Th17 cells are involved in mouse chronic obstructive pulmonary disease complicated with invasive pulmonary aspergillosis

Wan-Ru Geng1,2, Hang-Yong He1, Qing Zhang3, Zhao-Hui Tong1

1Department of Respiratory and Critical Care Medicine, Beijing Institute of Respiratory Medicine, Beijing Chao-Yang Hospital, Capital Medical University, Beijing 100020, China; 2Department of Respiratory Medicine, Affiliated Hospital of Inner Mongolia University for the Nationalities, Tongliao, Inner Mongolia 028000, China; 3Department of Respiratory and Critical Care Medicine, The Second Hospital of Jilin University, Changchun, Jilin 130041, China.

Abstract
Background: The incidence of chronic obstructive pulmonary disease (COPD) complicated with invasive pulmonary aspergillosis (IPA) has increased in the last two decades. The mechanism underpinning susceptibility to and high mortality of COPD complicated with IPA is unclear, and the role of T helper cells 17 (Th17 cells) in the compound disease remains unknown. Therefore, this study aimed to assess the function of Th17 cells in COPD combined with IPA.

Methods: COPD, IPA, and COPD+IPA mouse models were established in male wild type C57/BL6 mice. The amounts of Th17 cells and retinoic acid-related orphan receptors γ (RORγ) were tested by flow cytometry. Then, serum interleukin (IL)-17 and IL-23 levels were detected by enzyme-linked immunosorbent assay (ELISA) in the control, COPD, IPA and COPD+IPA groups. In addition, COPD+IPA was induced in IL-17 knockout (KO) mice, for determining the role of Th17 cells in COPD+IPA.

Results: Compared with the COPD group, the COPD+IPA group showed higher amounts of blood RORγ (35.09 ± 16.12)% vs. (17.92 ± 4.91)% [P = 0.02] and serum IL-17 (17.96 ± 9.59 pg/mL vs. 8.05 ± 4.44 pg/mL, P = 0.02), but blood ([5.18 ± 1.09]%) vs. [4.15 ± 0.87]%, P = 0.28) and lung levels of Th17 cells ([11.98 ± 0.83]% vs. [2.03 ± 0.98]%, P = 0.91), lung levels of RORγ ([9.58 ± 6.93]% vs. [9.63 ± 5.98]%, P = 0.49) and serum IL-23 (51.55 ± 27.82 pg/mL vs. 68.70 ± 15.20 pg/mL, P = 0.15) showed no significant differences. Compared with the IPA group, the COPD+IPA group displayed lower amounts of blood ([5.18 ± 1.09]% vs. [9.21 ± 3.56]%, P = 0.01) and lung Th17 cells ([11.98 ± 0.83]% vs. [6.29 ± 1.11]%, P = 0.01) and serum IL-23 (51.55 ± 27.82 pg/mL vs. 154.90 ± 64.60 pg/mL, P = 0.01) and IL-17 (17.96 ± 9.59 pg/mL vs. 39.81 ± 22.37 pg/mL, P = 0.02), while comparable blood ([35.09 ± 16.12]% vs. [29.86 ± 15.42]%, P = 0.25) and lung levels of RORγ ([9.58 ± 6.93]% vs. [15.10 ± 2.95]%, P = 0.18) were found in these two groups. Finally, Aspergillus load in IL-17 KO COPD+IPA mice was almost 2 times that of COPD+IPA mice (1851.687.69 ± 944.480.43 vs. 892,958.10 ± 686,808.80, τ = 2.32, P = 0.02).

Conclusion: These findings indicate that Th17 cells might be involved in the pathogenesis of COPD combined with IPA, with IL-17 likely playing an antifungal role.

Keywords: T helper cells 17; Chronic obstructive pulmonary disease (COPD); Invasive pulmonary aspergillosis (IPA)

Introduction
Aspergillus spp. can cause an opportunistic infection in the airway and pulmonary parenchyma, known as invasive pulmonary aspergillosis (IPA) in immunocompromised hosts.[1] Chronic obstructive pulmonary disease (COPD) patients, who were once considered non-classical immunosuppressive hosts, are less prone to IPA. However, reports of COPD patients complicated with IPA have increased during the last two decades.[2,3] According to previous reports, the incidence of IPA in patients with COPD is 16.3%.[4] Meanwhile, an incidence of IPA in critically ill COPD patients of up to 13% was found in our intensive care unit.[5] Severe COPD patients have been recognized as a susceptible population for IPA.[6,7] The mortality rate is as high as 70% to 100% for COPD patients with IPA.[6,3] However, the mechanism underlying susceptibility to and high mortality of COPD complicated with Aspergillus infection is unknown.

