care for this group of patients, leading to a poor prognosis, mostly requiring heart transplantation at the end-stage [3]. Exploring the pathogenesis of myocarditis might help to find potential new therapeutic targets to improve prognosis.

Myocarditis presents various pathological characters depending on its different etiologies. After the initial acute inflammatory phase, the progression from myocarditis to DCM, which is triggered by an autoimmune process, remains similar, regardless of the initial cause of myocardial damage [4]. In the process, innate immune response is essential, which initiates and parallels the adaptive immune response, resulting in a sustained inflammatory state [5]. Supporting this idea, data from the MalaCards database, which provides clinical and genetic information on human diseases, shows that the innate immune system is the pathway most strongly associated with myocarditis [6]. This finding implies that the innate immune system's reaction to initial heart injury serves as both a primary defense mechanism and a vital element in the subsequent autoimmune responses.

Pentraxin 3 (PTX3) is a member of the pentraxin family, which plays an important role in humoral innate immunity [7]. PTX3 has been reported to promote inflammation in many infectious and systemic autoimmune diseases [8]. Meanwhile, PTX3 has been found to play a cardioprotective role in many cardiovascular diseases, such as acute myocardial infarction, atherosclerosis, and heart failure [9].

Moreover, the cardioprotective role of PTX3 has also been mentioned in myocarditis. Paeschke A et al. demonstrated that more inflammatory infiltration and cardiomyocyte apoptosis were presented in PTX3-deficient myocarditis mice models, suggesting a protective role of PTX3 in tissue inflammatory injury in myocarditis [10]. However, the data on human samples was lacking, and the specific mechanism of PTX3 in cardio-protection remained to be elucidated.

Nod-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome plays a fundamental role in innate immunity and inflammation, serving as a critical regulator of immune responses. It is primarily activated by stimulation from danger-associated molecular patterns and pathogen-associated molecular patterns. Once activated, NLRP3 triggers the activation of caspase-1, which in turn promotes the cleavage and release of pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18 [11]. These cytokines are key mediators of inflammatory responses and are implicated in the pathogenesis of various inflammatory diseases, including cardiovascular diseases such as atherosclerosis and heart failure [12].

In the context of myocarditis, NLRP3 is considered to be a promoter of inflammation in the development of myocarditis, although the exact mechanism is still unclear [13]. Previous studies have highlighted potential interactions between PTX3 and NLRP3, Wang et al. demonstrated that PTX3 deficiency increased NLRP3 inflammasome activation in retinal pigmented epithelium [14], suggesting that PTX3 might regulate the inflammation through NLRP3.

Given the critical roles of both PTX3 and NLRP3 in the pathogenesis of myocarditis and their established interaction in other disease contexts, it is reasonable to hypothesize that PTX3 may similarly regulate inflammation through NLRP3 in myocarditis.

In this study, we investigated the expression of PTX3 and NLRP3 in myocardial biopsies and explored their role in myocarditis at the cardiomyocyte level in vitro.

Methods

Immunofluorescence staining

Autopsy pathological sections of left ventricular anterior walls from two deceased patients were used. One patient was a 56-year-old male with severe myocarditis, and the other was a 54-year-old male with a fall injury. The sections were generously donated by the General Hospital of the People's Liberation Army. Ethical approval (No. S2021-134-01) was obtained from the Institutional Review Board of the Chinese PLA General Hospital. All human specimens were irreversibly anonymized through coded labeling before analysis, in compliance with the Declaration of Helsinki. The sections were baked at 60℃ for 4 h to ensure adherence to the slides. Dewaxing was performed using xylene, followed by ethanol and ultrapure water. Antigen retrieval was carried out by incubating the sections in antigen retrieval buffer for 10 min. The sections were then treated with 0.3% Triton X-100 for 10 min and blocking buffer for 2 h. The sections were incubated with primary antibodies against PTX3 (Santa Cruz Biotechnology, Shanghai, China) and NLRP3 (Affinity Biosciences, Jiangsu, China) overnight at 4℃. After rinsing three times with phosphate buffer saline (PBS) for 5 min each, the sections were incubated with secondary antibodies: goat anti-rabbit IgG H&L 488 (Abcam, Boston, MA, USA) and goat anti-mouse IgG H&L 594 (Abcam, Boston, MA, USA), as well as DAPI (ASGB-BIO, Beijing, China). Each incubation step was followed by three rinses with PBS for 5 min. Fluorescence images were collected using a fluorescence microscope (Ci-S, Nikon, Japan).

