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Original Article

Effect and mechanism of pulmonary infection on immune function and renin-angiotensin-aldosterone system in patients with severe acute pancreatitis

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Abstract: Objective: To explore the effect and mechanism of pulmonary infection on immune function and renin-angiotensin-aldosterone system (RAAS) in patients with severe acute pancreatitis (SAP). Methods: The clinical data of patients with SAP admitted to the Affiliated Yantai Yuhuangding Hospital of Qingdao University from January 2016 to March 2018 were selected for retrospective analysis and randomized into the control group (n=64) and the pulmonary infection group (n=58) according to the presence or absence of pulmonary infection. In two groups of patients, serum levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), c-reactive protein (CRP), procalcitonin (PCT), interleukin-17 (IL-17), interleukin-23 (IL-23), angiotensin-I (Ang-I), angiotensin-II (Ang-II), renin (REN), aldosterone (ALD) were detected with enzyme-linked immunosorbent assay while the expression of Toll-like receptor 4 (TLR4) in peripheral blood mononuclear cells was measured using Western blot. Results: There was no statistical difference in the general data in two groups of patients (P>0.05). Compared with those in the control group, patients in the pulmonary infection group had higher levels of serum IL-1β, IL-6, TNF-α, CRP, PCT, IL-17, IL-23, Ang-I, Ang-II, REN and ALD (all P<0.05), and higher expression of TLR4 in mononuclear cells (P<0.001). Conclusion: RAAS can be activated and inflammatory responses be aggravated in SAP patients complicated with pulmonary infection, which may be related to the activation of TLR4/IL-23/IL-17 signaling pathway.

Keywords: Severe acute pancreatitis, pulmonary infection, immune function, renin-angiotensin-aldosterone system

Introduction

Severe acute pancreatitis (SAP) falls into acute response period and systemic infection period according to the course of disease; especially in the period of systemic infection, severe complications such as acute respiratory distress syndrome (ARDS), acute renal failure, pancreatic encephalopathy, septicemia can be accompanied, resulting in the mortality of patients up to 20%-30% [1, 2]. The early stage of SAP is susceptible to secondary pulmonary infection, leading to impaired immune function in the lung. In addition, a large number of infiltrating inflammatory cells such as lymphocytes, neutrophils, and macrophages can be observed and they release pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in the lung tissues [3, 4]. About 20% SAP patients tend to develop ARDS due to pulmonary infection, leading to a poor prognosis [3].

The TLR4/IL-23/IL-17 signaling pathway exerts an immunomodulatory effect in a series of diseases such as acute lung injury, ischemia-reperfusion injuries of the heart and brain, and hepatitis [5-7]. In a mouse model of paraquat-induced pulmonary inflammation and injury, Yan et al. found that serum IL-17 and IL-23 levels were prominently increased, and γδT cells which secreted IL-17 were outstandingly promoted; anti-γδT antibodies, anti-IL-23 antibodies and TLR4 knockout mice were adopted respectively, contributing to remarkedly reducing neutrophils infiltration and alleviating lung injury [8]. Besides, activation of renin-angiotensin-aldosterone system (RAAS) can constrict blood vessels, aggravate tissue edema and

Table 1. Comparison of general data

General data	Control group (n=64)	Pulmonary infection group (n=58)	t/χ^2	P
Age (year)	43.57±9.16	44.26±8.80	0.423	0.673
Male (case)	36	32	0.014	0.905
Female (case)	28	26		
BMI (kg/m^2)	23.31±1.94	23.55±2.03	0.668	0.506
Drinking history (case)	30	31	0.526	0.468

Note: BMI, body mass index.

