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Original Article

Interleukin-17A promotes paraquat-induced acute lung injury on mice

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Abstract: Objective: Interleukin-17A (IL-17A) is a potent proinflammatory factor. Our study aimed to investigate the role of IL-17A on acute lung injury (ALI) induced by paraquat (PQ) and the potential way. Methods: A total of 180 female mice were randomly and evenly divided into 9 groups: Control group (NS), PQ groups (4 groups) and PQ+A b groups (4 groups). NS group mice were treated with equal volume of physiological saline by gastric gavage. Mice in PQ and PQ+A b groups were exposed to PQ solution by gastric gavage (25 mg/kg). PQ+A b groups mice were intraperitoneally (i.p.) injected with IL-17A neutralizing antibody (5 mg/kg) 2 hours after PQ perfusion, all of managements above were one-off. Mice were executed respectively at 8 h, 24 h, 48 h and 72 h, NS group mice at 24 h. Lung pathological change was observed with HE staining, wet-to-dry (W/D) ratio and total protein content in broncho-alveolar lavage fluid (BALF) were determined, changes of cytokines were detected by ELISA, leukocyte and neutrophil in BALF were recorded, lung IL-17A and Nuclear Factor-κB p65 (NF-κB p65) were examined by immunohistochemical (IHC), q-PCR was used for the expression of IL-17A mRNA in lung tissue. Results: After PQ perfusion, levels and expression of IL-17A in mice increased ($P<0.05$); While blockade IL-17A with antibody, the ALI induced by PQ was attenuated, activation of NF-κB p65 and the percentage of neutrophil were reduced ($P<0.05$). Conclusion: IL-17A promotes the process of ALI induced by PQ, and probably by activating NF-κB p65 and recruiting neutrophil.

Keywords: Interleukin-17A, paraquat, acute lung injury, mouse, Nuclear Factor-κB p65, neutrophil

Introduction

Paraquat (PQ) is an organic heterocyclic contact defoliant and herbicide, which is widely used in agriculture around the world, especially in the developing countries [1]. PQ is highly toxic to human and animal, and oral is the main way of poisoning. Frequent PQ poisoning and high mortality makes it become a severe public health issue in developing countries since no specific treatment was effective at present, therefore, many countries try to limit the production and use of PQ. However, it is still widely used due to the effective weed control effect, and the poisoning therefore is staying at a high level.

PQ can cause multiple organ injury or even failure, but lung is the main target organ [2]. In spite of the mechanisms of PQ-induced lung injury stay not been fully elucidated, current research suggests that the generation of oxygen radicals is the initiating factor. PQ poisoning early produces oxygen radicals [3], which then activates a variety of effector cells and prompts the release of inflammatory mediators to damage lung tissues [4], causes the pathological characteristics of pulmonary interstitial edema, hemorrhage [5], clinically for Acute Respiratory Distress Syndrome (ARDS) as the major performance, the severe cases can quickly appear to death for respiratory failure. After the acute injury periods, pulmonary fibrosis results in respiratory failure to death [6]. Recent studies show that the participation of inflammation medium is an important part of the PQ poisoning lung injury.

IL-17A also known as IL-17, is a pro-inflammatory cytokine, which mainly produced by T helper cell 17 (Th17), natural killer cell and neutrophil [7]. Studies show that, since IL-17A could induce respiratory epithelial cells to secrete CXCL8, CXCL1, CXCL5, IL-6, GCSF, and GM-CSF, which in turn recruit neutrophils to the airways [8, 9], the cytokine implicates in a multitude of inflam-

matory lung diseases, both autoimmune and acquired [10]. Reports revealed that, IL-17AR (IL-17A receptor)-deficient mice or neutralized with IL-17A antibody reduced neutrophil influx, attenuated the early lung response to silica particles or bleomycin (BLM), and then alleviated the lung injury induced by silica particles or BLM [7, 11]. Recent evidences confirmed that elevated IL-17A expression closely correlated to the development of Acute Lung Injury (ALI), IL-17A-deficient mouse was resistant to the induction of ALI, in addition, blockade IL-17A with neutralizing antibody ameliorated the induction of ALI induced by lipopolysaccharide (LPS) or multiple-trauma [12, 13]. The above studies prompted us to speculate whether IL-17A implicates in the development of ALI induced by PQ poisoning.

In our study, we built a mouse model of acute PQ poisoning and blockade IL-17A in vivo with neutralizing antibody to investigate the role of IL-17A in the ALI process, and further to explore the possible ways.

Materials and methods

Animals

Healthy female ICR (Institute of Cancer Research) mice (SPF grade, 6-8 weeks, 26-30 g in body weight) were purchased from Liaoning Changsheng Biological Technology Company (Animal production license No.: SCXK (l) 2015-0001). The mice were kept under a 12-h light/12-h dark cycle with free access to food and water for 1 week prior to experimental procedures in the Animal Lab of China Medical University Science Experiment Center. Animal experiments were in accordance with ethical policies of the International Journal of Experimental Pathology and approved by the local ethical committee.

Animal grouping and model establishment

A total of 180 female mice were randomly and evenly divided into 9 groups: Control group (NS, n=20), PQ groups and PQ+A b groups, then both of PQ and PQ+A b groups divided into 4 subgroups (n=20) respectively according to 8 h, 24 h, 48 h, 72 h. Mice in NS group were treated with equal volume of physiological saline by gastric gavage. Mice in PQ and PQ+A b groups were exposed to PQ solution (Shaanxi Galen Crop Science co., LTD, CHN) by gastric gavage (25 mg/kg). Mice in PQ+A b groups were intraperitoneally (i.p.) injected with IL-17A neutralizing antibody (Catalog Number: 16-7173, eBiosicence, USA) (5 mg/kg) 2 hours after PQ perfusion, all of the managements above were one-off.

