



# Periostin Modulating *Mycoplasma pneumoniae* Pneumonia in Children Related to Th17 Cell Function

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## Abstract

**Objective** *Mycoplasma pneumoniae* pneumonia (MPP) is recognized as a significant respiratory tract infection in children. Periostin associates with airway remodeling, and the T helper 17 (Th17) cells play a crucial role against *M. pneumoniae* infection. This study investigates the effect of periostin in Th17 cells and the associated mechanism in MPP.

**Methods** The study investigated the role of periostin stimulated with pulmonary bronchoalveolar lavage fluid (BALF) from MPP. Levels of infection of *M. pneumoniae* were determined using quantitative real-time polymerase chain reaction. The periostin was cloned into vector, and siRNA fragment were synthesized. The Th17 cells were transfected with the vector and the fragment, and its expression and proinflammatory cytokines (interleukin [IL]-6, tumor necrosis factor [TNF]- $\alpha$ , and IL-1 $\beta$ ) were determined using western blot. The cell apoptosis, migration, and proliferation were measured using flow cytometer, transwell migration, and cell counting kit-8 assay, respectively.

**Results** The results showed that periostin expression had a positive correlation with MPP severity. Fluorescence-activated cell sorting analysis showed that the periostin inhibited the apoptosis of Th17 cells. Moreover, transwell migration showed a significant increased migration in Th17 cell was detected treated with BALF, and selective knockdown of periostin by specific siRNA had negative effect on cell migration. Western blot analysis showed the periostin induced the expression of the proinflammatory cytokines (IL-6, TNF- $\alpha$ , and IL-1 $\beta$ ), and downregulation of periostin could decrease the expression of cytokines in MPP group.

**Conclusion** The study suggested that periostin is required for Th17 cells migration, and it also has effect on Th17 apoptosis and proinflammatory cytokines expression in MPP.

## Keywords

- ▶ *Mycoplasma pneumoniae*
- ▶ Th17 cell
- ▶ periostin
- ▶ migration
- ▶ apoptosis
- ▶ inflammatory cytokine

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## Introduction

*Mycoplasma pneumoniae* pneumonia (MPP) is considered as one of the common etiologies of community-acquired pneumonia because of its presumed benign self-limiting nature and affects mainly children and young adults.<sup>1</sup> However, some patients with severe pulmonary manifestations experience long-term sequelae followed by a variety of complications, such as refractory *Mycoplasma pneumoniae* pneumonia (RMPP), acute respiratory distress syndrome, necrotizing pneumonia, and fulminant pneumonia.<sup>2,3</sup> *M. pneumoniae* infection induces host cell-mediated excessive immune stress responses that produce lung structural and/or functional impairment. Hence, studying the immune regulatory mechanisms of MPP might have an important social and economic significance for treatment.

At present, the mechanism of MPP immune response to lung injury is still unclear. The latter is believed to be caused by indirect tissue damage caused by excessive host immune response.<sup>4</sup> Current research suggests that after *M. pneumoniae* adhere to host cells, it synthesizes hydrogen peroxide that can cause oxidative stress in respiratory epithelial cells and secretes community-acquired respiratory distress syndrome (CARDS) toxins that damage the respiratory tract epithelium.<sup>5,6</sup> CARDS toxins simultaneously stimulate the expression of Th2 cytokines (interleukin [IL]-4 and IL-13), which brings to inflammatory responses and aggregation of T cells.<sup>7,8</sup> It is worth noting that the high expression of IL-4 and IL-13 in children with MPP can induce the production of periostin.<sup>9</sup> Periostin, as an extracellular matrix, is a member of the stromal cell, belonging to fasciclin family, and plays an important role in regulating and remodeling the inflammatory microenvironment in pathological conditions.<sup>10,11</sup> Changes in the expression of periostin were involved in many aspects of inflammation, airway remodeling, development of a Th2 phenotype, and increased expression of inflammatory mediators. For example, *M. pneumoniae* can adhere to host cells and its synthetic hydrogen peroxide can induce oxidative stress response in respiratory epithelial cells.<sup>12</sup>

The role of mucosal T cells in health has also been recently highlighted by numerous studies conducted on T helper 17 (Th17) cells. Numerous studies showed that Th17 cells may act as a double-edged sword in MPP, on the one hand to help immunity against MPP, and on the other hand, unchecked proliferation of Th17 cells also lead to autoimmunity and inflammatory conditions upon pathogenesis.<sup>13-15</sup> Studies have confirmed that periostin works by binding to Th17 cell surface receptor integrin, such as  $\alpha\text{v}\beta\text{1}$  and  $\alpha\text{v}\beta\text{3}$ .<sup>16</sup> Integrins can transmit extracellular signals into cells, thereby affecting and regulating the biological behavior of Th17 cells, such as cell adhesion, survival, proliferation, differentiation, migration, etc.<sup>17</sup>

In this regard, we want to know how does Th17 migrate into the lung tissue when *M. pneumoniae* causes infections. Meanwhile, the present study aimed to investigate the effect of periostin on Th17 cells and the associated mechanism in MPP, via detecting cell apoptosis, migration, and cytokine expression. We also delved into the mechanisms and relevant role of Th17 cells in progressing MPP.