Table 2. Comparison of serum inflammatory factors

Indicators	Control group (n=64)	Pulmonary infection group (n=58)	t	P
IL-1β (ng/L)	78.23±11.54	122.80±15.91	17.830	<0.001
IL-6 (ng/L)	9.51±6.04	12.48±7.22	2.472	0.015
TNF-α (ng/L)	18.09±7.87	26.35±8.11	5.706	<0.001
CRP (mg/L)	20.11±6.44	27.64±6.98	6.198	<0.001
PCT (μg/L)	0.29±0.11	0.40±0.23	3.420	0.001

Note: IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; CRP, c-reactive protein; PCT, procalcitonin.

necrosis, and plays an indispensable role in the pathogenesis of SAP [9]. Nevertheless, there are few studies on the role of the TLR4/IL-23/IL-17 signaling pathway and RAAS in pulmonary infection of patients with SAP. Therefore, this article aims to investigate the effects of secondary pulmonary infection on TLR4/IL-23/IL-17 signaling pathway and RAAS in SAP patients.

Materials and methods

General data

This study was approved by the Ethics Committee of the Affiliated Yantai Yuhuangding Hospital of Qingdao University and informed consents were obtained. The clinical data of patients with SAP admitted to the Affiliated Yantai Yuhuangding Hospital of Qingdao University from January 2016 to March 2018 were selected for retrospective analysis and randomized into the control group (n=64) and the pulmonary infection group (n=58) according to the presence or absence of pulmonary infection. General data such as age, gender, body mass index (BMI) and drinking history in two groups of patients were collected.

Inclusion and exclusion criteria

Inclusion criteria: 1) SAP: There were acute persistent abdominal pain, marked tenderness in the epigastrium, rebound tenderness, serum amylase ≥3x ULN, local complications such as pancreatic necrosis, pseudocyst, pancreatic abscess, and organ failure; the Balthazar grading of enhanced computed tomography (CT) scan was grades D and E, which meant obvious intrapancreatic and peripancreatic inflammatory changes, effusions, abscesses, or necrosis. 2) Pulmonary infections: There existed cough, expectoration, body temperature of more than 38°C, moist rales, infiltrative changes, white blood cell count $\geq 11*10^9$/L, and pathogens isolated from sputum cultures.

Exclusion criteria: 1) Mild acute pancreatitis: There was no local complications or organ dysfunction; the grading of enhanced CT ranged from grade A to grade C, which meant a large pancreas and an irregular contour. 2) Pulmonary infection occurred within 6 months before acute pancreatitis. 3) Patients complicated with hepatic renal dysfunction, respiratory diseases, cardiovascular and cerebrovascular diseases, tumors, etc.

Enzyme-linked immunosorbent assay (Elisa)

Five milliliters of venous blood were drawn from two groups of patients, left to stand for some time at room temperature and centrifuged at 3,000 rpm/min for 20 min. The supernatant was separated in a sterile centrifuge tube. Serum levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), c-reactive protein (CRP), procalcitonin (PCT), interleukin-17 (IL-17), interleukin-23 (IL-23), angiotensin-I (Ang-I), angiotensin-II (Ang-II), renin (REN) and aldosterone (ALD) were measured using the Elisa kit (Jingmei Biotechnology, Jiangsu). The kit was performed strictly as the manufacturer's instructions.

Western blot

Mononuclear cells were isolated from peripheral blood in two groups of patients with human peripheral blood lymphocyte separating medi-