Specimen harvest

At 8 h, 24 h, 48 h and 72 h after the above treatments, mice from each group were executed after anesthetized with 10% chloral hydrate (0.3 ml/100 g i.p.), NS group mice were executed at 24 h. Peripheral blood, bronchoalveolar lavage fluid (BALF) and lung tissues were obtained respectively.

Peripheral blood was centrifuged for 10 min (1500 rpm, 4°C) to obtain the upper serum. 6 mice tracheas were cannulated, and each mouse was lavaged with phosphate buffered saline (PBS) three times each mouse immediately after blood was collected, BALF was collected and the volume was recorded. Then the BALF was mixed well and centrifuged (1500 rpm, 4°C) for 10 min. Supernatants were stored at -80°C for total protein analysis and cytokines assay, pellets were prepared for inflammatory cell counts. Mice lungs which were not lavaged were used to do Wet-to-Dry (W/D) ratio calculation, histological study and q-PCR test.

Lung wet-to-dry (W/D) calculation

Lungs were cleared and weighed immediately after removal to obtain the “wet” weight, and then placed in an oven at 60°C for 72 h to obtain the “dry” weight.

ELISA for cytokines in serum and BALF

IL-17A, IL-6, TNF-α in serum and IL-17A in BALF were detected by Enzyme-linked (ELISA) kits (Bioss, Beijing, CHN) according to the manufacturer’s instructions.

Inflammatory cell counts in BALF

BALF pellets were resuspended in 0.3 ml saline solution, total number of leukocytes and neutrophils were calculated with a hemocytometer.

Total protein analysis in BALF

Total protein content in BALF was measured by Bradford method using Coomassie brilliant

Table 1. Changes of lung W/D

Groups	n	8 h	24 h	48 h	72 h
NS	6		3.86±0.04		
PQ	6	4.32±0.04[a]	4.72±0.07[a]	5.51±0.17[a]	5.11±0.10[a]
PQ+A b	6	4.23±0.09[a,b]	4.32±0.05[a,b]	4.96±0.14[a,b]	4.52±0.05[a,b]

Note: [a]*P*<0.05, vs. NS group; [b]*P*<0.05, vs. PQ group.

Table 2. Total protein in BALF

Groups	n	Total protein in BALF (μg/ml)
NS	6	50.317±7.027
PQ		
8 h	6	100.208±10.977[a]
24 h	6	152.392±15.696[a]
48 h	6	190.278±13.460[a]
72 h	6	178.822±17.881[a]
PQ+A b		
8 h	6	78.863±9.420[a,b]
24 h	6	113.107±15.512[a,b]
48 h	6	156.438±14.661[a,b]
72 h	6	148.847±6.913[a,b]

Note: [a]*P*<0.001, vs. NS group; [b]*P*<0.001, vs. PQ group.

blue G-250 kits (Solarbio, Beijing, China) in accordance with the manufacturer's instructions.

Tissue preparation and histological study

Left upper lung tissues were fixed with 10% neutral formalin, embedded in paraffin, and sliced at thickness of 5 μm.

Histology of lung was examined under the microscope with Hematoxylin-Eosin staining. To grade the lung injury, five visual fields per section were randomly selected with 200× magnification under bright-field viewing, and performed by measuring the thickness of the alveolar septa as well as semi-quantitative scoring, as described earlier [14] for 5 different aspects: Pulmonary edema, inflammatory infiltration, hemorrhage, atelectasis and hyaline membrane formation: 0 for no injury, 1 for injury <25%, 2 for injury ranging from 25% to 50%, 3 for 50% to 75%, and 4 for injury >75%.

Immunohistochemistry (IHC) for IL-17A and NF-κB p65

Lung slides were dewaxed and hydrated. The antigens were repaired in sodium citrate at pH 6.0 by microwave for 10 min. Endogenous peroxidase was inactivated by 3% H_2O_2 for 15 min. Goat serum (SL2-10, Solarbio, Beijing, CHN) was used to block non-specific antigen sites for 15 min. Then, the slides were incubated successionally with diluted primary antibody (IL-17A for 1:50; NF-κB p65 for 1:100) (IL-17A:130-82-1-AP; NF-κB p65:8242P. Proteintech, Wuhan, CHN) over night at 4°C. The sections were then washed with PBS and incubated with biotin-conjugated secondary antibody (ZB-2301, Beyotime, Shanghai, CHN) (1:200 diluted) for 30 min at 37°C, then, streptavidin/HRP (ZB-2301, Beyotime, Shanghai, China) was added and 100 μl diaminobenzidine (DAB) was added successively until reaction was terminated by the tap water, dyeing time was controlled under microscope. Finally, the slides were re-dyed with hematoxylin (Solarbio, Beijing, China), dehydrated, vitrified and mounted before observed under light microscope. Negative controls were generated by omitting the primary antibodies. The results were evaluated semi-quantitatively according to the percentage of positive cells in 5 randomly selected fields under 400-fold magnification and then take pictures [15]. For the score of positive cell ratio, 0-1%, 1-10%, 10-50%, 50-80% and 80-100% were scored as 0, 1, 2, 3 and 4, respectively. For intensity score, negative, weakly positive, positive and strongly positive were scored as 0, 1, 2, and 3, respectively. IHC score value = positive cell ratio score × intensity score.

All histology procedures were performed by two experienced pathologist who were blinded to the treatment group.