## Materials and Methods

### Serum and Bronchoalveolar Lavage Fluid Samples

Ten patients with MPP from Guangzhou Women and Children's Medical Center were recruited in this study. All patients showed positive results for *M. pneumoniae* DNA by polymerase chain reaction (PCR) in throat swabs or/and bronchoalveolar lavage fluid (BALF) on admission. Exclusion criteria were: (1) immune-compromised patients (due to solid or hematological malignancies, immunosuppressive treatments, or genetic disorders); (2) patients with chronic pulmonary, cardiac, metabolic, or neurological conditions, whether congenital (e.g., cystic fibrosis, tetralogy of Fallot, or hypoxic ischemic encephalopathy) or acquired (e.g., hyperreactive airway disease); (3) laboratory tests showing the presence of other pathogens, such as bacteria and virus; and (4) missing clinical record data. Severity was classified at the end of the enrollment. Severe patients were defined as follows<sup>18</sup>: (1) major criteria: invasive mechanical ventilation, fluid refractory shock, acute need for noninvasive positive pressure ventilation, and hypoxemia requiring fraction of inspired oxygen ( $\text{FiO}_2$ ) greater than the inspired concentration or flow feasible in the general care area; (2) minor criteria: respiratory rate greater than the World Health Organization (WHO) classification for age, apnea, increased work of breathing (e.g., retractions, dyspnea, nasal flaring, and grunting),  $\text{PaO}_2/\text{FiO}_2$  ratio < 250, multilobar infiltrates, Pediatric Early Warning Score > 6, altered mental status, hypotension, presence of effusion, comorbid conditions (e.g., hemoglobin SS disease, immunosuppression, and immunodeficiency), and unexplained metabolic acidosis. Ten healthy children were used as control group. The study approval was granted by the Ethical Committee of the Guangzhou Women and Children's Medical Center, and the guardians of all children recruited in the study provided written informed consent (grant no. 2021278B00).

### Flexible Bronchoscopy

Bronchoscopy and bronchoalveolar lavage (BAL) for diagnosis and treatment were performed immediately after admission if there were no contraindications. Patients were prepared for bronchoscopy using inhalant lidocaine to minimize cough reflex, intravenous midazolam for moderate sedation, and atropine to reduce airway secretions. BAL was performed in the most affected area, which was identified radiologically and/or endoscopically. The lavage consisted in introducing three to five aliquots of sterile saline solution warmed to 37°C, followed by immediate aspiration. BAL was recovered by aspiration into a suction trap under a negative pressure of 6.65 to 13.3 kPa (50–100 mm Hg). The recovery volume of the BALF was larger than 40%, which is considered acceptable.

### Quantitative Real-Time PCR Analysis

RNA extraction from swabs or/and BALF samples with Trizol were performed according to the manufacturer's instructions (Takara, China). Extracted RNA was dissolved in RNase-free double distilled water, and reverse transcribed to cDNA

using the PrimeScript RT kit (Takara, China). Real-time PCR (RT-PCR) was performed using the SYBR Premix Ex TaqTMII kit with *M. pneumoniae* primers (forward: 5'-GATACTAGCTGTCGGGGCGCAT-3', 5'-AATTTCATTAG TAGCAGTCTCGCTAG-3') and actin beta primers (forward: 5'-GAGGTATCCTGACCCTGAA GTA-3', reverse: 5'-CACACGCAGCTCATTGTAGA-3') as an internal reference.

#### Enzyme-Linked Immunosorbent Assay Analysis

Serum concentrations of periostin in the samples of 5 children with mild MPP, 5 with severe MPP, and 10 healthy children were determined according to the manufacturers' protocols from the enzyme-linked immunosorbent assay (ELISA) kits. Absorbance was measured at 450 nm using an ELISA reader. All samples were analyzed in triplicate, and the average concentration for each patient was calculated.

#### Western Blot Analysis

Proteins were extracted from the samples of 10 patients (5 mild cases, 5 severe cases) and 10 healthy children and Th17 cells were stimulated by the supernatant of BALF of MPP patients which was separated by centrifugation at 1,000 g for 10 minutes for BALF of MPP patients. The total protein was extracted with the conventional method and was followed by determination of protein concentration with bicinchoninic acid method. Subsequently, 30 µg of proteins were subjected to 12% polyacrylamide-SDS gel electrophoresis and electroblotted onto nitrocellulose membranes and blocking overnight. The primary antibody periostin (cat# ab14041), IL-1β (cat# ab9787), IL-6 (cat# ab9324), and tumor necrosis factor (TNF)-α (cat# ab221921) were all from Abcam (Cambridge, UK) and then added and incubated for 1 hour, followed by phosphate buffered saline washing. Subsequently, an antibody (goat anti-rabbit IgG H&L (HRP), cat# ab97051) was applied to the primary antibody-treated polyvinylidene difluoride membranes at room temperature for 2 hours. The specific bands on membranes were visualized using the supersignal chemiluminescence system (Promega Corporation) and exposed to X-ray film. Band intensities were quantitated using Image J software (Version 2.0) and statistical analysis was performed with GraphPad Prism (Version 5.04).

#### Cell Preparation and Transfection

Mouse cell line Th17 was obtained from Cell Library, China Academy of Science (Shanghai, China), and the cells were cultured in RPMI 1640 (Sigma-Aldrich, St. Louis, Missouri, United States), with 10% fetal bovine serum (FBS) (Gibco-BRL, Grand Island, New York, United States). Cells were cultured in a 37°C incubator filled with 5% CO<sub>2</sub> and routinely passaged at 90% intensity. The culture medium was supplemented with 10% FBS and 100 units/mL penicillin and streptomycin. It was stimulated by BALF of MPP patients. Subsequently, Th17 cells were stimulated by the supernatant of BALF of MPP patients for 24 hours.

#### Transfection of Plasmid and siRNA

Human full-length periostin was cloned into the pEX-m02 vector (FulenGen Guangzhou, China). siRNAs fragment

synthesis was obtained from GenePharma company (GenePharma, Shanghai, China), Th17 cells were seeded onto six-well culture plates at a density of  $2 \times 10^6$  cells/well and transfected with either overexpression plasmid or the specific siRNAs for periostin or controls at a concentration of 50 nM by using Lipofectamine 2000 (Invitrogen, Carlsbad, California, United States) according to the manufacturer's protocol. The day before transfection, cells were placed in a 500-µL antibiotic-free growth medium; 20 pmol siRNA diluted in 50 µL serum-free Gibco Opti-MEM I reduced serum medium and lightly mixed. Then took 1 µL Lipofectamine 2000 and diluted it into 50-µL Opti-MEM I reduced serum medium. Mixed gently and incubated at room temperature for 5 minutes. After incubation for 5 minutes, dilute oligonucleotides and dilute Lipofectamine 2000 were mixed gently, then incubated at room temperature for 20 minutes.