The immune response is the main host defense mechanism against Aspergillus infection. Among the pool of T cells that participate in immune response against Aspergillus, Thelper cells 17 (Th17 cells) are the most important subset.[8] In the past two decades, studies have found that Th17 cells, with the dominant secretion of the cytokine interleukin 17 (IL-17), play an important role in the host
response to fungal infections.\[^{9}\]\ Retinoic acid-related orphan receptor \(\gamma t\) (ROR\(\gamma t\)), a specific nuclear transcription factor in Th17 cells, and IL-23 play an important role in stabilizing and promoting the differentiation of Th17 cells.\[^{10}\]\ Th17 cells are involved in inflammatory responses by secreting a variety of cytokines, including their most studied cytokine IL-17A.\[^{11}\]\ Moreover, Th17 cells also act as an important part of inflammation during the pathogenesis of COPD.\[^{12,13}\]\ Meanwhile, Th17 cell amounts are inversely correlated with forced expiratory volume in 1 s (FEV\(_1\)) percentage in COPD.\[^{14,15}\]\ In addition, it was demonstrated that dysregulation in Th17 cell compartment, creating an imbalance of Th17/regulatory T cells, contributes to COPD-associated pulmonary hypertension.\[^{16}\]\

The above findings demonstrate that Th17 cells have critical functions in Aspergillus infection and COPD. However, the role of Th17 cells in COPD combined IPA remains unknown. We hypothesized that COPD has lower Th17 cell amounts when combined with Aspergillus infection, with Th17 cells playing a protective role in response to Aspergillus infection in COPD. Therefore, the present study aimed to assess the function of Th17 cells in COPD combined with IPA. To this end, we built an IPA animal model in COPD mice, and assessed the amounts of Th17 cells and their transcription factor ROR\(\gamma t\), IL-17 and IL-23 as compared with the control, COPD and IPA groups. The specific role of IL-17 in COPD combined with IPA was evaluated in IL-17 knockout (KO) mice.

**Methods**

**Ethical approval**

Animal studies were reviewed and approved by the ethics committee of Beijing Chao-Yang Hospital, Capital Medical University (No. 2015-9-18). All animal procedures were carried out in strict accordance with the recommendations of the Chinese Laboratory Animal Requirements of Environment and Housing Facilities.

**Mice**

Male wild-type C57/BL6 mice, 6 to 8 weeks old, were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). IL-17a\(-/-\) (IL-17 KO) mice in a C57BL/6 background were provided by Dr. Iwakura (University of Tokyo, Tokyo, Japan) as previously described.\[^{17}\]\ The animals were housed in individual ventilated cages (IVC) under standard laboratory conditions with a 12/12h light/dark cycle. The full system was kept in an environmentally controlled room at (22 ± 1)°C and (55 ± 5)% humidity. Clean food and water were provided ad libitum. All mice were maintained in the Beijing Institute of Respiratory Medicine. The following five groups were set up: control, IPA, COPD, COPD+IPA, and IL-17 KO COPD+IPA groups.

**COPD models**

Cigarette smoke induced COPD: The mice of the COPD, COPD+IPA, and IL-17 KO COPD+IPA groups were exposed to cigarette smoke intermittently for 10 min at 120 min/day for consecutive 5 days with 2 days of rest for 16 weeks; meanwhile, the IPA and control groups were exposed to filtered air correspondingly. The experiments were performed with a nose-only inhalation generator (SIBATA technology company, Toyota, Japan), which was composed of a smoke generating device SG-300 (SIBATA) inhaled chamber and the connecting transfer pipe. Baisha cigarettes (Tar at 20 mg, 1.0 mg nicotine and 14 mg carbon monoxide) were put in the smoke generating device, and the parameters were set to reach a smoke concentration of about 2.5%.