Expression of IL-17A mRNA in lung tissue

Lung tissues used for q-PCR were stored at -80°C, and total RNA was isolated from lung homogenates with Trizol reagent (15596026, Invitrogen, San Diego, CA, USA), then used RT kit (TaKaRa, Dalian, China) perform the reverse transcription (RT) reaction in 20 μl system. q-PCR reaction was performed in 20 μl system containing 10 μl SYBR® Premix Ex TaqTM, 0.8 μl forward primer (10 μM), 0.8 μl reverse primer (10 μM), 1.6 μl cDNA template, and 6.8 μl dis-

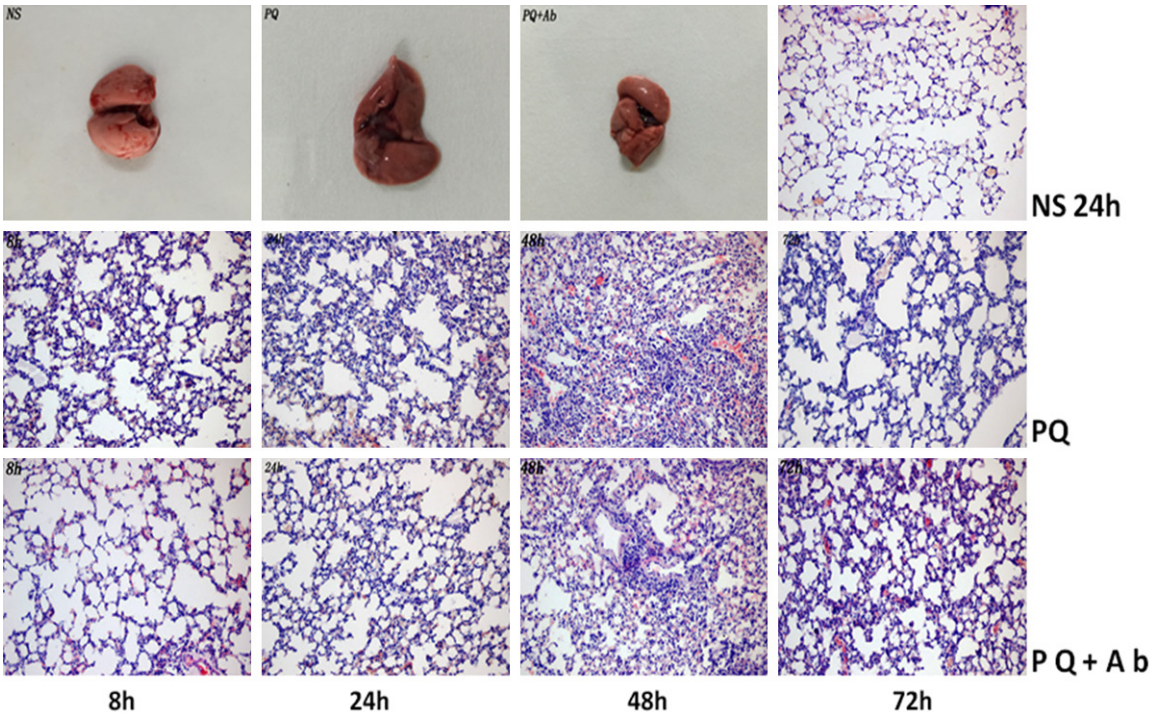

Figure 1. Gross inspection was at 24 h, and pulmonary hemorrhage and swelling were observed in PQ and PQ+A b groups. The hemorrhage and swelling in PQ+A b group were milder than in PQ group, NS group mice lung shows no significant changes. Histology observed with HE staining under light microscope (×200), lungs of mice from NS group showed normal structure and no evident lesion. While in the PQ and PQ+A b groups, there were great changes in tissue structure characterized by the infiltration of inflammatory cells in the widened alveolar septum, alveolar hemorrhage, full of edema fluid in the alveolar cavity, and obvious alveolar structure collapse were observed, all of the poisoning mice lung tissues showed pathological changes of ALI.

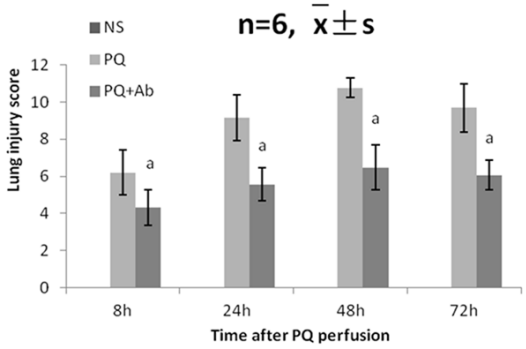

Figure 2. Lung injury score increased after PQ perfusion, and highest at 48 h, while the scores in PQ+A b groups were lower than those in PQ groups at each time. (^aP<0.01, vs. PQ groups).

tilled H_2O (dH_2O). PCR program: 37°C reverse transcription 15 min, 85°C pre-denaturation 2 min, 95°C denaturation 30 s, 40°C annealing 30 s, 40 cycles on 96 Real-Time Quantitative lightcycle 480II (Roche, Switzerland/German). GAPDH was used for an internal control, relative gene expression was calculated using the ratios that IL-17A mRNA/GAPDH mRNA. The sequences were referred from PUBMED database, all the primers (Sangon, Shanghai, China) were designed with Primer 5.0. Dissolve curve was drew at the same time to ensure the specificity of fluorescent q-PCR. The primers used were listed as follows: IL-17A (344 bp): forward: 5'-TGTCAATGCGGAGGGAAAG-3', reverse: 5'-GCAGTTTGGGACCCCTTTAC-3'; GAPDH (183 bp): forward: 5'-GGTTGTCTCCTGCGACTTCA-3', reverse: 5'-TGGTCCAGGGTTTCTTACTC-3'.