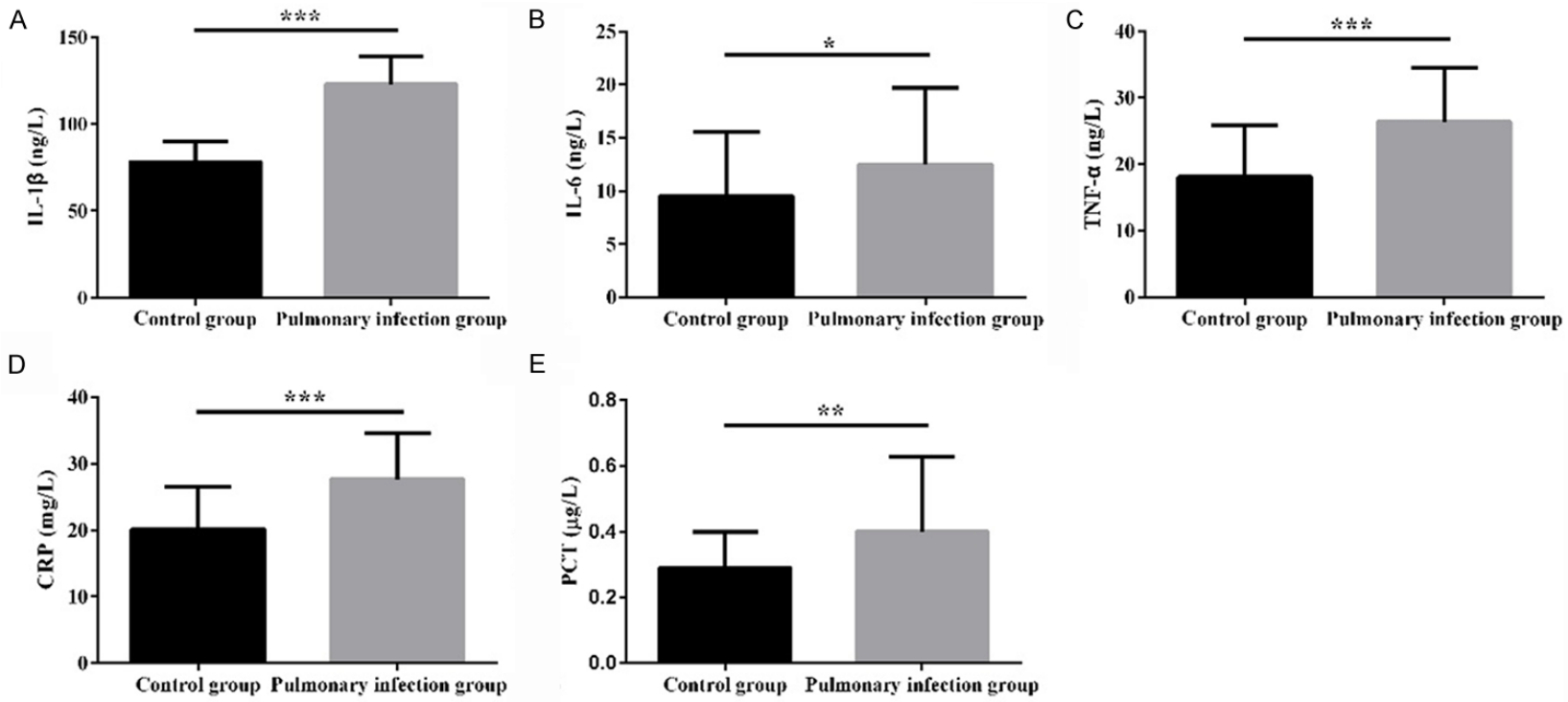

Figure 1. Comparison of serum inflammatory factors in two groups of patients. Detections of serum IL-1β, IL-6, TNF-α, CRP and PCT by Elisa were showed in (A-E) (*P<0.05, **P<0.01, ***P<0.001). IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; CRP, c-reactive protein; PCT, procalcitonin.

Table 3. Effect of pulmonary infection on the expressions of IL-23 and IL-17

Indicators	Control group (n=64)	Pulmonary infection group (n=58)	t	P
IL-17 (ng/L)	17.59±4.89	20.86±5.33	3.534	0.001
IL-23 (ng/L)	12.44±3.15	17.09±3.85	7.328	<0.001

Note: IL-17, interleukin-17; IL-23, interleukin-23.