Confirmation of COPD model establishment: Weight changes in the four groups were based on individual animals assessed separately at different times. Lung function (Flexi Vent rodent animal breath tester, SCIREQ, Montreal, PQ, Canada) was analyzed for respiratory mechanics parameters, including pulmonary compliance, airway resistance and elastic resistance.\[^{18,19}\]\ Then, lung samples were fixed with 10% neutral buffered formalin (Sigma, St. Louis, MO, USA), parafin embedded, sliced at 5-µm and stained with hematoxylin and eosin (H&E) for histological analysis. In combination with histological evidence of emphysema in the lung, increased airway resistance reflected successful COPD model establishment.

**Establishment of COPD combined with IPA**

Aspergillus fumigatus isolate 204305 (ATCC, Manassas, VA, USA) was maintained on potato dextrose agar for 5 to 7 days at 37°C. Conidia were harvested by washing the culture flask with 50 mL of sterile phosphate-buffered saline (PBS) supplemented with 0.1% Tween 20. The conidia were then passed through a sterile 40 µm nylon membrane to remove hyphal fragments and enumerated on a hemocytometer. The final density of Aspergillus fumigatus spore suspension was 1 × 10^9/mL.

Methylprednisolone (Pfizer Pharmaceuticals Limited, New York, NY, USA) was suspended in sterile water containing 0.1% Tween 80. Doses were 100 mg/kg injected subcutaneously in 0.5 mL or less saline. This dose was given on three alternate days per week (beginning on Monday with infection on Friday) for the three-dose regimen.

COPD mice were slightly anesthetized with 1.5% pentobarbital (5 mL/kg). An incision of 0.5 to 1 cm was made along the midline of the mouse neck, and muscle tissues were separated bluntly to expose the trachea. Then, 30 µL of the previously obtained Aspergillus fumigatus spore suspension was intratracheally administered, followed by 10 µL filtered air to ensure all the suspension was pushed into the trachea. With histological evidence of invasive growth of Aspergillus in the lung, a successful COPD+IPA model establishment was confirmed.\[^{120}\]

**Flow cytometry for cell detection**

Cells were washed and resuspended at a density of 10^7 cells/mL in fluorescent antibody (FA) buffer (Difco) and 0.1% NaN3. Fc receptors were blocked by addition of unlabeled anti-CD16/32 antibody (Fc block; BD Pharmingen, San Diego, CA, USA). After Fc receptor blocking, 0.5 × 10^6 to 1 × 10^6 cells were stained in a final volume of...
Flow cytometry for extracellular and intracellular molecules

Prior to intracellular cytokine staining, cells were stained in vitro for 5 h with phorbol 12-myristate 13-acetate (PMA) (50 ng/mL) and ionomycin (1 μg/mL) in the presence of brefeldin A (BD Pharmingen) to promote intracellular accumulation of cytokines. After stimulation, cells were washed twice with PBS and resuspended in 100 μL saline, and an equal volume of 4% formalin was added for fixation. A minimum of 20,000 events were acquired on a FACS Calibur flow cytometer (BD Biosciences, Piscataway, NJ, USA) using the Cell-Quest software (BD Biosciences, Franklin Lakes, NJ, USA). The acquired data were analyzed with the FlowJo software (Tree Star, Ashland, OR, USA).

ELISA

Serum IL-17 and IL-23 levels were tested. First, whole blood was centrifuged at 3000 r/min for 10 min for serum preparation. Then, ELISA kits (BD Biosciences) were used to measure serum IL-17 and IL-23 levels according to the manufacturer’s protocols.

Quantitative real-time polymerase chain reaction (PCR)

Fresh lung samples were removed and snap frozen in liquid nitrogen. Total RNA was extracted with RNAiso Plus (TaKaRa, Dalian, China) and reverse transcribed into complementary DNA (cDNA) with the PrimeScript RT reagent kit (TaKaRa). Quantitative real-time PCR was performed with SYBR Premix Ex Taq II reagents from TaKaRa, according to the manufacturer’s instructions. PCR reactions were performed in 20 μL mixtures containing 2 μL of cDNA. The 26S RNA of Aspergillus fumigatus ATCC204305 was amplified using specific primers (TaKaRa) (26S rRNA-F: CCGTCTAACGTCCTGGAAC; 26S rRNA-R: CTTGTGCGCTATCGGTCTCC). The standard curve was drawn according to the Ct value and copy number of the standard. The Ct value in the test sample was obtained, and the concentration of Aspergillus fumigatus 26S RNA in the sample was derived according to the standard curve.