Statistical analysis

Data are expressed as $\bar{x}$ (mean) ± s (standard derivation). Statistical analysis was performed with SPSS 22.0, statistical differences were determined by one-way analysis of variance (one-way ANOVA), followed by Least Significant Difference test (LSD-t) or Games-Howell test for multiple comparis ons, P<0.05 was considered to be statistically significant.

Table 3A. Levels of IL-17A in Serum and BALF (pg/ml)

Group	n	Serum IL-17A	n	BALF IL-17A
NS	8	14.558±1.108	6	5.253±1.063
PQ				
8 h	8	28.819±3.399[a]	6	14.597±1.499[a]
24 h	8	63.216±3.718[a]	6	35.338±4.194[a]
48 h	8	87.808±5.138[a]	6	58.470±5.202[a]
72 h	8	111.623±5.469[a]	6	91.358±4.660[a]
PQ+A b				
8 h	8	19.689±2.093[a,b]	6	9.063±0.949[a,b]
24 h	8	23.285±3.729[a,b]	6	18.172±4.481[a,b]
48 h	8	49.424±6.081[a,b]	6	31.312±3.544[a,b]
72 h	8	69.741±2.031[a,b]	6	50.538±3.756[a,b]

Note: [a]P<0.05, vs. NS group; [b]P<0.05, vs. PQ group.

Table 3B. Levels of IL-6 and TNF-α in Serum (pg/ml)

Group	n	IL-6	TNF-α
NS	8	22.489±2.165	57.857±5.919
PQ			
8 h	8	37.743±2.836[a]	77.459±4.006[a]
24 h	8	53.716±4.419[a]	176.316±13.234[a]
48 h	8	98.646±8.471[a]	140.376±6.690[a]
72 h	8	72.298±10.316[a]	100.664±9.029[a]
PQ+A b			
8 h	8	31.115±3.233[a,b]	66.805±3.081[a,b]
24 h	8	44.906±3.562[a,b]	120.570±12.388[a,b]
48 h	8	82.464±7.264[a,b]	106.287±8.156[a,b]
72 h	8	57.871±4.757[a,b]	84.722±8.904[a,b]

Note: [a]P<0.05, vs. NS group; [b]P<0.05, vs. PQ group.

Results

Symptoms on the mouse

There were no evident symptoms on the NS group mice. In the PQ and PQ+A b groups, the mice successively appeared series of symptoms including listlessness, somnolence, tachypnea and oral secretions increased. The poisoning symptoms became progressively alleviate from 8 h to 48 h after PQ perfusion. Compared with PQ group, mice in PQ+A b group showed milder symptoms such as respiratory symptoms and oral secretion.

Death of mice

After PQ perfusion, 18 mice died in PQ group (4 at 8-24 hours, 8 at 24-48 hours, 6 at 48-72 hours) and 13 mice died in PQ+A b groups (3 at 8-24 hours, 6 at 24-48 hours, 4 at 48-72 hours). But the mortality between PQ and PQ+A b groups showed no different by Kaplan-Meier analysis (P>0.05). No mice die in NS group.

Mice lung W/D

Lung W/D showed the content of water in lung tissues after the treatments, reveals the lung tissue edema. After PQ perfusion, W/D increased significantly (all P<0.05) and peaked at 48 h; Compare with PQ groups, W/D in PQ+A b groups decreased at each time point (all P<0.05). Shown in **Table 1**.

Total protein content in BALF

As shown in **Table 2**, total protein in BALF increased markedly in PQ and PQ+A b groups after PQ perfusion compared to NS group, (all P<0.001), and highest at 48 h; It was lower in PQ+A b groups than those of in PQ groups (all P<0.001).

Histopathological change of lung tissues

Pulmonary hemorrhage and swelling were observed in PQ and PQ+A b groups, displays in the **Figure 1**. The hemorrhage and swelling in PQ+A b group were milder than in PQ group, NS group mice lung shows no significant changes.

Observed under light microscope, lung tissue from NS group showed normal structure and no evidently lesion. While in the PQ and PQ+A b groups, there were great changes in tissue structure, characterized by the infiltration of inflammatory cells in the widened alveolar septum, the alveolar hemorrhage, the full of edema fluid in the alveolar cavity, and obvious alveolar structure collapse were observed, all of the poisoning mice lung tissues presented pathological changes of ALI. The injury was milder in PQ+A b groups than that in PQ groups, as shown in **Figure 1**, and the pathological scores showed in **Figure 2**.

Cytokines change in serum and BALF

As shown in **Table 3A**, **3B**, in serum, after PQ perfusion, levels of IL-17A, IL-6 and TNF-α increased significantly (all P<0.05), TNF-α and IL-6 respectively peaked at 24 h and 48 h, but the IL-17A continuously elevated in the observed time. Compare with PQ groups, levels of