Cell culture

H9C2 cardiomyoblasts cells purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China) were maintained in RPMI 1640 medium (Gibco, California, USA) supplemented with 10% fetal bovine serum, 100μ/mL penicillin, and 100μ/mL streptomycin. Cells were passaged at 80–90% confluency using 0.25% trypsin-EDTA. For experiments, cells (passage 3–5) were seeded at a density of 5×10^3 cells/cm² and treated at 70–80% confluency.

Rat bone marrow-derived macrophages purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China) were maintained in the manufacturer-provided complete medium. Cultures were incubated at 37 °C in a 5% CO_2 humidified atmosphere, with medium refreshed every 48 h. According to Procell's technical specifications, these macrophages were not subjected to polarization protocols. The population represents a mixed state of naïve/unpolarized macrophages with possible spontaneous polarization due to microenvironmental cues during isolation and expansion. No additional inflammatory stimuli were applied before Transwell migration assays. Macrophages were used in their basal state to mimic physiological recruitment under endogenous inflammatory signals from LPS-treated cardiomyocytes.

Transfection

Control small interfering RNA (siRNA), si-PTX3, si-NLRP3, pcDNA3.1, and pcDNA3.1-PTX3 were designed by Gene Line Bioscience Ltd. (Beijing, China). H9C2 cells were inoculated in a 24-well culture plate with a density of $1.5*10^5$ cells/well. Transfection was performed according to the experimental groups as follows: (1) Control group: pcDNA3.1 + control siRNA; (2) LPS group: pcDNA3.1 + control siRNA; (3) LPS + PTX3 group: pcDNA3.1-PTX3 + control siRNA; (4) LPS + Si-PTX3 group: pcDNA3.1 + si-PTX3; (5) LPS + PTX3 + Si-NLRP3 group: pcDNA3.1-PTX3 + si-NLRP3. Transfection efficiency was validated by real-time quantitative polymerase chain reaction (qPCR), showing > 75% target gene knockdown at 24 h post-transfection (Supplementary Fig. 1). Cells were transfected for 6 h, followed by an 18-hour recovery period before treatment with LPS (100 ng/mL) for 24 h. The control group was then treated with PBS for another 24 h. Cell viability was validated by morphological monitoring by phase-contrast microscopy and inferred from consistent protein yields.

Real-time quantitative polymerase chain reaction (qPCR)

Real-time qPCR was performed using the fluorescence quantitative Kit (Yeasen Biotechnology, Shanghai, China). The cycling parameters consisted of an initial denaturation at 95 °C for 5 min, followed by 40 amplification cycles of 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 20 s. Relative transcriptional levels of PTX3 and NLRP3 were calculated by the Livak method ($2^{-}\Delta\Delta Ct$) with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the normalizing gene. The reactions were monitored using a Real-time PCR system (Molarray, MA-6000). Measurements were performed in triplicate.

Western blots

Total proteins were extracted from each group of H9c2 cells after lysis in radioimmunoprecipitation assay (RIPA) extraction buffer (Beyotime Biotechnology, Shanghai, China). Protein concentrations were determined using a bicinchoninic acid protein assay kit (Solarbio Life Science, Beijing, China). Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membranes. The membranes were blocked with 5% milk in Tris-buffered saline with Tween for 2 h at room temperature. After blocking, membranes were incubated with primary antibodies against PTX3 (1:1000, Santa Cruz Biotechnology, Shanghai, China), NLRP3 (1:1000, Affinity Biosciences, Jiangsu, China), and GAPDH (1:5000, Proteintech, Wuhan, China) overnight at 4 °C. The following day, the membranes were incubated with the horseradish peroxidase secondary antibodies (1:5000, Bioss, Beijing, China) for 1 h at room temperature. Band intensity was quantified using ImageJ v1.8.0 (National Institutes of Health, Bethesda, MD, USA) and normalized to GAPDH.

Enzyme-Linked immunosorbent assay (ELISA)

Concentrations of interleukin-1β (IL-1β) and IL-18 concentration in cell culture supernatants were determined using an ELISA kit (Ruixin Biotech, Quanzhou, China) according to the manufacturer's instructions.

Transwell

Transfected H9C2 cells (1.5×10^5/well) from different subgroups were seeded in lower chambers of Transwell with 6.5 mm membrane inserts (Costar, Corning, NY, USA). Macrophages (2×10^5) were added to the upper chamber of Transwell. Migration was allowed to proceed for 24 h. After migration, the macrophages that had migrated to the lower side were fixed by 4% paraformaldehyde for 10 min and 100% methanol for 20 min, followed by staining with 2.5% crystal violet solution for 15 min. The membranes were rinsed with PBS, and the number of migrated macrophages was counted in three random fields per well using an inverted biological microscope (Model XD-RFL, Ningbo Sunny Instruments Co., Ltd, China) with a digital camera.