#### Cell Apoptosis Assay

Briefly, Th17 cells ( $1 \times 10^6$ /sample) were stained with 4 mL of phycoerythrin-conjugated anti-CD4 MAB (Becton Dickinson, California, United States) for 30 minutes at 4°C, washed, and then stained with annexin V-FITC/PI kit. Fluorescence-activated cell sorting analysis for annexin-V and PI staining was performed using a C6 flow cytometer (BD Biosciences, United States).

#### Cell Proliferation Assay

Cell proliferation was measured by cell counting kit-8 (CCK-8) assay (Biyuntian, China) according to the manufacturer's instructions. Experiments were performed in 96-well plates. Th17 cells were seeded at a density of  $1 \times 10^6$  cells/mL; 20 µL CCK-8 solution was added into each well (containing 200 µL medium) and further incubated for 2 hours at 37°C. The absorbance of each group at 450 nm was detected using an absorbance microplate reader (iMark, China). This absorbance is directly proportional to the count of living cells.

#### Cell Migration Assay

The migration assay was designed using transwell plates (Corning Costar, Cambridge, Massachusetts, United States) that were 6.5 mm in diameter with 8 µm pore filters. Th17 cells were collected and resuspended in serum-free culture medium. A total of  $1 \times 10^6$  cells in serum-free culture medium were seeded into the upper chamber, while culture medium supplemented with 10% FBS was added to the lower chamber. After incubation for 24 hours at 37°C, the chambers were washed three times and fixed with 4% paraformaldehyde for 10 minutes. Finally, the chambers were stained with 0.5% crystal violet and the cells which passed through the membrane were counted visually under a microscope. The counts of cells were quantified using Image J.

#### Statistical Analysis

Clinical data are expressed as median and range. The non-parametric Mann-Whitney's *U* test was used to compare continuous variables between the two groups. Categorical variables were assessed using the Fisher's exact test. For the quantitative RT-PCR data, relative miRNA expression levels were calculated by comparative  $2^{-\Delta\Delta Ct}$  method. Normally

**Table 1** Baseline characteristics of patients with MPP in the study

Variable	Mild	Severe	p-Value
	(n = 5)	(n = 5)	(Mild vs. severe)
Demographic			
Age (mo), median (range)	7 (3–11)	7 (4–10)	0.9841
Male gender, n (%)	3 (60.00)	3 (60.00)	1.0000
Symptoms			
Total days with fever (d), median (range)	9 (7–10)	15 (14–20)	<b>0.0079</b>
LOS (d), median (range)	11 (7–12)	12 (7–18)	0.3889
Laboratory findings <sup>a</sup>			
WBC ( $\times 10^9/L$ ) (5–12), median (range)	5.7 (4.7–8.4)	9.2 (6.0–15.8)	<b>0.0238</b>
Hs-CRP (mg/L) (<5), median (range)	30.4 (5.23–37.33)	40.96 (12.94–69.80)	<b>0.1508</b>
LDH (U/L) (159–322), median (range)	295 (211–316)	708 (521–1,442)	<b>0.0079</b>
Radiology <sup>b</sup>			
Consolidation, n (%)	5 (100.00)	5 (100.00)	1.0000
Hydrothorax, n (%)	0 (0)	5 (100.00)	<b>0.0079</b>

Abbreviations: Hs-CRP, high-sensitive C-reactive protein; LDH, lactate dehydrogenase; LOS, length of stay; MPP, *Mycoplasma pneumoniae* pneumonia; WBC, white blood cell.

<sup>a</sup>The data of laboratory findings were collected from patients with acute exacerbation of MPP.

<sup>b</sup>Judged by chest radiograph or computed tomography scan in whole course of the patients.

distributed data are presented as means  $\pm$  standard deviation. Statistical significance was determined using Student's *t*-test. Multiple comparisons among test groups were conducted by one-way analysis of variance test. A  $p < 0.05$  was considered statistically significant.

## Results

### Expression of Periostin in MPP Patients

As shown in **Table 1** and **Supplementary Table S1** (available in the online version), a total of 10 children hospitalized with MPP were enrolled in the study, ranging from ages 3 to 11 years, including 4 females and 6 males. There were five children with mild MPP and five children with severe MPP. Compared with the mild cases, severe cases had longer duration of fever and higher levels of LDH in serum ( $p < 0.05$ ), and had unequivocal focal or segmental consolidation with pleural effusion (**Table 1** and **Fig. 1A, B**).

At first, quantitative RT-PCR showed that the content of *M. pneumoniae* was significantly increased in MPP patients, as about twofold compared with healthy children (**Fig. 2A**). The previous study had showed that periostin was a significant changed protein in serum samples from MPP. In this study, the ELISA assay (**Fig. 2B**) and western blot analysis (**Fig. 2C, D**) were used to detect the expression levels of periostin in serum. The data showed that the levels of periostin were increased in MPP patients, particularly in severe cases ( $n = 3$ ,  $p < 0.05$ ). These findings suggested that periostin might participate in the MPP immune response process.

### Periostin Modulates Apoptosis in Th17 Cells

To investigate the role of periostin in MPP patients, mouse cell line Th17 was selected and severe as an infection cellular

model to be used to test the periostin correlation with the extent and severity of the lung lesions in MPP. The specific process was that the Th17 cells were cultured and transfected with either periostin overexpression plasmid or periostin targeting siRNA. Followed, the Th17 cells were incubated with BALF from MPP patients for 24 hours.

As shown in **Fig. 3A**, the results showed that all the transfected plasmid was effectively worked and had no side effects on Th17 cells. Moreover, it is demonstrated that the number of Th17 cells were related to the periostin expression levels. The percentage of apoptotic cells was equally increased in CD4+ cells at 24 hours after periostin overexpression plasmid was introduced in both control group and MPP group (**Fig. 3B, C**). In contrast, the silence plasmids of periostin decreased CD4+ cells apoptosis in both control group and MPP group.