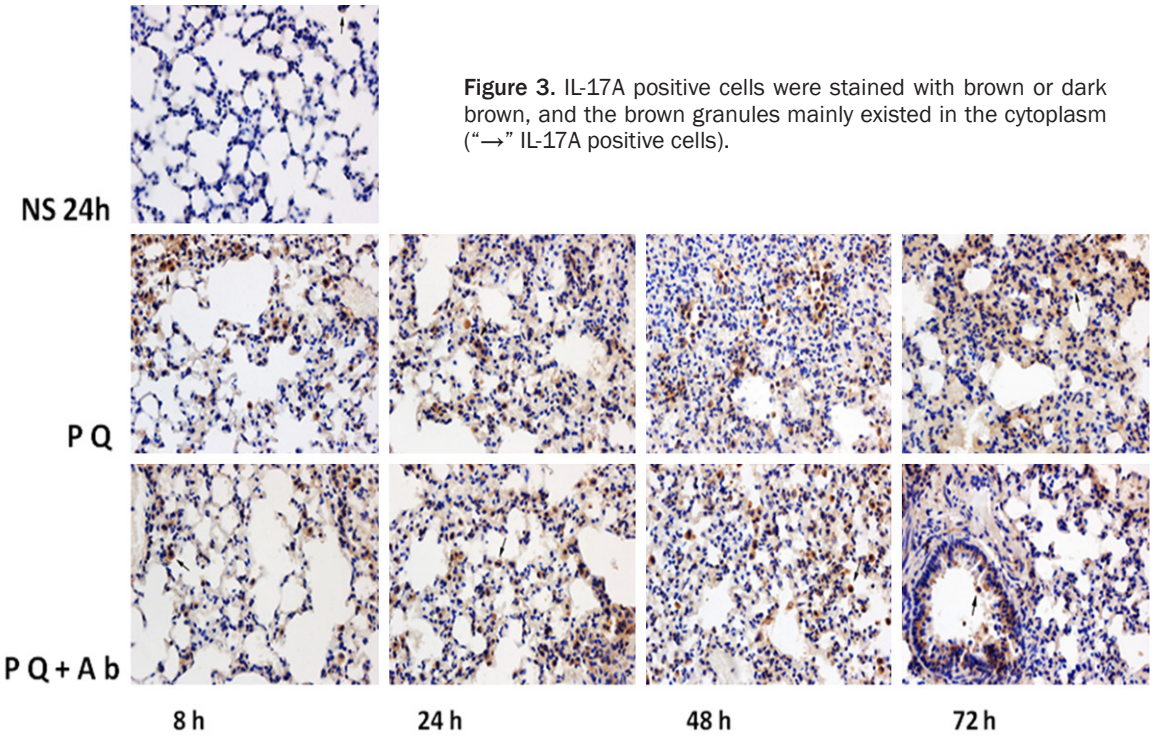

Figure 3. IL-17A positive cells were stained with brown or dark brown, and the brown granules mainly existed in the cytoplasm ("→" IL-17A positive cells).

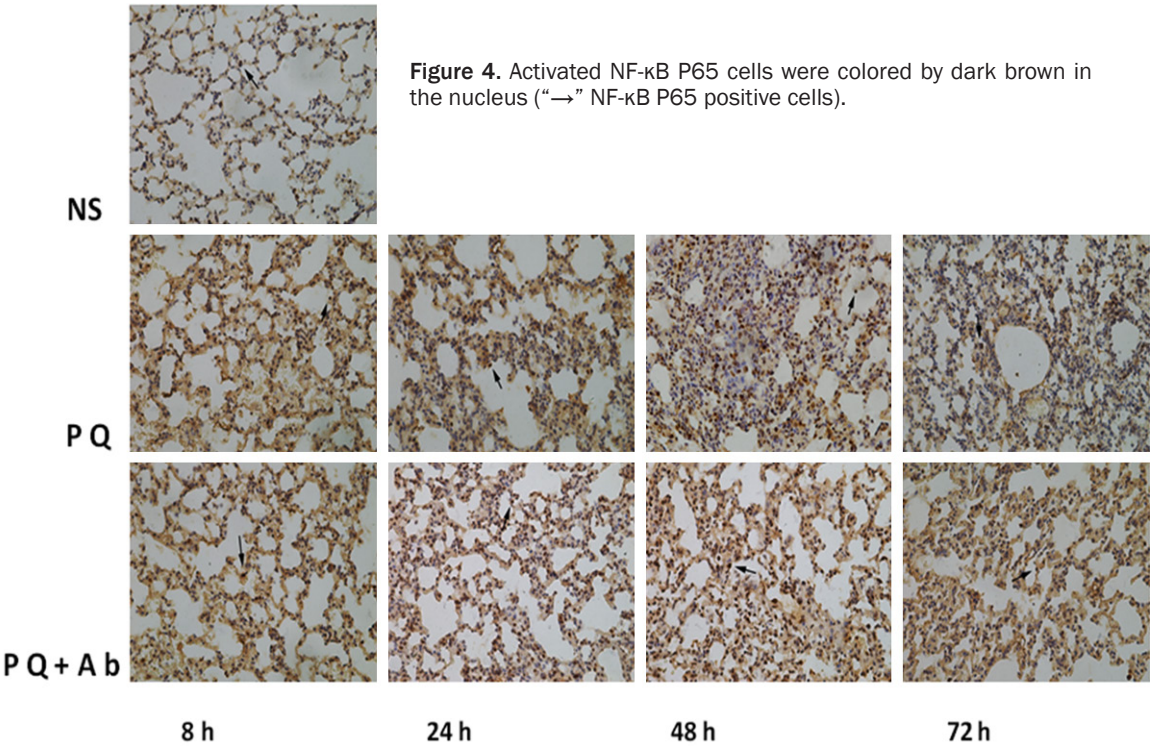

Figure 4. Activated NF-κB P65 cells were colored by dark brown in the nucleus ("→" NF-κB P65 positive cells).

IL-17A, IL-6, TNF-α in PQ+A b groups mice decreased (all $P<0.05$); In BALF, levels of IL-17A in PQ and PQ+A b groups were higher than those in NS groups (all $P<0.05$), while compared to PQ groups, IL-17A decreased significantly in PQ+A b groups (all $P<0.05$).