Statistical analysis

R version 4.4.2 (The R Foundation for Statistical Computing) and GraphPad-Prism 8.0 (GraphPad, San Diego,

CA, USA) were used for statistical analysis. Data are expressed as mean ± standard error of the mean (SEM). Normality was assessed using the Shapiro-Wilk test ($P > 0.05$ for all groups). For normally distributed data with homogeneous variances (Levene's test), two-group comparisons were analyzed using the two-tailed Student's t-test. For multi-group comparisons, one-way ANOVA followed by Bonferroni correction was applied. Non-normally distributed data were analyzed using the Kruskal-Wallis test with Dunn's correction. Statistical significance was defined as $P < 0.05$.

Results

Expression of PTX3 and NLRP3 is increased in myocarditis patients

Cardiac autopsy specimens were obtained and analyzed from two deceased patients: a 54-year-old male who died from a fall injury and a 56-year-old male who died from severe myocarditis. Compared to the patient with fall injury, elevated myocardial expression of both PTX3 and NLRP3 was detected in the patient with severe myocarditis. Furthermore, colocalization of PTX3 and NLRP3 was observed in the myocardium of the patient who died from myocarditis (Fig. 1).

Upregulated expression of PTX3 and NLRP3 in LPS-stimulated cardiomyocytes

Building on these findings, we further investigated the expression of PTX3 and NLRP3 in cardiomyocytes stimulated with LPS. Our results demonstrated that LPS stimulation significantly increased the expression of PTX3 compared to the control group, with statistical significance observed both at the gene level ($P = 0.0132$) and protein level ($P < 0.0001$). Similarly, NLPR3 expression was also markedly upregulated in the LPS group, as evidenced by Western blot ($P < 0.0001$) and qPCR ($P = 0.0115$) (Fig. 2).

PTX3 promotes the expression of NLRP3 and its downstream cytokines IL-1β and IL-8

Given the concurrent upregulation and colocalization of PTX3 and NLRP3 in myocarditis, we further investigated the potential relationship between these two. Compared to the LPS-only treatment group, the expression of NLRP3 was upregulated in the LPS + PTX3 co-treated group, as evidenced by qPCR ($P = 0.0708$) and Western blot analysis ($P < 0.0001$) (Fig. 2B and E). Consistently, a reduction in both NLRP3 protein ($P = 0.0001$) and RNA ($P = 0.0734$) levels was observed in the LPS + Si-PTX3 group compared to the LPS group (Fig. 2B and E). Notably, the knockdown of NLRP3 using siRNA did not result in any significant alteration in either PTX3 RNA ($P = 0.1962$) and protein ($P > 0.9999$) expression levels (Fig. 2A and D). These findings suggest a potential positive regulatory effect of PTX3 on NLRP3 expression in cardiomyocytes under inflammatory conditions.

We further explored the downstream inflammatory factors regulated by PTX3 and NLRP3. The expression patterns of IL-1β and IL-18 were found to align with those of PTX3. A trend of upregulation of both IL-1β and IL-18 was observed in the LPS + PTX3 group compared to the LPS group. This increase was abrogated upon the knockdown of NLRP3 expression. Furthermore, a reduction in PTX3 expression was also correlated with decreased levels of IL-1β and IL-18 (Fig. 3).