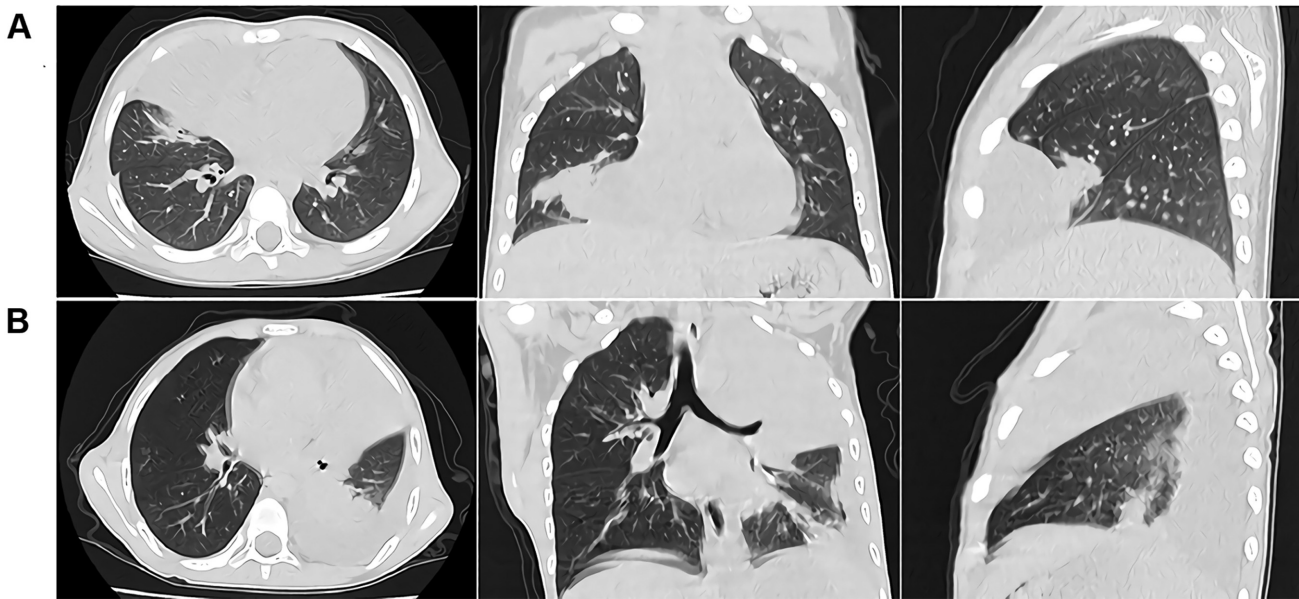
These results suggested that knockdown the periostin expression may play a role in suppressed Th17 cell proliferation and promoted apoptosis especially in MPP patients.

### Periostin Promoted Th17 Cells Migration

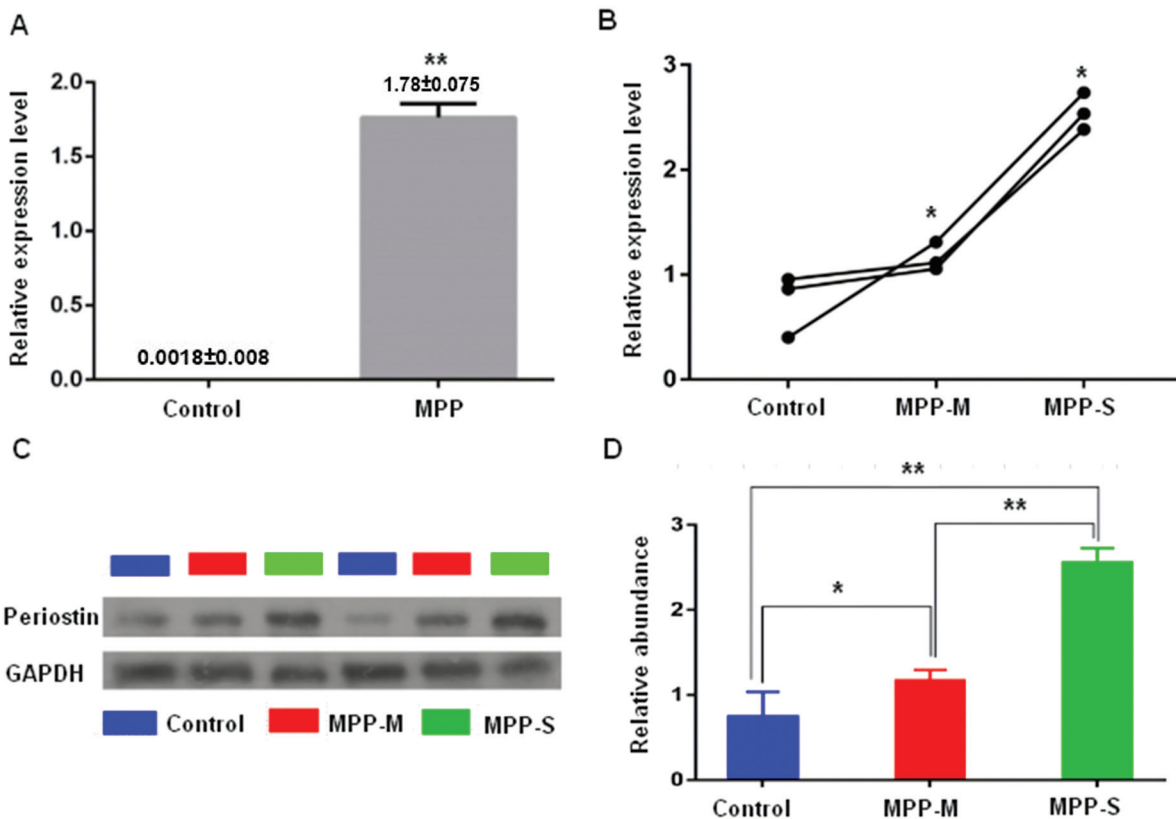
We also studied the functional role of periostin in Th17 cell migration by using a transwell migration assay. The cell migration and invasion of Th17 were significantly promoted in periostin overexpression in MPP group when compared with cells transfected with siRNA negative control vector (**Fig. 4**,  $p < 0.01$ ). These results suggested that upregulation of periostin promoted the migration of Th17 cell with stimulation of BALF from MPP patients.