Table 4. IHC Scores of IL-17A and NF-κB p65

Group	n	IL-17A	NF-κB p65
NS	6	2.467±0.767	2.667±0.576
PQ			
8 h	6	4.333±1.270[a]	5.733±0.729[a]
24 h	6	6.033±1.033[a]	8.567±0.817[a]
48 h	6	7.733±0.832[a]	9.467±0.610[a]
72 h	6	6.633±1.926[a]	7.433±0.817[a]
PQ+A b			
8 h	6	3.567±0.865[a,b]	4.567±0.385[a,b]
24 h	6	4.567±1.033[a,b]	6.133±1.188[a,b]
48 h	6	4.867±1.559[a,b]	7.067±1.228[a,b]
72 h	6	4.400±0.970[a,b]	6.467±0.879[a,b]

Note: [a]$P<0.05$, vs. NS group; [b]$P<0.05$, vs. PQ group.

Expression of IL-17A and NF-κB p65 examined by IHC

IL-17A positive cells were stained with brown or dark brown and the brown granules mainly existed in the cytoplasm (in **Figure 3**). As for NF-κB p65, in the NS group, brown granules were mainly in cytoplasm, while in PQ and PQ+A b groups, the brown granules were mainly existed in cell nucleus (in **Figure 4**).

Compared with NS group, IL-17A IHC scores in PQ and PQ+A b groups were significantly increased (all $P<0.05$) after PQ perfusion and peaked at the 48 h, while in PQ+A b groups, IL-17A IHC scores were lower than those in PQ groups at 24 h, 48 h and 72 h (all $P<0.05$). The IHC scores of NF-κB p65 in PQ and PQ+A b groups were higher than those in NS groups (all $P<0.05$), compared to PQ groups, the scores in PQ+A b groups decreased at each time in the observation period (all $P<0.05$), (in **Table 4**).

q-PCR for the expression of IL-17A mRNA in lung tissue

Compared with NS groups, the expression of IL-17A mRNA in PQ and PQ+A b groups was significantly increased from 8 to 72 hours after PQ perfusion (all $P<0.01$), and highest at 48 h. While the IL-17A mRNA level in PQ+A b groups was lower than those of in PQ groups at 24 h, 48 h and 72 h (all $P<0.05$) (in **Table 5**).

Inflammatory cell counts in BALF

After PQ perfusion, leukocytes and the percentage of neutrophils in BALF increased significantly compared to NS groups (all $P<0.01$); While in PQ+A b groups, leukocytes and the percentage of neutrophils were lower than those of in PQ groups (all $P<0.05$) (in **Table 6**).

Discussion

As is well known, intentional or accidental ingestion of commercial liquid formulations of PQ is the leading cause of PQ-intoxication that induced millions of human death every year in the world, it causes multiple organs injury once was poisoned. Previous studies have shown that the major target organ in PQ poisoning is the lung [2], both of ALI and pulmonary fibrosis can lead to respiratory failure and eventually to death. The mechanisms of PQ-induced lung injury are complex. Even though free radicals has been deemed to play a crucial role in the lung injury [16], several inflammatory and chemotactic cytokines have been reported to play a very important role in the course, such inflammatory cytokines as IL-6, TGF-β1, TNF-α are found to be involved in the pathogenesis of lung injury [17].

IL-17A is a potent pro-inflammatory cytokine that has been verified to play a crucial role in the development of ALI induced by various reasons (LPS, Bleomycin, silicon and multiple-trauma) [7, 11, 13, 18] and was suggested to be a potential application for IL-17A-based therapy in clinical practice [12]. Herein, we used IL-17A neutralizing antibody to blockade IL-17A in mouse after PQ perfusion to prove if IL-17A plays the same role in the PQ-induced ALI and the possible way.

In our study, toxic symptoms presented on mouse after PQ perfusion, successively as restless, somnolence, tachypnea, and oral secretions increased. But the symptoms appeared to be milder after IL-17A was blockaded with antibody, although the mortality showed no decrease between PQ and PQ+A b groups mice by the Kaplan-Meier analysis, but the death in the PQ+A b group tend to be reduced.

Pulmonary edema is a change of lung injury after PQ perfusion, and we measured the wet-to-dry ratios of lung tissues to quantify the pulmonary edema extent. Our experiments showed that treatment with IL-17A antibody inhibited pulmonary edema, as the wet-to-dry ratios in the PQ+A b groups were significantly

Table 5. Expression of IL-17A mRNA examined by q-PCR (IL-17A mRNA/GAPDH mRNA) ($\bar{x}\pm s$)

Group	n	8 h	24 h	48 h	72 h
NS	6		1.00		
PQ	6	1.46±0.40[a]	2.87±0.39[a]	3.50±0.38[a]	3.02±0.52[a]
PQ+A b	6	1.46±0.38[a]	1.97±0.30[a,b]	2.51±0.50[a,b]	2.15±0.37[a,b]

Note: [a]$P<0.01$, vs. NS group; [b]$P<0.05$, vs. PQ group.

Table 6. Changes of Inflammatory Cells in BALF ($\bar{x}\pm s$)

Group	n	Leukocytes count ($\times10^6$/ml)	Neutrophil percentage (%)
NS	6	0.183±0.019	21.500±1.521
PQ			
8 h	6	0.252±0.016[a]	35.555±7.096[a]
24 h	6	0.312±0.028[a]	44.655±9.827[a]
48 h	6	0.517±0.017[a]	52.917±11.109[a]
72 h	6	0.442±0.018[a]	45.883±8.912[a]
PQ+A b			
8 h	6	0.207±0.016[a,b]	27.633±4.812[a,b]
24 h	6	0.267±0.016[a,b]	35.672±7.891[a,b]
48 h	6	0.367±0.030[a,b]	40.367±9.402[a,b]
72 h	6	0.345±0.015[a,b]	34.367±6.173[a,b]

Note: [a]$P<0.01$, vs. NS group; [b]$P<0.05$, vs. PQ group.

lower than those in PQ groups. Pathological changes in the lung reflected the injury; we observed that lungs from poisoning mice looked swelling and congestive. Under the light microscope, ALI pathological alterations in lung tissues present as alveolar edema, hemorrhage, inflammatory cell infiltration, and perfusion alveolar collapse accompanied with wall thickening. Administrated PQ poisoning mice with IL-17A antibody significantly alleviated the pathological changes. As an evaluation criteria, total protein content in BALF was also used to assess the injury of PQ poisoning mice [19], in our study, total proteins in BALF increased after PQ perfusion and decreased after IL-17A was blockaded with antibody in mice.