Statistical analysis

All experiments were repeated three times, and the results were pooled. Continuous data with normal distribution were presented as mean ± standard deviation (SD) and analyzed using Student’s t test. Non-normally distributed data were expressed as median (IQR). The Mann-Whitney U test was used for comparing non-normally distributed data. Multiple group comparison of normally and non-normally distributed data was performed by analysis of variance (ANOVA) and the Kruskal-Wallis H test, respectively. Student-Newman-Keuls (SNK) test was performed for comparison between two groups. All tests were carried out with the SPSS statistics 22.0 System Software (SPSS Inc., Chicago, IL, USA). Statistical significance was set at P < 0.05.

Results

Confirmation of the establishment of the animal model with COPD combined IPA

First, COPD model establishment was confirmed by individual weight recording, lung function parameters and lung histology. In the process of modeling, weights in the COPD and COPD+IPA groups were declined compared with those of the control and IPA groups [Figure 1A]. Weight change was the lowest in the IPA+COPD group, followed by the COPD and IPA groups, with the control group showing the highest values [Figure 1B].

Lung function tests showed that pulmonary compliance (0.027 ± 0.008 mL/cmH2O vs. 0.016 ± 0.005 mL/cmH2O, t = 4.26, P = 0.01) and airway resistance (Raw) (1.90 ± 0.22 cmH2O·s/mL vs. 1.54 ± 0.15 cmH2O·s/mL, t = 3.12, P = 0.02) in the COPD groups were significantly higher than those of the control group, while elastic resistance (Er) was significantly lower (34.73 ± 10.75 cmH2O·cm/L vs. 61.89 ± 16.54 cmH2O·cm/L, t = −4.30, P = 0.03) [Figure 2A–C].

Histologically, compared with the control group, COPD mice showed incomplete mucosal epithelial structure, with occasionally few alveolar fusion fractures observed. Furthermore, bronchial cilia were shorter and partially exfoliated; the alveolar wall became thinner or ruptured into larger alveoli. The alveolar structure of the IPA group was similar to that of the control group, but there were necrosis and Aspergillus hyphal growing in the IPA [Figure 3A and 3B]. Meanwhile, increased small airway destruction, and emphysema formation were found in the process of modeling, weights in the IPA+COPD group 

Amounts of Th17 cells and RORγt in blood and lung

Compared with the COPD group, the COPD+IPA group showed higher amounts of blood RORγt ([35.09 ± 16.12]% vs. [17.92 ± 4.91]%, P = 0.02), but blood ([5.18 ± 1.09]% vs. [4.15 ± 0.87]%, P = 0.28) and lung levels of Th17 cells ([1.98 ± 0.83]% vs. [2.03 ± 0.98]%, P = 0.91) and lung level of RORγt ([9.58 ± 6.93]% vs. [9.63 ± 5.98]%, P = 0.49) showed no significant differences [Figure 4, Table 1]. Compared with the IPA group, the COPD+IPA group displayed significantly lower amounts of blood Th17 cells ([5.18 ± 1.09]% vs. [9.21 ± 3.56]%, P = 0.01) and lung Th17 cells ([1.98 ± 0.83]% vs. [6.29 ± 1.11]%, P = 0.01), while
Serum IL-17 and IL-23 levels

Compared with the COPD group, the COPD+IPA group showed significantly higher amounts of serum IL-23 (51.55 ± 27.82 pg/mL vs. 15.90 ± 64.60 pg/mL, P = 0.01) and IL-17 (17.96 ± 9.59 pg/mL vs. 39.81 ± 22.37 pg/mL, P = 0.02). In comparison with the control group, the COPD+IPA group had a significantly higher IL-17 (17.96 ± 9.59 pg/mL vs. 5.24 ± 0.59 pg/mL, P = 0.01) and a similar IL-23 (51.55 ± 27.82 pg/mL vs. 65.19 ± 8.93 pg/mL, P = 0.22) when compared with the control group. Finally, serum IL-17 (39.81 ± 22.37 pg/mL vs. 5.24 ± 0.59 pg/mL, P < 0.01) and IL-23 levels (154.90 ± 64.60 pg/mL vs. 65.19 ± 8.93 pg/mL, P = 0.01) were significantly higher in the IPA group than in the control group [Figure 5, Table 2]. Compared with COPD+IPA group, IL-17 KO COPD+IPA mice had no IL-17 detected in serum.