### Periostin Promotes Production of Proinflammatory Cytokines

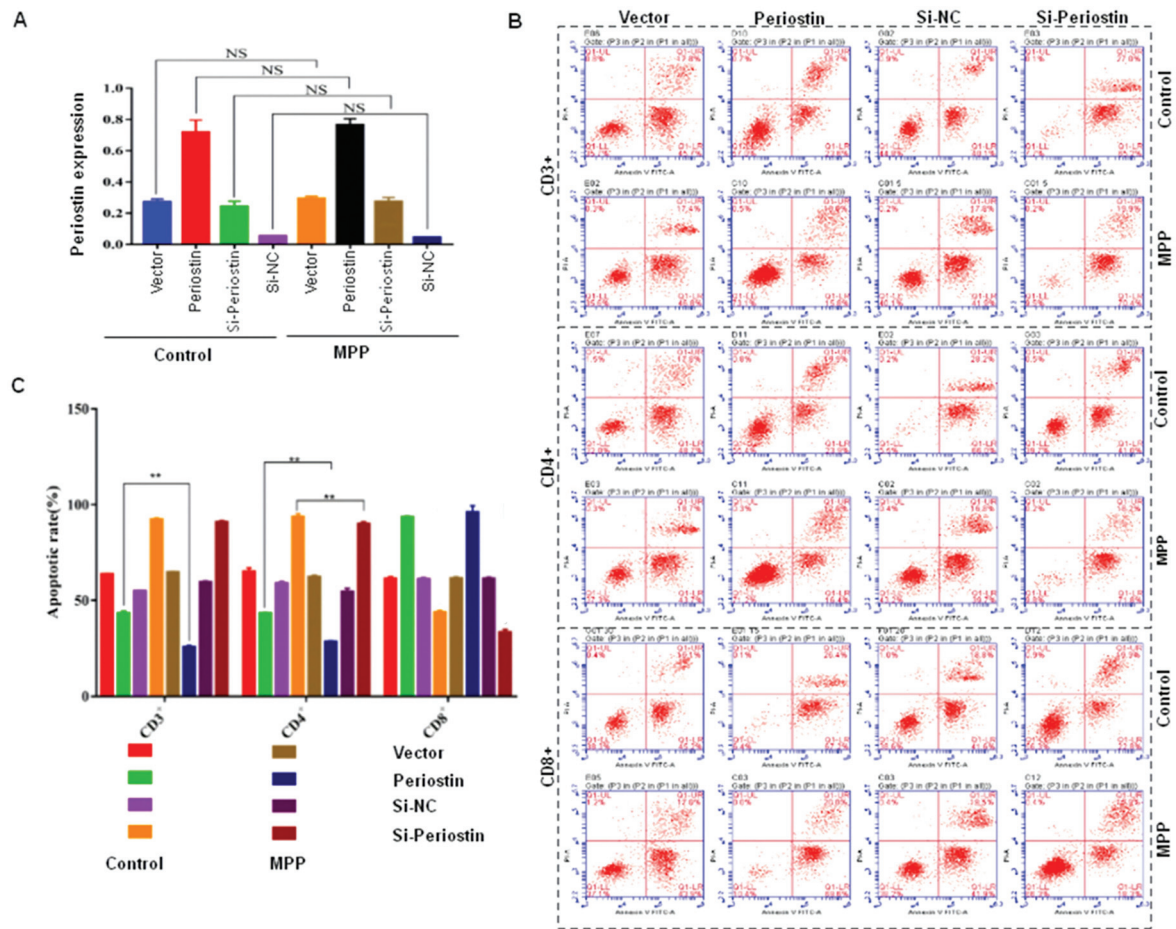
To further investigated the relationship between the expression pattern of proinflammatory cytokines (IL-6, TNF- $\alpha$ , and



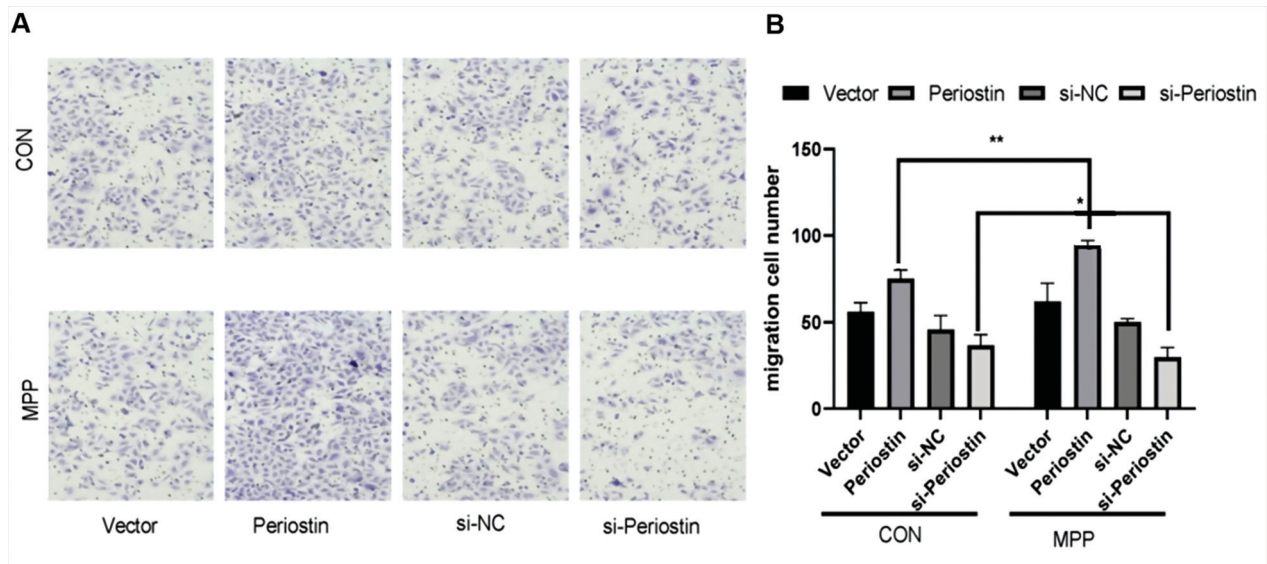
**Fig. 1** Imaging features of lung injury associated with MPP in children. (A) High-resolution CT scan of the chest revealed areas of consolidation in right middle lobe in a 3-year-old child with mild MPP. (B) High-resolution CT scan of the chest revealed areas of airspace consolidation and pleural effusion in left lobes in a 6-year-old child with severe MPP. CT, computed tomography; MPP, *Mycoplasma pneumoniae* pneumonia.