Studies showed that IL-17A plays an important role in triggering an inflammatory response [20], such as IL-17A recruits and/or activates neutrophils to damage lung tissues in LPS-induced pulmonary inflammation and bleomycin-induced lung injury [11, 12, 21, 22]. In our experiments, the contents of IL-17A in serum and BALF significantly increased immediately after mice were perfused with PQ, and the levels of IL-17A in PQ+A b groups mice decreased after neutralized with antibody. We also found that, the expression of IL-17A determined by IHC and q-PCR elevated in the PQ-poisoning mice lung tissues. The numbers of cell which secret IL-17A increased and the expression of IL-17A mRNA elevated simultaneously after PQ perfusion, suggesting that IL-17A implicated in the PQ-induced ALI. However, the IL-17A positive cell numbers and IL-17A mRNA levels decreased at 24 h after administrated with antibody, not fully in line with the levels in serum and BALF, we speculate that there may be some feedback pathways between IL-17A and the IL-17A-derived cells, but we failed to investigate the pathway in the work.

Previous studies suggest that PQ induces alveolar macrophage and neutrophil infiltrate to lung, recruit and release cytokines to damage lung tissues [4], cytokines as IL-6, TGF-β1, TNF-α play a definite but complex role in lung injury including PQ-induced [5, 23, 24]. These cytokines can initiate, amplify, and perpetuate the inflammatory response during the ALI process [17]. In our study, levels of IL-6 and TNF-α in serum increased after PQ was perfused, meanwhile, leucocytes and the percentage of neutrophil in BALF increased, which was consistent with previous studies. While blockade with IL-17A antibody, levels of above-mentioned cytokines in serum and the leucocytes in BALF were lower. Moreover, the percentage of neutrophil in BALF significantly decreased after IL-17A was neutralized, therefore, we speculated that IL-17A also recruits neutrophil to exacerbate the PQ-induced injury as that in LPS/bleomycin-induced lung injury [25].

As a nuclear transcription factor, NF-κB plays a crucial role in regulating gene transcription in inflammatory reaction [26]. p65 protein is a main subunit of NF-κB which activates cytokines to enlarge the cascade effect in the response by promoting related gene transcription [27]. In resting state, p65 protein combines with the inhibiting protein IκB formed a complexity which existing in the cytoplasm in an inactive form, when stimulated by infection or cytokines, IκB be predominate phosphorylation and separated from IκB p65 predominate, the free p65 quickly transferred to the nucleus and

bonded the specificity of gene sequences into the NF-κB, which prompts the cytokines release [26, 28]. IL-17A has been reported to activate NF-κB p65 in mice lung which results the largely secretion of TNF-α to damage the lung [29, 30]. In our experiments, thimbleful activated NF-κB in nucleus in NS group mice lung tissue while a mass of activated NF-κB presented in lung tissues after the mice were perfused with PQ, but activated NF-κB in PQ+A b groups was less than that in the PQ groups. The result indicated NF-κB p65 involved in the ALI induced by PQ, and blockade IL-17A hindered the activation of NF-κB p65, and was likely to reduce TNF-α.

In conclusion, this study investigated for the first time that IL-17A implicated in the ALI induced by PQ in a mouse model, blockade IL-17A with the neutralizing antibody attenuates the injury and was likely to reduce the activation of NF-κB p65 and recruitment of neutrophil. Our experiments provide a new idea for the treatment of ALI induced by PQ poisoning.

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Disclosure of conflict of interest

None.

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References

[1] Mainwaring G, Lim LF, Antrobus K, Swain C, Clapp M, Kimber I, Orphanides G, Moggs JG. Identification of early molecular pathways affected by paraquat in rat lung. Toxicology 2006; 225: 157-72.

[2] Rose MS, Smith LL, Wyatt I. Evidence for energy-dependent accumulation of paraquat into rat lung. Nature 1974; 252: 314-5.

[3] Frank L. Prolonged survival after paraquat. Role of the lung antioxidant enzyme systems. Biochem Pharmacol 1981; 30: 2319-24.

[4] Wegener T, Sandhagen B, Chan KW, Saldeen T. N-acetylcysteine in paraquat toxicity: toxicological and histological evaluation in rats. Ups J Med Sci 1988; 93: 81-9.

[5] Chen D, Jiao GY, Ma T, Liu XW, Yang C, Liu Z. The mechanism of rapamycin in the intervention of paraquat-induced acute lung injury in rats. Xenobiotica 2015; 45: 538-46.

[6] Orito K, Suzuki Y, Matsuda H, Shirai M, Akahori F. Chymase is activated in the pulmonary inflammation and fibrosis induced by paraquat in hamsters. Tohoku J Exp Med 2004; 203: 287-94.

[7] Lo Re S, Dumoutier L, Couillin I, Van Vyve C, Yakoub Y, Uwambayinema F, Marien B, van den Brûle S, Van Snick J, Uyttenhove C, Ryffel B, Renauld JC, Lison D, Huaux F. IL-17A-producing gammadelta T and Th17 lymphocytes mediate lung inflammation but not fibrosis in experimental silicosis. J Immunol 2010; 184: 6367-77.