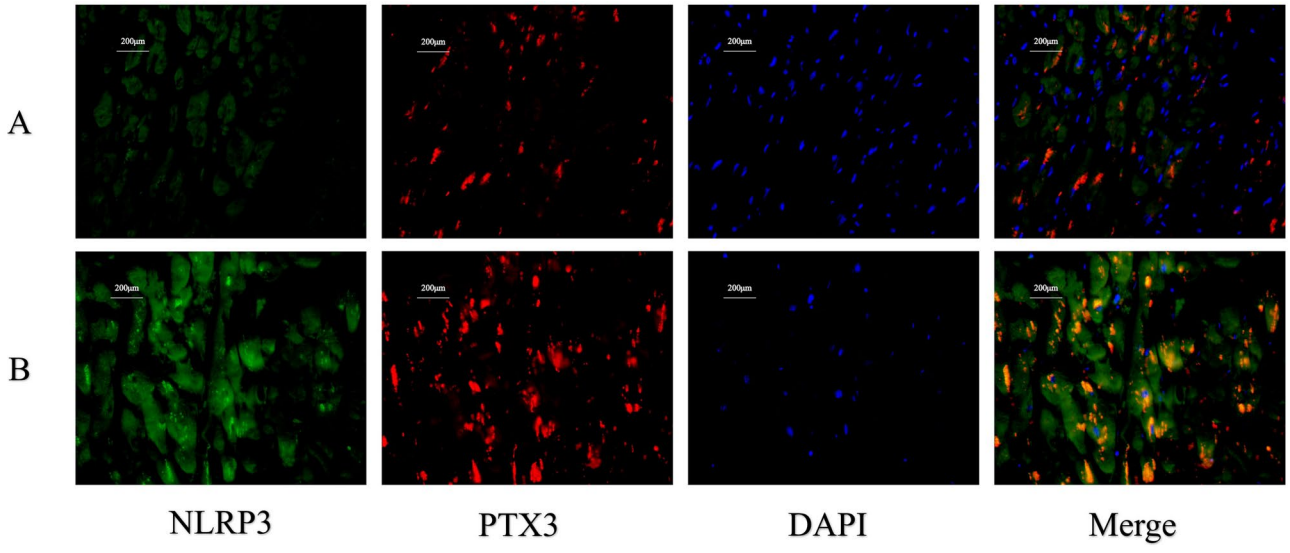

Fig. 1 (**A**) Immunofluorescence staining of PTX3 (red) and NLRP3 (green) in myocardial sections from a patient with fall injury. (**B**) Immunofluorescence staining of PTX3 (red) and NLRP3 (green) in myocardial sections from a patient with severe myocarditis. PTX3 and NLRP3 colocalized in cardiomyocyte (merged in orange). (original magnification×400)