**Fig. 2** Expression of periostin in the MPP patients. (A) The *M. pneumoniae* was confirmed by RT-PCR; (B) periostin levels in the MPP were measured by the relative absorbance from ELISA; (C) western blot analysis showed the correlation between periostin expression in mild group, severe group, and healthy control group; (D) quantification analysis of western blot. All data are shown as the mean ± standard deviation and were analyzed by one-way analysis of variance with Student's *t*-test ( $*p < 0.05$ ,  $**p < 0.01$ ). ELISA, enzyme-linked immunosorbent assay; MPP, *Mycoplasma pneumoniae* pneumonia; RT-PCR, real-time polymerase chain reaction.

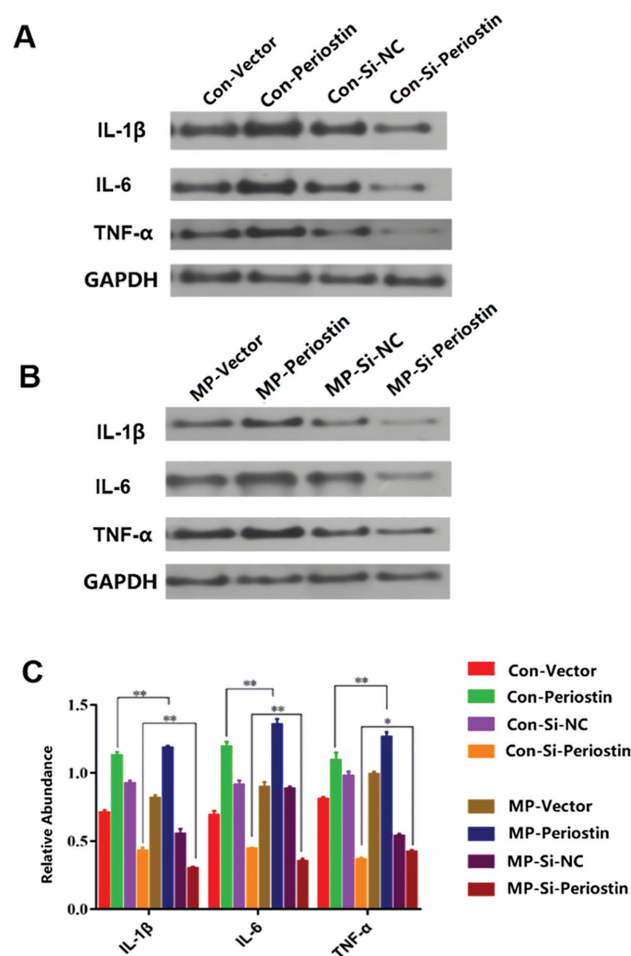


**Fig. 3** FACS analysis of Th17 cell subtypes apoptosis with periostin overexpression and silencing after BALF treatment. (A) The BALF extract from control group and MPP patients were applied to murine Th17 cells and transfected with periostin overexpression vector and periostin siRNA fragment separately. The viability of Th17 cells was measured by CCK-8 kit. (B) The Th17 cells were stained with FITC-conjugated mAb to CD4+ and the apoptosis amount of various immune cell subtypes were counted. The dot-plot patterns were shown for the representative flow cytometric profile for the targeted cells. (C) Quantification analysis of (B) treatment groups was assessed by ANOVA test followed by Student's t-test ( $p < 0.05$ ,  $**p < 0.01$ ). ANOVA, analysis of variance; BALF, bronchoalveolar lavage fluid; CCK-8, cell counting kit-8; FACS, fluorescence-activated cell sorting; MPP, *Mycoplasma pneumoniae pneumoniae*; Th17, T helper 17.



**Fig. 4** Cell migration assay in Th17 cells. (A) Periostin expression effect cell invasion in murine Th17 cells, analyzed by transwell invasion assay. The cells were stained with 0.5% crystal violet (magnification,  $\times 100$ ). (B) Statistical analysis of migrated cells. The data are presented as the mean  $\pm$  SD ( $p < 0.05$ ,  $**p < 0.01$ ). SD, standard deviation; Th17, T helper 17.

IL-1 $\beta$ ) in MPP patients and the periostin, we measured the proinflammatory factors protein expression level from Th17 cells transfected with periostin overexpression and periostin silence plasmids. All the three cytokines showed a positive correlation with periostin expression (IL-6, TNF- $\alpha$ , IL-1 $\beta$ ). The *M. pneumoniae*-periostin group also showed relatively mild expression of both IL-6 and IL-1 $\beta$  (**Fig. 5A, B**). Moreover, *M. pneumoniae*-periostin group showed higher TNF- $\alpha$  cytokine levels. Although all three proinflammatory cytokines (IL-6, TNF- $\alpha$ , and IL-1 $\beta$ ) levels were found to be in highly consistence with the periostin expression, those cytokines expression also exhibited a significant difference ( $p < 0.05$ ) between *M. pneumoniae*-periostin and the control groups (**Fig. 5C**). These findings indicate that periostin modulates the secretion of proinflammatory cytokines of Th17 cells affecting the MPP progress.