um (Beijing solarbio science & technology co., ltd.). After cell counting, 150 μL radio immunoprecipitation assay (RIPA) lysate (Beyotime, Shanghai) and 1 mmol/L phenylmethanesulfonyl fluoride (PMSF) protease inhibitor (Beyotime, Shanghai) were added to the chosen 10^6 cells. After full lysis, the concentration of protein was measured with Bicinchoninic acid (BCA) protein quantification kit (Beyotime, Shanghai). Twenty micrograms of total proteins were taken for polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membrane in an ice-water bath at 200 mA for 120 min. It was then blocked with 5% skim milk at room temperature for 120 min and diluted 1:1,000 and 1:2,000 with anti-Toll-like receptor 4 (TLR4) (Abcam, Britain) and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibodies (Abcam, Britain), respectively. After incubation overnight at 4°C and washing for three times, horseradish peroxidase-labelled goat anti-rabbit secondary antibodies (Boster, Wuhan) were diluted 1:2,000; after exposure, images were obtained using a gel imager. The grayscales of the Western blot images were measured with Image Pro Plus 6.0 software and the gray scale value of the control group was considered as one.

Statistical analysis

All statistical analyses were performed with SPSS20.0 software. The measurement data which met normal distribution were expressed as mean ± standard deviation and the independent sample t test was adopted in two sets of measurement data. The quantitative data were presented as case/percentage (n/%) and the Chi-square test was used in comparisons between groups. P<0.05 suggested a significant difference.

Results

Comparison of general data

There was no statistically significant difference between the two groups in terms of age, gender, BMI and drinking history, which were comparable (all P>0.05). See **Table 1**.

Comparison of serum inflammatory factors

The serum levels of IL-1β, IL-6, TNF-α, CRP and PCT in the pulmonary infection group were higher than those in the control group (P<0.001, P=0.015, P<0.001, P<0.001, P=0.001). See **Table 2** and **Figure 1**.

Effect of pulmonary infection on the expressions of IL-23 and IL-17

Compared with those in the control group, the serum levels of IL-17 and IL-23 of patients in the pulmonary infection group were much higher (P=0.001; P<0.001). See **Table 3** and **Figure 2**.

Effect of pulmonary infection on the expression of TLR4

The expression of TLR4 in the mononuclear cells was higher in the pulmonary infection group than that in the control group (P<0.001). See **Figure 3**.

Comparison of RAAS indicators in two groups of patients

Compared with those in the control group, patients in the pulmonary infection group had higher serum contents of Ang-I, Ang-II, REN, ALD (P=0.017, P=0.003, P=0.029, P<0.001). See **Table 4** and **Figure 4**.

Discussion

The infiltration of inflammatory cells such as neutrophils, lymphocytes and macrophages and the release of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, CRP and PCT were noted in SAP complicated with pulmonary infection, resulting in the cascade of inflammatory factors, systemic inflammatory response syndrome and multiple organ failures, the pathological mechanism of which was the main cause of high mortality in patients with SAP complicated with pulmonary infection [10, 11]. In this article, we also found that the serum levels of inflammatory factors in SAP patients without pulmonary infection were higher than those in normal persons while contents of IL-1β, IL-6, TNF-α, CRP and PCT in serum of SAP