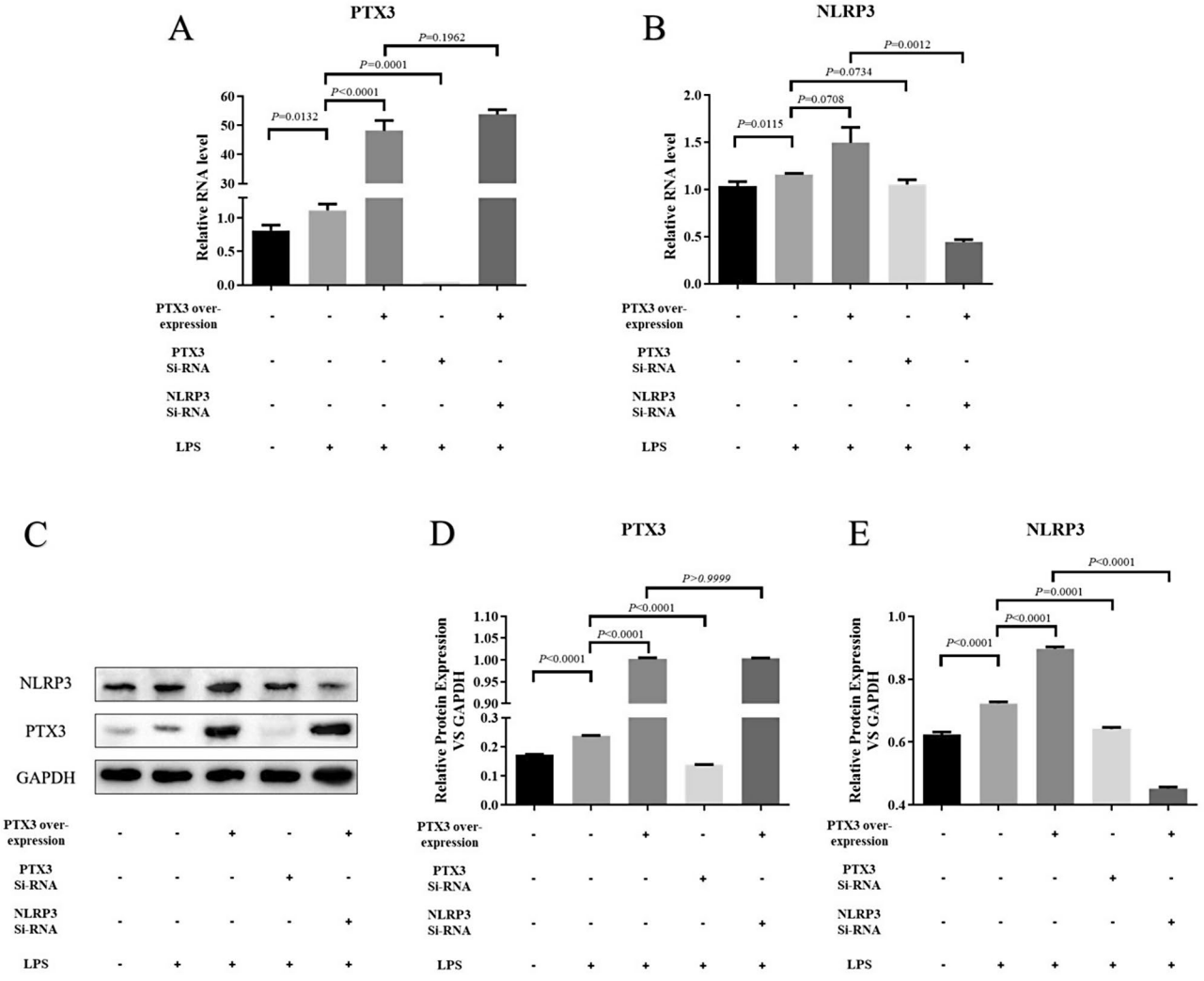

Fig. 2 (**A**) qPCR analysis of PTX3 mRNA expression in cardiomyocytes, normalized to GAPDH ($n=3$ biological replicates). (**B**) qPCR analysis of NLRP3 mRNA expression in cardiomyocytes, normalized to GAPDH ($n=3$ biological replicates). (**C**) Representative Western blot image showing PTX3 and NLRP3 protein expression in cardiomyocytes. (**D**) Quantitative analysis of PTX3 protein expression relative to GAPDH ($n=3$ independent experiments). (**E**) Quantitative analysis of NLRP3 protein expression relative to GAPDH ($n=3$ independent experiments). Data are presented as mean ± SEM

PTX3 promotes the migration of macrophages to cardiomyocytes under an inflammatory state via NLRP3

The Transwell assay (Fig. 4) showed that the number of macrophages in the lower chamber was significantly greater in the LPS group than in the control group ($P=0.0232$). When PTX3 expression was enhanced, the number of migrating macrophages significantly increased compared to the LPS group ($P=0.0439$). This increase was reversed by interfering with the expression of PTX3 ($P=0.0003$) or NLRP3 ($P=0.0021$) (Fig. 4).

Discussion

In this study, we identified a novel cardiomyocyte-autonomous mechanism linking PTX3 to NLRP3 inflammasome activation in myocarditis. We found that PTX3 and NLRP3 were co-localized in cardiomyocytes of human myocarditis specimens, with expression levels significantly higher than in trauma controls. LPS-stimulated H9C2 cardiomyocytes overexpressing PTX3 exhibited upregulated NLRP3, IL-1β, and IL-18, while PTX3 knockdown attenuated these effects. PTX3-enhanced NLRP3 activity promoted macrophage migration in Transwell assays. These results collectively suggest that cardiomyocyte-derived PTX3 may drive inflammatory cascades in myocarditis via NLRP3 activation.

Pentraxins are a family of multimetric proteins that include C-reactive protein (CRP), serum amyloid P component (SAP) and pentraxins 3 (PTX3), which play crucial roles in humoral innate immunity [7]. Among them, CRP and PTX3 are the most prominent acute-phase proteins. While CRP and SAP are primarily produced in the liver [15], PTX3 is produced locally by a variety of cell types, including immune and vascular cells [16, 17]. As