[8] McAllister F, Henry A, Kreindler JL, Dubin PJ, Ulrich L, Steele C, Finder JD, Pilewski JM, Carreno BM, Goldman SJ, Pirhonen J, Kolls JK. Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-alpha and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis. J Immunol 2005; 175: 404-12.

[9] Nembrini C, Marsland BJ, Kopf M. IL-17-producing T cells in lung immunity and inflammation. J Allergy Clin Immunol 2009; 123: 986-94.

[10] Holloway TL Schwacha MG. The Th-17 response and its potential role in post-injury pulmonary complications. Int J Burns Trauma 2012; 2: 11-7.

[11] Braun RK, Ferrick C, Neubauer P, Sjoding M, Sterner-Kock A, Kock M, Putney L, Ferrick DA, Hyde DM, Love RB. IL-17 producing gammadelta T cells are required for a controlled inflammatory response after bleomycin-induced lung injury. Inflammation 2008; 31: 167-79.

[12] Li Q, Gu Y, Tu Q, Wang K, Gu X, Ren T. Blockade of interleukin-17 restrains the development of acute lung injury. Scand J Immunol 2016; 83: 203-11.

[13] Dai H, Xu L, Tang Y, Liu Z, Sun T. Treatment with a neutralising anti-rat interleukin-17 antibody after multiple-trauma reduces lung inflammation. Injury 2015; 46: 1465-70.

[14] Smith KM, Mrozek JD, Simonton SC, Bing DR, Meyers PA, Connett JE, Mammel MC. Prolonged partial liquid ventilation using conventional and high-frequency ventilatory techniques: gas exchange and lung pathology in an animal model of respiratory distress syndrome. Crit Care Med 1997; 25: 1888-97.

[15] Kaemmerer D, Peter L, Lupp A, Schulz S, Sänger J, Baum RP, Prasad V, Hommann M. Comparing of IRS and Her2 as immunohistochemical scoring schemes in gastroenteropancreatic neuroendocrine tumors. Int J Clin Exp Pathol 2012; 5: 187-94.
[16] Kim JH, Gil HW, Yang JO, Lee EY, Hong SY. Effect of glutathione administration on serum levels of reactive oxygen metabolites in patients with paraquat intoxication: a pilot study. Korean J Intern Med 2010; 25: 282-7.
[17] Hemmati AA, Nazari Z, Motlagh ME, Goldasteh S. The role of sodium cromolyn in treatment of paraquat-induced pulmonary fibrosis in rat. Pharmacol Res 2002; 46: 229-34.
[18] Kim SR, Kim HJ, Kim DI, Lee KB, Park HJ, Jeong JS, Cho SH, Lee YC. Blockade of interplay between IL-17A and endoplasmic reticulum stress attenuates LPS-induced lung injury. Theranostics 2015; 5: 1343-62.
[19] Jin W, Rong L, Liu Y, Song Y, Li Y, Pan J. Increased claudin-3, -4 and -18 levels in bronchoalveolar lavage fluid reflect severity of acute lung injury. Respirology 2013; 18: 643-51.
[20] Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 cells. Annu Rev Immunol 2009; 27: 485-517.
[21] Fujie H, Niu K, Ohba M, Tomioka Y, Kitazawa H, Nagashima K, Ohrui T, Numasaki M. A distinct regulatory role of Th17 cytokines IL-17A and IL-17F in chemokine secretion from lung microvascular endothelial cells. Inflammation 2012; 35: 1119-31.
[22] You QH, Zhang D, Niu CC, Zhu ZM, Wang N, Yue Y, Sun GY. Expression of IL-17A and IL-17F in lipopolysaccharide-induced acute lung injury and the counteraction of anisodamine or methylprednisolone. Cytokine 2014; 66: 78-86.
[23] Zhang Z, Ding L, Wu L, Xu L, Zheng L, Huang X. Salidroside alleviates paraquat-induced rat acute lung injury by repressing TGF-β1 expression. Int J Clin Exp Pathol 2014; 7: 8841-7.
[24] Dong XS, Hu XB, Liu W, Sun YQ, Liu Z. Effects of RNA interference-induced Smad3 gene silencing on pulmonary fibrosis caused by paraquat in mice. Exp Biol Med (Maywood) 2012; 237: 548-555.
[25] Miyamoto M, Prause O, Sjöstrand M, Laan M, Lötvall J, Lindén A. Endogenous IL-17 as a mediator of neutrophil recruitment caused by endotoxin exposure in mouse airways. J Immunol 2003; 170: 4665-72.
[26] Brennenstuhl H, Armento A, Braczysnki AK, Mittelbronn M, Naumann U. IκBζ, an atypical member of the inhibitor of nuclear factor kappa B family, is induced by γ-irradiation in glioma cells, regulating cytokine secretion and associated with poor prognosis. Int J Oncol 2015; 47: 1971-80.
[27] Baeuerle PA, Baltimore D. A 65-kappaD subunit of active NF-kappaB is required for inhibition of NF-kappaB by I kappaB. Genes Dev 1989; 3: 1689-98.
[28] Metelev VG, Kubareva EA, Oretskaya TS. Regulation of activity of transcription factor NF-κB by synthetic oligonucleotides. Biochemistry (Mosc) 2013; 78: 867-78.
[29] Song X, Qian Y. IL-17 family cytokines mediated signaling in the pathogenesis of inflammatory diseases. Cell Signal 2013; 25: 2335-47.
[30] Mi S, Li Z, Liu H, Hu ZW, Hua F. Blocking IL-17A protects against lung injury-induced pulmonary fibrosis through promoting the activation of p50NF-kappaB. Yao Xue Xue Bao 2012; 47: 739-44.