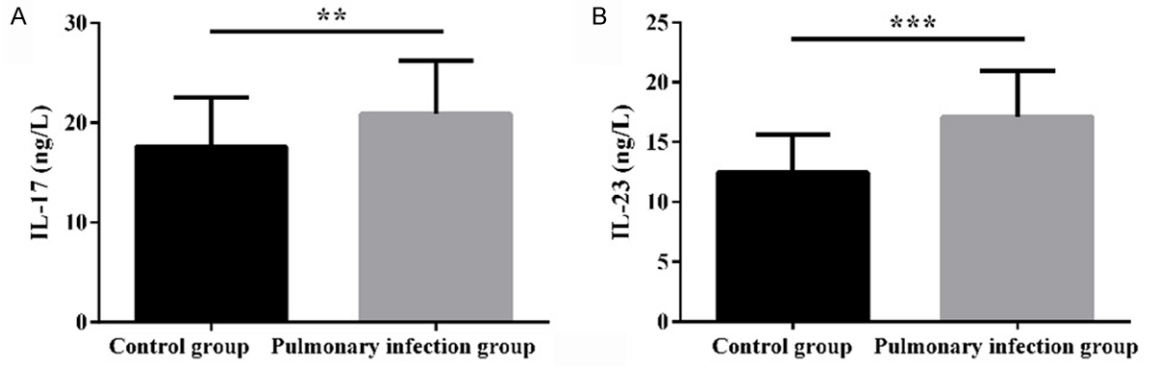

Figure 2. Effect of pulmonary infection on the expressions of IL-23 and IL-17. Detections of serum IL-17 and IL-23 by Elisa were showed in (A, B) (**P<0.01, ***P<0.001). IL-17, interleukin-17; IL-23, interleukin-23.

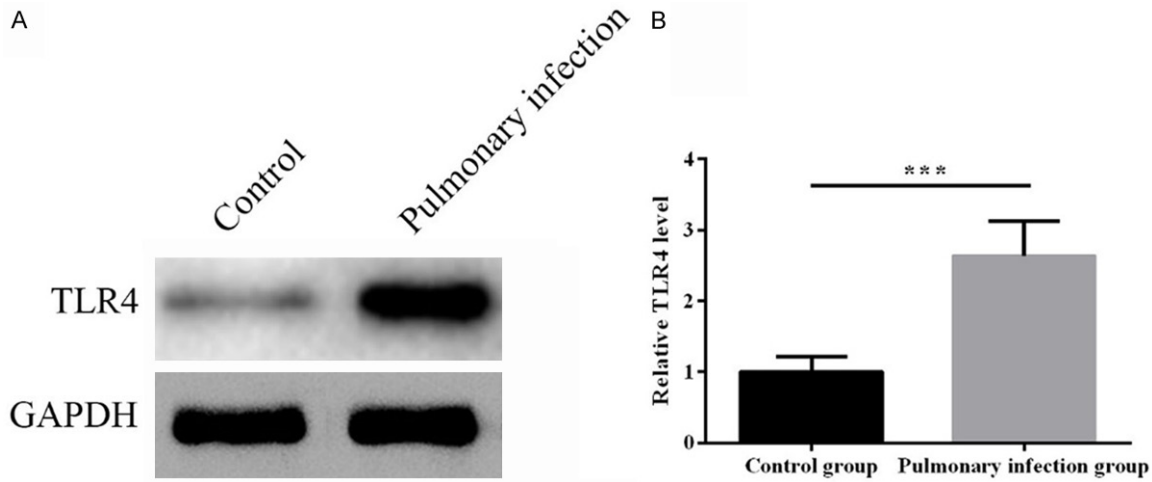

Figure 3. Effect of pulmonary infection on the expression of TLR4. A: Grayscale of TLR4 expression detected by Western blot; B: Statistical chart of Western blot grayscale (***P<0.001). TLR4, Toll-like receptor 4; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 4. Comparisons of RAAS indicators in two groups of patients

Indicators	Control group (n=64)	Pulmonary infection group (n=58)	t	P
Ang-I (μg/L)	14.25±1.57	15.02±1.94	2.419	0.017
Ang-II (μg/L)	87.24±10.11	93.18±11.60	3.022	0.003
REN (μg/L)	1.62±0.38	1.79±0.47	2.206	0.029
ALD (ng/L)	208.55±17.81	224.08±25.03	3.976	<0.001

Note: Ang-I, angiotensin-I; Ang-II, angiotensin-II; REN, renin; ALD, aldosterone.

patients complicated with pulmonary infection were further elevated, suggesting that the pulmonary infection aggravated the inflammatory responses of the body. In a study of Sailai et al., alveolar macrophages in SAP secreted a series of pro-inflammatory factors such as TNF-α; by inhibiting the expression of key factor NFκB, the release of pro-inflammatory factors could be reduced and the degree of lung injury was alleviated, elucidating that the inhibition of the upstream signal pathway of inflammation might be the next step of the treatment research [12].