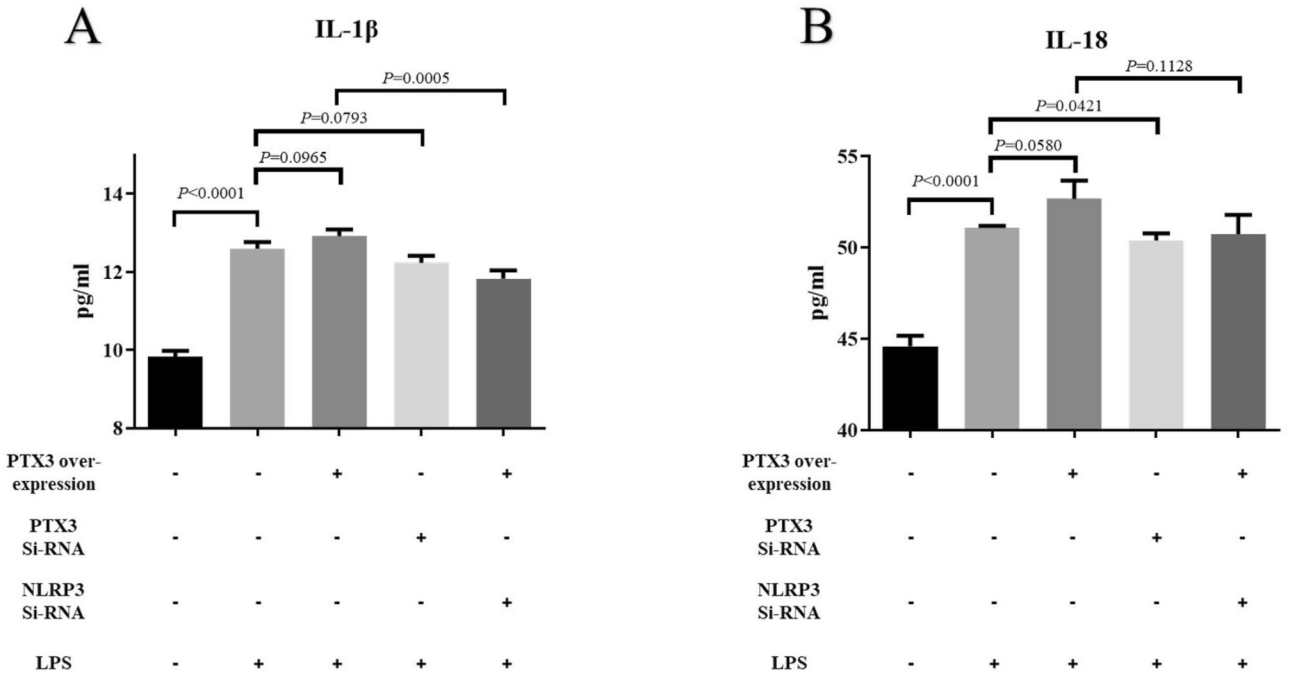

Fig. 3 (**A**) ELISA analysis showing IL-1β levels in cell culture supernatants of different groups of cardiomyocytes. (**B**) ELISA analysis showing IL-18 levels in cell culture supernatants of different groups of cardiomyocytes. Data are presented as mean ± SEM ($n = 4$ biological replicates)

an acute phase protein, PTX3 exists at low systemic levels in healthy humans but can increase rapidly after tissue injury or inflammation, peaking earlier than CRP [18]. Moreover, the strict evolutionary conservation of PTX3 between mice and humans makes it possible to study its role in innate immunity using gene-modified mice [19]. Therefore, PTX3 has been extensively studied in various inflammation-related diseases and has been implicated in the pathogenesis and prognosis of many conditions. In the field of cardiovascular disease, previous studies have focused on atherosclerosis, myocardial infarction, and heart failure [20]. GD Norata et al. found that PTX3-knockout mice exhibited a greater area of atherosclerosis along with increased inflammation [21]. Salio M et al. found that PTX3-deficient mice had increased neutrophil infiltration, elevated complement 3 (C3) deposition, more apoptotic cardiomyocytes, and a larger infarct size [22]. W Chen et al. demonstrated that recombinant PTX3 injection reduces myocardial injury, tissue fibrosis, and pro-inflammatory factor expression in spontaneously hypertensive heart failure rats [23]. However, the role of PTX3 in myocarditis has rarely been studied. In our study, we observed a simultaneous increase in PTX3 and NLRP3 expression in a myocarditis patient. The colocalization of these two factors suggests their potential joint involvement in the pathogenesis of myocarditis. PTX3 has been reported to synergize with complement C1q in promoting NLRP3 inflammasome overactivation in rheumatoid arthritis [24], but this synergy has not yet been established in myocarditis. In viral myocarditis, a variety of cytokines significantly increase in the acute phase before immune cell infiltration, suggesting that intrinsic cells of the myocardium, such as cardiomyocytes, play an important role in the secretion of these cytokines and the subsequent recruitment of immune cells [25]. Therefore, we further investigated the effects of PTX3 and NLRP3 on LPS-stimulated H9C2 cardiomyocytes.

Our results showed that PTX3 increased the expression of NLRP3 when acting on cardiomyocytes under inflammatory conditions, as well as the expression of downstream cytokines of NLRP3. In contrast, NLRP3 did not affect PTX3 expression in cardiomyocytes during inflammation.