COPD model in IL-17 KO mice and Aspergillus load comparison between IL-17 KO COPD+IPA and COPD+IPA mice

In the process of establishing a COPD model in IL-17 KO mice, animal weights in the control and IL-17 KO COPD groups increased with time, but the IL-17 KO group showed significantly lower values compared with control mice from the second week [Figure 6A]. This resulted in an
overall decrease of body weight change in the IL-17 KO group compared with controls [Figure 6B].

Next, Aspergillus load was tested by culture on potato dextrose agar. While the control group showed no growth, the IL-17 KO COPD+IPA group showed Aspergillus colonies covering the whole plate [Figure 6C]. This was confirmed by RT-PCR. Indeed, Aspergillus 26S RNA load in the IL-17 KO COPD+IPA group was about two times that of the COPD+IPA mice (1,851,687.69 ± 944,480.43 vs. 892,958.10 ± 686,808.80, t = 2.32, P = 0.02) [Figure 6D].

Discussion

The main strengths of this study were the establishment of a COPD+IPA model in mice for the first time, and investigating the role of Th17 cell based on this model. This enabled us to discriminate the effects of Th17 cells in COPD and/or IPA. In addition, we found that IL-17 played a protective role in IL-17 KO COPD+IPA mice by reducing fungal load. Upon infection of COPD mice with IPA, Th17 cell amounts were reduced in the blood or lung compared with control mice infected with IPA; this lower amount of Th17 cells may not be directly related to inflammation in COPD itself. Some other pathways maybe responsible for the inappropriately reduced levels of Th17 cells in COPD mice infected with IPA, which deserves further investigation.

In this study, compared with the COPD group, the COPD+IPA group showed higher amounts of blood RORγt as well as serum levels of IL-17, while blood level of Th17 cells was comparable in both groups. RORγt is the key...
transcription factor which directs the differentiation program of proinflammatory Th17 cells.\[14\] Acute exacerbations of COPD are associated with decreased CD4+ T cells, which may be related to the immunocompromised status of COPD.\[15\] Another study also reported that stimulated lung CD4+ T cells from COPD subjects displayed significantly impaired interferon-\(\gamma\) (IFN-\(\gamma\)) production.\[16\] In patients with COPD, chronic pulmonary inflammation is accompanied by the induction of defective immune responses that contribute to intermittent respiratory infections, worsening the inflammatory lung microenvironment and disease severity.\[17-19\] Also, reduced immune cell infiltration into the lung, along with decreased levels of the inflammatory cytokine IL-17, was also noted in a previous report.\[20\] Therefore, when the amount of CD4+ T cells decreased or its function was inhibited in COPD, although ROR\(\gamma\)t level increased, the blood level of Th17 cells did not increase accordingly.

Furthermore, compared with the IPA group, the COPD+IPA group showed lower levels of blood and lung Th17 cells and serum IL-23 and IL-17 amounts, although blood ROR\(\gamma\)t was similarly elevated. Several explanations for mechanism of ineffective immune reaction in COPD are possible for these findings. Firstly, immune imbalance in COPD may affect secondary immune responses against infection.\[21\] A previous study found that chronic exposure to smoke and high doses of influenza virus could suppress immune response and decrease survival.\[22\] Another study by Bhat \textit{et al}\[12,23\] suggested that smoke exposure reduces the adaptive immune response to \textit{Haemophilus influenzae}. Second, “T cell depletion” was recently proposed as the exhaustion of T cells gradually after long-term exposure to persistent antigens and inflammation.\[24-26\] Bhat \textit{et al}\[12,23\] also observed that the mouse model of COPD combined with \textit{Haemophilus influenzae} infection has increased number of inflammatory cells in the lung tissue, but decreased amounts of lymphocytes, especially T lymphocytes, as well as reduced IFN-\(\gamma\) and IL-4 in the lung.\[23\] Moreover, the effect of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) blockade on T cell responses in COPD may represent a
source for immunosuppression of antibacterial immunity.\(^{[12,23]}\) On the other hand, blocking of immune checkpoint receptors CTLA-4 and PD-1 resulted in increased T cell proliferation and IFN-\(\gamma\) production, which in turn strengthens the over expression of these molecules on COPD T cells.\(^{[27]}\) Therefore, our findings suggested that the Th17 cell-associated immune pathway may be involved in cytokine production/secretion in the pathogenesis of COPD combined with IPA. Meanwhile, Th17 cells did not effectively react to \textit{Aspergillus} infection in the lung in the context of COPD.