**Fig. 5** Proinflammatory cytokines assay in Th17 cells. Differential protein expression of IL-6, IL-1 $\beta$ , and TNF- $\alpha$  was analyzed in BALF of control (A) and MPP (B) patient groups treated with Th17 cells. The cells were cultured to 90% confluence in Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum at 37°C and in 5% CO<sub>2</sub>. (C) Quantification analysis of (A) and (B) after transfection for 24 hours. All data are shown as the mean  $\pm$  SD and were analyzed by one-way ANOVA with Student's *t*-test (\* $p < 0.05$ , \*\* $p < 0.01$ ). ANOVA, analysis of variance; BALF, bronchoalveolar lavage fluid; IL, interleukin; MPP, *Mycoplasma pneumoniae* pneumonia; SD, standard deviation; Th17, T helper 17; TNF, tumor necrosis factor.

## Discussion

Inflammatory responses induced by *M. pneumoniae* coordinate with the injuries to the endothelium, which leads to high permeability of the alveolar-capillary barrier and alveolar edema, thereby inducing pneumonia.<sup>19,20</sup> The periostin, a nonstructural extracellular matrix protein, highly expressed at sites of injury or inflammation, may play a role in mediating the MPP. However, the underlying mechanism of this process remains unclear.

A previous study showed that periostin protein expression increased T lymphocyte cell differentiation as well as induction of host protective immune response.<sup>21</sup> Periostin has been considered as a promising clinically candidate biomarker to predict the response to treatments in both lung-related disease such as asthma<sup>22</sup> and idiopathic pulmonary fibrosis.<sup>23</sup> However, the clinical effects of periostin in predicting MPP are limited by the poor understanding of the underlying mechanisms of MPP. In this study, our results showed that the periostin was expressed in MPP patients, and the level of periostin protein was positively correlated with the severity of MPP, which may function to promote wound healing.

Th17 cells are involved in respiratory *Mycoplasma* infection in animals and contributes to a *Mycoplasma*-induced lung inflammation.<sup>24</sup> In previous studies, the level of Th17 cells was remarkably increased in children with *M. pneumoniae* infection, and patients with hepatic and cardiovascular extrapulmonary manifestations showed a higher frequency of Th17 cells when compared with those without extrapulmonary manifestations, suggesting that Th17 cells may be involved in the clearance of *M. pneumoniae* but excessive Th17 responses may contribute to the immunopathological damage.<sup>14</sup> Combined with previous studies, which showed that Th17 cell surface expresses periostin receptor integrin  $\alpha\beta3$ ,<sup>16</sup> this study hypothesize that the airway epithelial secretion periostin, which binds to integrin on the Th17 cell surface, allows Th17 cells to migrate into the alveoli and participates in the immune response against *M. pneumoniae* in the lungs, maintaining the mucosal barrier integrity against *M. pneumoniae* infections. Therefore, we investigated the role of periostin in regulating Th17 cells apoptosis, migration, and inflammatory cytokines in approaching MPP. The results demonstrated that the periostin inhibits apoptosis of CD4+ subtype Th17 cells. Moreover, periostin promoted Th17 cell migration, especially in the MPP group. Periostin also induced expression of proinflammatory cytokines (IL-6, TNF- $\alpha$ , and IL-1 $\beta$ ) of Th17 cells.

There is evidence that periostin has a protective role in inhibiting a variety of damage-induced cells apoptosis, such as stretch-induced apoptosis,<sup>25</sup> melatonin-induced apoptosis,<sup>26</sup> and hypoxia-induced apoptosis,<sup>27</sup> which in consistence with our data that periostin inhibits CD4+ apoptotic lymphocytes apoptosis in *M. pneumoniae*-induced Th17 cells. The correlation numbers of T cell subsets CD4+ are linked with illness duration, such as the levels of CD4+ were significantly dropped after effective treatment for chronic renal insufficiency

people.<sup>28</sup> Our data showed the same trend that periostin overexpression downregulated the apoptosis CD4+ in MPP group.

Previous data showed that the level of IL-6 in the acute stage was significantly higher than those in the recovery stage, and it could be used as a reference index for the assessment of disease severity.<sup>29</sup> Maturation and releasing of IL-1 $\beta$  also related with cytoadherence ability of *M. pneumoniae* upon infection. This result suggested that the cytoadherence ability of *M. pneumoniae* activated immune responses and was involved in the pathogenesis of *M. pneumoniae* infection.<sup>30</sup> In addition, the high expression of TNF- $\alpha$  not only mediated MPP inhibition through epithelial apoptosis but also worked as a good diagnostic biomarker for differentiating children with RMPP and general MPP.<sup>31,32</sup> Based on the above analysis, our results, that periostin-induced expression of proinflammatory cytokines, might provide potential targets for understanding the cause or mechanism of MPP.

This experiment found that the periostin may play an important role in mediating the course of MPP, but the underlying mechanism of this process had not been confirmed and elaborated. Therefore, advanced research needs to establish a rat model of MPP and probe into the relationship between the periostin expression and Th17 activation in MPP. The pathogenic mechanism in MPP may provide new targets for MPP therapy.

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#### Conflict of Interest

None declared.