IL-17 is mainly secreted by Th17 cells, and $CD8^+$ T cells and other innate immune cells, such as NKT cells, γδT cells, neutrophils can also secrete in small amounts. With IL-17, inflammatory cells such as neutrophils, lymphocytes and macrophages can be induced to migrate toward tissue lesion sites, and involved in cell activation. Therefore, it plays a dispensable immunomodulatory role in the pulmonary infection, asthma, chronic obstructive pulmo-

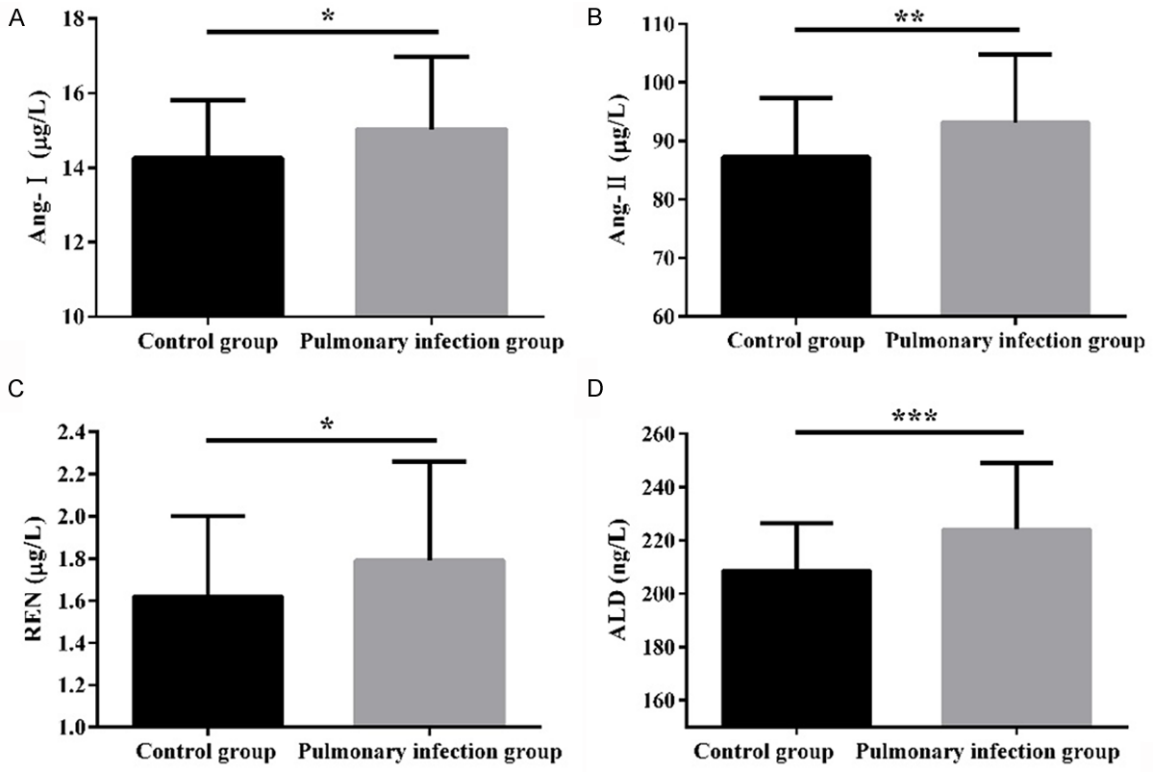

Figure 4. Comparison of RAAS indicators. Detections of serum Ang-I, Ang-II, REN and ALD by Elisa were showed in (A-D) (*P<0.05, **P<0.01, ***P<0.001). Ang-I, angiotensin-I; Ang-II, angiotensin-II; REN, renin; ALD: aldosterone.