In this study, compared with the control group, the COPD group only showed a trend of higher amounts of blood Th17 cells, serum levels of IL-17 and IL-23. This result is similar to that reported in several previous studies. Paats \textit{et al}.\(^{[28]}\) found that CD4+Th17 cells/IL-17 level in blood were similar in COPD patients and non-smokers in control group. IL-17 level in sputum sample and Th17 cells in lung tissue were reported similar in COPD patients and non-smokers.\(^{[22,25,100]}\) However, elevated levels of Th17 cells, IL-17 and IL-23 in blood and lung of COPD patients were reported in other studies.\(^{[81,25]}\) This elevation may correlate with IL-18 induced endothelial activation and enhanced neutrophil recruitment to the sites of inflammation.\(^{[13]}\) Furthermore, these differences may be caused by the different methods used in evaluation of Th17 and IL-17 levels.\(^{[22]}\) A study suggested that Th17 and IL-17 levels were prone to be similar between COPD and non-COPD groups when evaluated with immunohistochemical analysis.\(^{[34]}\)

Finally, to assess whether the Th17 cell pathway is totally ineffective in COPD mice infected by \textit{Aspergillus}, and whether IL-17 has a protective role against \textit{Aspergillus} infection, we established a COPD+IPA animal model in IL-17 KO mice. As shown above, \textit{Aspergillus} load in IL-17 KO COPD+IPA mice was almost two times that of COPD+IPA mice. This finding indicates that in IL-17 KO mice, recruitment, migration and activation of granulocytes may be reduced, leading to increased \textit{Aspergillus} load and more severe infection. This study demonstrated that IL-17 has an important protective immune function in the context of COPD complicated with IPA, although Th17 cells were partially depleted in the lung of COPD mice. This is consistent with findings reported by previous studies showing that IL-17-deficient mice have increased fungal load after invasive fungal infection, with the mechanism involving altered neutrophil recruitment and cytokine expression.\(^{[35,36]}\)

Our findings suggested that in COPD murine model, Th17/IL-17 induces a protective immune response in COPD +IPA. However, the chronic pulmonary inflammation of
COPD is accompanied by the induction of defective immune responses with the chronic depletion of T cells, which worsens the inflammatory lung microenvironment and leads to a damaged antifungal immunity, and a high mortality of IPA in COPD.

In summary, this study provided evidence that the Th17 cell-associated immune pathway may be involved but inhibited in the immunopathological process of COPD combined with IPA. We also demonstrated the infection-protection role of IL-17 in IL-17 KO COPD combined with IPA animal model. However, further studies, including clinical trials, are required to validate these findings.

Acknowledgements
We thank Dr. Dong-Xu Cao for technical assistance and Dr. Yu Wang for editorial assistance.

Funding
This study was supported by a grant from the National Natural Science Foundation of China (No. 81400003).

Conflicts of interest
None.