#### References

- Bajantri B, Venkatram S, Diaz-Fuentes G. *Mycoplasma pneumoniae*: a potentially severe infection. *J Clin Med Res* 2018;10(07):535–544
- Wu HM, Wong KS, Huang YC, et al. Macrolide-resistant *Mycoplasma pneumoniae* in children in Taiwan. *J Infect Chemother* 2013;19(04):782–786
- Hsieh YC, Tsao KC, Huang CG, et al. Life-threatening pneumonia caused by macrolide-resistant *Mycoplasma pneumoniae*. *Pediatr Infect Dis J* 2012;31(02):208–209
- Zhao JL, Wang X, Wang YS. Relationships between Th1/Th2 cytokine profiles and chest radiographic manifestations in childhood *Mycoplasma pneumoniae* pneumonia. *Ther Clin Risk Manag* 2016;12:1683–1692
- Becker A, Kannan TR, Taylor AB, et al. Structure of CARDS toxin, a unique ADP-ribosylating and vacuolating cytotoxin from *Mycoplasma pneumoniae*. *Proc Natl Acad Sci U S A* 2015;112(16):5165–5170
- Zhang Z, Wan R, Yuan Q, et al. Cell damage and neutrophils promote the infection of *Mycoplasma pneumoniae* and inflammatory response. *Microb Pathog* 2022;169:105647
- Medina JL, Coalson JJ, Brooks EG, et al. *Mycoplasma pneumoniae* CARDS toxin induces pulmonary eosinophilic and lymphocytic inflammation. *Am J Respir Cell Mol Biol* 2012;46(06):815–822
- Medina JL, Coalson JJ, Brooks EG, et al. *Mycoplasma pneumoniae* CARDS toxin exacerbates ovalbumin-induced asthma-like inflammation in BALB/c mice. *PLoS One* 2014;9(07):e102613
- Takayama G, Arima K, Kanaji T, et al. Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J Allergy Clin Immunol* 2006;118(01):98–104
- Conway SJ, Izuhara K, Kudo Y, et al. The role of periostin in tissue remodeling across health and disease. *Cell Mol Life Sci* 2014;71(07):1279–1288
- Izuhara K, Conway SJ, Moore BB, et al. Roles of periostin in respiratory disorders. *Am J Respir Crit Care Med* 2016;193(09):949–956
- Halasz E, Townes-Anderson E, Wang W. LIM kinases in synaptic plasticity and their potential as therapeutic targets. *Neural Regen Res* 2020;15(08):1471–1472
- Guo H, He Z, Li M, Wang T, Zhang L. Imbalance of peripheral blood Th17 and Treg responses in children with refractory *Mycoplasma pneumoniae* pneumonia. *J Infect Chemother* 2016;22(03):162–166
- Wang X, Chen X, Tang H, et al. Increased frequency of Th17 cells in children with *Mycoplasma pneumoniae* pneumonia. *J Clin Lab Anal* 2016;30(06):1214–1219
- Li QL, Wu YY, Sun HM, et al. The role of miR-29c/B7-H3/Th17 axis in children with *Mycoplasma pneumoniae* pneumonia. *Ital J Pediatr* 2019;45(01):61
- Du F, Garg AV, Kosar K, et al. Inflammatory Th17 cells express integrin  $\alpha\beta3$  for pathogenic function. *Cell Rep* 2016;16(05):1339–1351
- Butcher JT, Norris RA, Hoffman S, Mjaatvedt CH, Markwald RR. Periostin promotes atrioventricular mesenchyme matrix invasion and remodeling mediated by integrin signaling through Rho/PI 3-kinase. *Dev Biol* 2007;302(01):256–266
- Bradley JS, Byington CL, Shah SS, et al; Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2011;53(07):e25–e76
- Shimizu T. Inflammation-inducing factors of *Mycoplasma pneumoniae*. *Front Microbiol* 2016;7:414
- Jiang Z, Li S, Zhu C, Zhou R, Leung PHM. *Mycoplasma pneumoniae* infections: pathogenesis and vaccine development. *Pathogens* 2021;10(02):119
- Sonnenberg-Riethmacher E, Mieke M, Riethmacher D. Periostin in allergy and inflammation. *Front Immunol* 2021;12:722170
- Matsumoto H. Roles of periostin in asthma. *Adv Exp Med Biol* 2019;1132:145–159
- Uchida M, Shiraishi H, Ohta S, et al. Periostin, a matricellular protein, plays a role in the induction of chemokines in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2012;46(05):677–686
- Luo Y, Li C, Zhou Z, Gong Z, Zhu C, Lei A. Biological functions of IL-17-producing cells in mycoplasma respiratory infection. *Immunology* 2021;164(02):223–230
- Yu KW, Yao CC, Jeng JH, Shieh HY, Chen YJ. Periostin inhibits mechanical stretch-induced apoptosis in osteoblast-like MG-63 cells. *J Formos Med Assoc* 2018;117(04):292–300
- Meng X, Zhu Y, Tao L, Zhao S, Qiu S. Periostin has a protective role in melatonin-induced cell apoptosis by inhibiting the eIF2 $\alpha$ -ATF4 pathway in human osteoblasts. *Int J Mol Med* 2018;41(02):1003–1012
- Liu HL, Chen Z. Periostin inhibits hypoxia-induced apoptosis in human periodontal ligament fibroblasts via the Akt/PKB signaling pathway. *Int J Clin Exp Med* 2017;10:9171–9179
- Lin JR, J ZXu YW. Effects of losartan on immune function, t cell subsets and micro-inflammatory status in patients with chronic renal insufficiency. *Acta Med Mediter* 2020;220(02):321–329



- 29 Fang KN, Wang J, Ni JW. [Correlation between *Mycoplasma pneumoniae* DNA replication level and disease severity in children with severe *Mycoplasma pneumoniae* pneumonia]. Zhongguo Dang Dai Er Ke Za Zhi 2019;21(09):876–880
- 30 Shimizu T, Kida Y, Kuwano K. Cytoadherence-dependent induction of inflammatory responses by *Mycoplasma pneumoniae*. Immunology 2011;133(01):51–61
- 31 Li G, Fan L, Wang Y, et al. High co-expression of TNF- $\alpha$  and CARDS toxin is a good predictor for refractory *Mycoplasma pneumoniae* pneumonia. Mol Med 2019;25(01):38
- 32 Sun JH, Sun F, Yan B, Li JY, Xin L. Data mining and systematic pharmacology to reveal the mechanisms of traditional Chinese medicine in *Mycoplasma pneumoniae* pneumonia treatment. Biomed Pharmacother 2020;125:109900