nary disease, inflammatory bowel disease, coronary atherosclerosis and rheumatoid arthritis [13, 14]. The secretion of IL-17 is mainly regulated by IL-23 through a variety of mechanisms: 1) IL-23 can promote the differentiation of primitive $CD4^{+}$ T cells into Th17 cells. 2) Stimulations of IL-23 and anti-CD3 antibodies synergistically lead to an increase in IL-17 secreted by NKT cells. 3) Both IL-23 and IL-1 can promote secretion of IL-17 secreted by γδT cells [8]. Thus, IL-23 and IL-17 are involved in the progress of pulmonary inflammation, and the study showed that the use of the corresponding inhibitory molecules could notedly reduce the damage caused by pulmonary inflammation [15]. We also found in this article that the serum levels of IL-17 and IL-23 in SAP patients with pulmonary infection were higher than those in SAP patients without pulmonary infection, illustrating that pulmonary infection activated IL-23/IL-17 signaling pathway.

Upstream regulatory factors of the IL-23/IL-17 signaling pathway have not yet been clearly elucidated, but numerous studies have shown that TLR4 is closely related to the secretion of IL-23 [6, 16]. Mainly expressed in antigen presenting cells, TLR4, a pattern recognition receptor, can recognize the conserved molecular ligands on the external microbes or self-degradation products, thereby activating the body's immune responses [17]. By isolating peripheral blood mononuclear cells from patients, we found that the expression of TLR4 was markedly promoted in SAP patients complicated with pulmonary infection, which might be a key factor in stimulating the activation of IL-23/IL-17 signaling pathway.

RAAS, mainly involved in the regulation of blood pressure, serum sodium and water, is an important pathological mechanism of diseases such as hypertension, diabetes, and coronary heart diseases [18, 19]. In recent years, it has been discovered that RAAS also exerts a role in lung injury of SAP [9, 20]. For example, in a lung injury rat model of SAP by retrograde biliopancreatic duct injection of 5% sodium taurocholate, Yu et al. found that the expressions of Ang-II and its receptor AT-I in the lung tissues were obviously increased, serum contents of Ang-II

and amylase were remarkably reduced after the administration of angiotensin converting enzyme inhibitor captopril, resulting in inhibition of the expressions of RhoA, ROCK and MLCK, reduction of pulmonary vascular permeability, alleviation of pulmonary edema and pneumonia, the therapeutic effect of which might be related to Ang-II and its Rho/ROCK signaling pathway [9]. We also found in this paper that serum levels of Ang-I, Ang-II, REN, and ALD in SAP patients complicated with pulmonary infection were higher than those in SAP patients without pulmonary infection, indicating RAAS could be activated in SAP patients with pulmonary infection.

Although the effects of SAP complicated with pulmonary infection on TLR4/IL-23/IL-17 signaling pathway and RAAS are preliminarily discussed, there are still many imperfections in our research: 1) A study found that high-mobility group box 1 protein (HMGB1), the upstream regulator of the TLR4/IL-23/IL-17 signaling pathway, could stimulate the secretion of IL-23 and IL-17 by TLR4 [21, 22]. However, this article did not investigate whether HMGB1 was involved in the pathological mechanism of SAP complicated with pulmonary infection. 2) Whether the specific inhibition of the high expressions of TLR4, IL-23 and IL-17 reduces the pulmonary and systemic inflammatory responses and alleviates the symptoms of SAP need establish the corresponding animal model to verify. 3) Does pulmonary infection affect the downstream Rho/ROCK signaling pathway of RAAS? Can angiotensin converting enzyme inhibitors such as captopril exert protective effects? 4) Whether the TLR4/IL-23/IL-17 signaling pathway and the activation of RAAS have mutually promotive effects and the mechanism of interactions between the two systems still need further molecular experiments. In the future, we will further study the pathogenesis of SAP with pulmonary infection, and to specifically block TLR4, IL-23, IL-17, and RAAS, antibodies or medication will be adopted for therapeutic investigation.

Collectively, RAAS can be activated and inflammatory responses are aggravated in SAP patients complicated with pulmonary infection, which may be associated with the activation of the TLR4/IL-23/IL-17 signaling pathway.

Disclosure of conflict of interest

None.

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