Previous studies revealed the correlation between NLRP3 activation and myocarditis. Y Wang et al. found that NLRP3 was involved in coxsackievirus B3 (CVB3)-induced inflammasome activation, playing a crucial role in viral myocarditis (VMC) [26]. They also suggested that Cathepsin B may exacerbate CVB3-induced VMC by activating NLRP3 inflammasome and promoting pyroptosis [27]. Y Yu et al. found that calpain inhibition could suppress the NLRP3 inflammasome/caspase-1-mediated pyroptosis pathways, thereby attenuating CVB3-induced myocarditis [28]. Overall, the activation of the NLRP3 inflammasome is closely linked to pyroptosis in myocarditis, resulting in the release of pro-inflammatory cytokines that expand or sustain inflammation in the innate immune response [13]. Our study found that PTX3 increased the expression of NLRP3 and its downstream

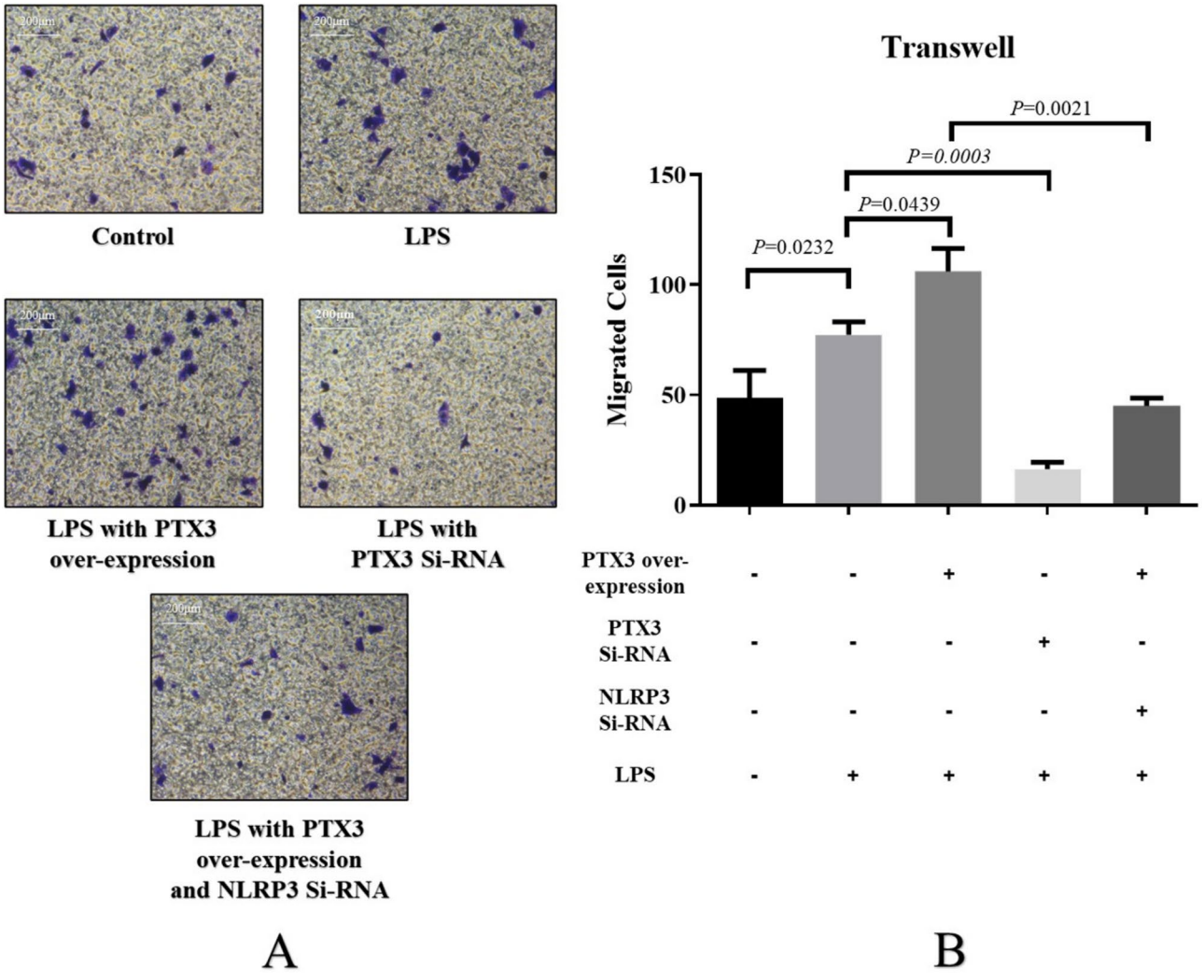

Fig. 4 (**A**) Transwell assay demonstrating the effect of PTX3 on macrophage migration. Cardiomyocytes from each experimental group were plated in the lower compartment, while macrophages were introduced into the upper compartment. Migrated macrophages were stained with crystal violet. (original magnification×100). (**B**) Quantitative assessment of macrophage migration in the transwell assay. Data are expressed as mean ± SEM ($n = 3$ independent experiments)

cytokines, IL-1β and IL-18, indicating PTX3's role in the innate immune response.