References
1. Kanj A, Abdallah N, Soubani AO. The spectrum of pulmonary aspergillosis. Respir Med 2018;141:1-13. doi: 10.1016/j. rmed.2018.06.029.
2. Bulpa P, Dive A, Sible Y. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease. Eur Respir J 2007;30:782-800. doi: 10.1183/09031936.00062206.
3. Bulpa P, Dive AM, Garrino MG, Delos MA, Gonzalez MR, Evrard P, et al. Chronic obstructive pulmonary disease patients with invasive pulmonary aspergillosis: benefits of intensive care. Intensive Care Med 2001;27:59-67. doi: 10.1007/s001340000076.
4. Guine J, Torres-Narbona M, Gijón P, Muñoz P, Pozo F, Peláez T, et al. Pulmonary aspergillosis in patients with chronic obstructive pulmonary disease: incidence, risk factors, and outcome. Clin Microbiol Infect 2010;16:870-877. doi: 10.1111/j.1469-0691.2009.03015.x.
5. He H, Ding L, Li F, Zhan Q. Clinical features of invasive bronchial pulmonary aspergillosis in critically ill patients with chronic obstructive respiratory diseases: a prospective study. Crit Care 2011;15:R5. doi: 10.1186/cc9402.
6. Blot SI, Taccone FS, Van den Abeele AM, Bulpa P, Meersseman W, Brusselsers N, et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. Am J Resp Crit Care Med 2012;186:56-64. doi: 10.1164/rcrm.201111-1978OC.
7. Taccone FS, Van den Abeele AM, Bulpa P, Misser B, Meersseman W, Cardoso TC, et al. Epidemiology of invasive aspergillosis in critically ill patients: clinical presentation, underlying conditions, and outcomes. Crit Care 2015;19:7. doi: 10.1186/s13054-014-0722-7.
8. Camargo IE, Husain S. Immune correlates of protection in human invasive aspergillosis. Clin Infect Dis 2014;59:569-577. doi: 10.1093/cid/ciu337.
9. Zelante T, Bozza S, De Luca A, D’Angelo C, Bonifazi P, Moretti S, et al. Th17 cells in the setting of Aspergillus infection and pathology. Med Mycol 2009;47 (Suppl 1):S162-S169. doi: 10.1080/ 13693780802140766.
10. Zelante T, De Luca A, Bonifazi P, Montagnoli C, Bozza S, Moretti S, et al. IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. Eur J Immunol 2007;37:2695-2706. doi: 10.1002/eji.200737409.
11. Bozza S, Zelante T, Moretti S, Bonifazi P, De Luca A, D’Angelo C, et al. Lack of Toll IL-1R8 exacerbates Th17 cell responses in fungal infection. J Immunol 2008;180:4022-4031. doi: 10.4049/jimmunol.180.6.4022.
12. Bhat TA, Panzaia L, Kalathil SG, Thanavala Y. Immune dysfunction in patients with chronic obstructive pulmonary disease. Ann Am Thorac Soc 2015;12 (Suppl 2):S169-S175. doi: 10.1513/Annal sATS.201503-126AW.
13. Zhu X, Gadgil AS, Givelber R, George MP, Stoner MW, Scurba FC, et al. Peripheral T cells function correlaters with the severity of chronic obstructive pulmonary disease. J Immunol 2009;182:3270-3277. doi: 10.4049/jimmunol.0802622.
14. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lafaille JJ, et al. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 2006;126:1121-1133. doi: 10.1016/j.cell.2006.07.035.
15. Freeman CM, Martinez CH, Tod JC, Martinez FJ, Han MK, Thompson DL, et al. Acute exacerbations of chronic obstructive pulmonary disease are associated with decreased CD4+ & CD8+ T cells and increased growth & differentiation factor-15 (GDF-15) in peripheral blood. Respir Med 2015;109:212-220.
16. Freeman CM, McCubbrey AL, Crudgington S, Nelson J, Martinez FJ, Han MK, et al. Basal gene expression by lung CD4+ T cells in chronic obstructive pulmonary disease identifies independent molecular correlates of amphysema extent. PLoS One 2014;9:e96421. doi: 10.1371/journal.pone.0096421.
17. Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation: cellular and molecular mechanisms. J Dent Res 2012;91:142-149. doi: 10.1177/0022034511421120.
18. Brusselle GG, Joos GF, Bracke KR. New insights into the immunology of chronic obstructive pulmonary disease. Lancet 2011;378:1015–1026. doi: 10.1016/S0140-6736(11)60886-4.
19. Rovina N, Koutsoukou A, Koulouras NG. Inflammation and immune response in COPD: where do we stand? Mediators Inflamm 2013;2013:413735. doi: 10.1155/2013/413735.
20. Lugade AA, Bogner PN, Murphy TF, Thanavala Y. The role of TLR2 and bacterial lipoprotein in enhancing airway inflammation and immunity. Front Immunol 2011;2:10. doi: 10.3389/ fimmu.2011.00010.
21. Sales-Campos H, Tonani L, Cardoso CR, Kress MR. The immune interplay between the host and the pathogen in Aspergillus fumigatus lung infection. Biomed Res Int 2013;2013:693023. doi: 10.1155/2013/693023.
22. Doe C, Bafadhel M, Siddiqui S, Desai D, Mistry V, Rugman P, et al. Expression of the T helper 17-associated cytokine IL-17A and IL- 17F in asthma and COPD. Chest 2010;138:1140-1147. doi: 10.1378/chest.09-3058.
23. Bhat TA, Kalathil SG, Bogner PN, Miller A, Lehmann PV, Thatcher TH, et al. Second Hand Smoke Induces Inflammation and Impairs Immunity to Respiratory Infections. J Immunol 2018;200:2927-2940. doi: 10.4049/jimmunol.1701417.
24. Lugade AA, Bogner PN, Thatcher TH, Sime PJ, Phripp RP, Thanavala Y. Cigarette smoke exposure exacerbates lung inflammation and compromises immunity to bacterial infection. J Immunol 2012;189:2526-2535. doi: 10.4049/jimmunol.1302584.
25. McKinney EF, Lee JC, Jayne DR, Lyons PA, Smith KG. T cell exhaustion, co-stimulation and clinical outcome in autoimmunity and cancer. Nature 2015;523:612-616. doi: 10.1038/nature14468.
26. Hashimoto M, Kamphorst AO, Im SJ, Kissick HT, Pillai RN, Ramalingam SS, et al. CD8+ T cell exhaustion in chronic infection and cancer: opportunities for interventions. Annu Rev Med 2018;69:301-318. doi: 10.1146/annurev-med-012017-043208.
27. Grundy S, Plumb J, Lea S, Kaur M, Ray D, Singh D. Down regulation of T cell receptor expression in COPD pulmonary CD8+ cells. PLoS One 2015;8:e71629. doi: 10.1371/journal.pone.0071629.
28. Paats MS, Bergen IM, Hoogsteden HC, van der Eenden MM, Hendrikx RW. Systemic CD4+ and CD8+ T-cell cytokine profiles correlate with GOLD stage in stable COPD. Eur Respir J 2012;40:330-337. doi: 10.1183/09031936.00079611.
29. Eustace A, Smyth L, Mitchell L, Williamson K, Plumb J, Singh D. Identification of cells expressing IL-17A and IL-17F in the lungs of patients with COPD. Chest 2011;139:1089-1100. doi: 10.1378/ chest.10-0779.
30. Di Stefano A, Caramori G, Gennemi I, Contoli M, Vicari C, Capelli A, et al. T helper type 17-related cytokine expression is increased in the bronchial mucosa of stable chronic obstructive pulmonary disease patients. Clin Exp Immunol 2009;157:316-324. doi: 10.1111/ j.1365-2249.2009.03965.x.
31. Li XN, Pan X, Qiu D. Imbalances of Th17 and Treg cells and their respective cytokines in COPD patients by disease stage. Int J Clin Exp Med 2014;7:5324–5329.