Various types of innate immune cells are involved in myocarditis, such as natural killers, neutrophils, eosinophils, and monocytes/macrophages [29]. Among them, macrophages are the predominant immune cells infiltrating the myocardium during myocarditis [30]. The recruitment of macrophages to the site of inflammation characterizes the innate immune response [31]. Kim Y et al. reported that PTX3 silencing in human dental pulp cells significantly decreased the chemotactic migration of macrophages [32]. Our study also found that PTX3 overexpression increased the migration of macrophages in myocarditis, an effect that has not been previously demonstrated. Meanwhile, this effect was significantly attenuated by the inhibition of NLRP3. On the other hand, interfering with PTX3 expression could reduce macrophages migration. Thus, we propose that PTX3 enhances the inflammatory response in the innate immune system and promotes the recruitment of inflammatory cells through NLRP3 during myocarditis.

Interestingly, PTX3, which enhances the innate immune response and increased inflammatory cell infiltration at the cardiomyocyte level, has been shown to be protective in viral myocarditis rodent models by inhibiting inflammatory cell aggregation and mitigating cardiomyocyte injury [10], although the underlying mechanism remains to be elucidated. Continuous exposure of intracellular self-antigens is an essential part of maintaining myocardial-specific autoimmune response in myocarditis [33]. Cell-bound PTX3 could facilitate the rapid clearance of apoptotic cells, thereby reducing excessive

inflammation [34]. Pyroptosis, which can be enhanced by NLRP3, may also contribute to the clearance of autoantigens in lupus [35]. It is reasonable to speculate that PTX3 may contribute to the clearance of damaged cardiomyocytes by enhancing the innate immune response in the acute phase, thereby acting as a cardioprotective role in myocarditis by avoiding an over-activated immune response induced by continuous self-antigen exposure. Additionally, PTX3 may activate the innate immune system along with other negative feedback pathways of inflammation, thus mitigating excessive activation of inflammation. Deban L et al. found that PTX3 could dampen leucocyte recruitment in acute lung injury [36]. Previous studies also suggested that PTX3 could limit immune responses against autoantigens by counteracting the pattern recognition receptors of apoptotic cells [37]. Many articles have highlighted the dual complexity of PTX3 in inflammation [38, 39], a complexity that is also evident in myocarditis. Further investigation is needed to fully understand the bidirectional role of PTX3 in myocarditis.

While our findings provide novel insights into the role of PTX3 in myocarditis, several limitations should be acknowledged. First, the small human cohort limits generalizability, although this was partially addressed by in vitro replication. Second, we used unpolarized macrophages to mimic physiological recruitment, but subtype-specific effects (M1/M2) remain to be explored. Third, the 24-hour LPS treatment captures sustained inflammation but may overlook the early regulatory roles of PTX3. Future studies should include time-course experiments to delineate the precise kinetics of PTX3/NLRP3 signaling in cardiomyocytes.

Conclusion

In conclusion, our findings suggest that PTX3-mediated NLRP3 activation may contribute to inflammatory responses and macrophage recruitment in myocarditis. While these results highlight a potential cardiomyocyte-driven mechanism, further validation in complex physiological systems is required. This study provides a foundation for future investigations into PTX3-targeted therapies for myocarditis.

Abbreviations

C3	Complement 3
CRP	C-reactive protein
CVB3	Coxsackievirus B3
DCM	Dilated cardiomyopathy
ELISA	Enzyme-linked immunosorbent assay
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
IL-1β	Interleukin-1β
LPS	Lipopolysaccharide
NLRP3	Nod-like receptor family pyrin domain-containing 3
PBS	Phosphate buffer saline
PTX3	Pentraxin 3
qPCR	Real-time quantitative polymerase chain reaction
RIPA	Radioimmunoprecipitation assay
SAP	Serum amyloid P component
SEM	Standard error of the mean
Si-PTX3	Small interfering RNA targeting PTX3
VMC	Viral myocarditis

Supplementary Information

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Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions

ZTY conducted the primary experiments, analyzed the data, and drafted the manuscript. DY contributed to the study design, performed statistical analysis, and helped in drafting the manuscript. WXH assisted in conducting experiments. LY participated in data collection and interpretation. CGL involved in the design of the study and provided technical support. WN contributed to the acquisition and analysis of data. YQ assisted in the collection of data. ZWC participated in the design of the study. CXZ contributed to the study design, provided funding acquisition, and supervised the research. LXH participated in its design and coordination and provided funding acquisition. All authors reviewed the manuscript.

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Data availability

The data of the manuscript are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

Ethical approval (No. S2021-134-01) was obtained from the Institutional Review Board of the Chinese PLA General Hospital. All human specimens were irreversibly anonymized through coded labeling before analysis, in compliance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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