32. Imani S, Salimian J, Fu J, Ghanei M, Panahi Y. Th17/Treg-related cytokine imbalance in sulfur mustard exposed and stable chronic obstructive pulmonary (COPD) patients: correlation with disease activity. Immunopharmacol Immunotoxicol 2016;38:270–280. doi: 10.1080/08923973.2016.1188402.

33. Roussel L, Houle F, Chan C, Yao Y, Bérubé J, Olivenstein R, et al. IL-17 promotes p38 MAPK-dependent endothelial activation enhancing neutrophil recruitment to sites of inflammation. J Immunol 2010;184:4531–4537. doi: 10.4049/jimmunol.0903162.

34. Le Rouzic O, Pichavant M, Frealle E, Guillon A, Si-Tahar M, Gosset P. Th17 cytokines: novel potential therapeutic targets for COPD pathogenesis and exacerbations. Eur Respir J 2017;50:1602434. doi: 10.1183/13993003.02434-2016.

35. Conti HR, Shen F, Nayar N, Stocum E, Sun JN, Lindemann MJ, et al. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. J Exp Med 2009;206:299–311. doi: 10.1084/jem.20081463.

36. Huang W, Na L, Fidel PL, Schwarzenberger P. Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice. J Infect Dis 2004;190:624–631. doi: 10.1086/422329.

How to cite this article: Geng WR, He HY, Zhang Q, Tong ZH. Th17 cells are involved in mouse chronic obstructive pulmonary disease complicated with invasive pulmonary aspergillosis. Chin Med J 2021;134:555–563. doi: 10.1097/CM9.0000000000001183