Increased IL-33 expression in chronic obstructive pulmonary disease

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Abstract

Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease characterized by inflammatory cell activation and the release of inflammatory mediators. Interleukin-33 (IL-33) plays a critical role in various inflammatory and immunological pathologies, but evidence for its role in COPD is lacking. This study aimed to investigate the expression of IL-33 in COPD and to determine whether IL-33 participates in the initiation and progression of COPD. Levels of serum IL-33 and its receptors were measured by ELISA, and serum levels of IL-33, ST2, and IL-1 receptor accessory protein were elevated in patients with COPD compared with control subjects. Flow cytometry analysis further demonstrated an increase in peripheral blood lymphocytes (PBLs) expressing IL-33 in patients with COPD. Immunofluorescence analysis revealed that the main cellular source of IL-33 in lung tissue was human bronchial epithelial cells (HBEs). Cigarette smoke extract and lipopolysaccharide could enhance the ability of PBLs and HBEs to express IL-33. Furthermore, PBLs from patients with COPD showed greater IL-33 release in response to the stimulus. Collectively, these findings suggest that IL-33 expression levels are increased in COPD and related to airway and systemic inflammation. Therefore, IL-33 might contribute to the pathogenesis and progression of this disease.

Key words: chronic obstructive pulmonary disease; interleukin-33; human bronchial epithelial cells; peripheral blood lymphocytes

Chronic obstructive pulmonary disease (COPD) is a leading public health problem, and the incidence of COPD has recently increased; it is projected to rank third worldwide for mortality (37). COPD is typically characterized by irreversible and slowly progressive shortness of breath upon exertion, which is caused by airway obstruction resulting from airway inflammation. Bronchial biopsies from patients with COPD show infiltration with increased numbers of neutrophils, T cells, and macrophages (6, 7, 13), which induce the production of various cytokines in the airways, including IL-6, IL-8, tumor necrosis factor-α (TNF-α), and neutrophil-associated cytokines (5, 16). These cytokines are associated with different patterns of inflammation in the airways and eventually cause chronic bronchitis and emphysema by inducing mucus production, alveolar destruction, and remodeling of the airways.

IL-33 is a new member of the IL-1 family that has attracted great attention because of its function in immune responses. It is mainly expressed by a variety of cells and tissues in healthy and inflamed tissues. Immune cells, macrophages, and dendritic cells can also produce IL-33 in response to specific stimuli (46). IL-33 is known to be a ligand for ST2 and plays an important role in the immune response and inflammatory diseases. Apart from Th2-type immune response, recent studies indicate that IL-33 has a remarkable effect on Th1-type responses by promoting the secretion of Th1-type cytokines by natural killer and natural killer T cells (48, 55, 56). IL-1 receptor-related protein ST2 and IL-1 receptor accessory protein (IL-1RACp) are required for IL-33-mediated signal transduction because these molecules form parts of its receptor complex (14).

It has been reported that changes in the serum concentration of IL-33 and ST2 occur in allergic and heart diseases (11, 32, 50). Additionally, high expression levels of IL-33 have been detected in lung tissues, which play an important role in respiratory diseases. IL-33 expression levels are significantly elevated in bronchial asthma, and bronchial epithelium cells are an important source of IL-33 (36, 41, 42). Although elevated ST2 levels have been reported in patients with COPD (17), the relationship and function of the IL-33 pathway in the pathogenesis of this disease remain unknown.

To investigate the role and source of IL-33 in COPD, we measured the serum levels of IL-33, the receptors ST2 and IL-1RACp in patients with COPD and controls, and the intracellular expression levels of IL-33 in peripheral blood and lung tissues. IL-33 and its soluble receptors were increased in COPD, and the cellular sources of IL-33 in the peripheral blood and lung tissues included peripheral blood lymphocytes (PBLs) and human bronchial epithelial (HBE) cells. Moreover, we found that IL-33 production by these cells could be stimulated by cigarette smoke extract (CSE) and LPS, which were major risk factors for COPD.

Materials and Methods

Subjects. A total of 112 patients with COPD and 115 sex-, age-, and race-matched controls were chosen for inclusion in our study. The clinical characteristics of these populations are described in detail in Table 1. COPD was diagnosed according to the American Thoracic Society criteria and the Global Initiative for COPD (GOLD) criteria (57) (postbronchodilator forced expiratory volume in 1 s/forced vital...
capacity ratio <70%). Patients with COPD and controls all had a history of smoking with a pack-year index >20. Plasma cotinine concentrations were measured by a direct ELISA kit (Immunalysis, Pomona, CA) to confirm smoking status. The sensitivity of the cotinine assay is 1 ng/ml, Plasma cotinine levels for patients with COPD and controls were all above the recognized minimum criterion of active smoke exposure (9.5 ng/ml) (23). Subjects who suffered from asthma or other obstructive lung diseases or who exhibited disease exacerbation or respiratory infection in the 4 wk before recruitment were excluded from this study. Patients receiving inhaled corticosteroids or oral theophylline, anti-inflammatory therapy, or oral steroids for chronic inflammatory diseases during the previous 4 wk were also excluded.

In these patients with COPD and healthy individuals, lung tissues were collected during surgical resection of solitary pulmonary nodules, and lung cancer was excluded by pathology after surgery. A total of 12 lung specimens was collected, including six COPD specimens and six control specimens. In these subjects, the 12 participants who supplied lung tissue specimens also provided venous blood simultaneously, and others only provided a blood sample.

All study subjects were recruited from Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology in Wuhan (Hubei, China) and signed a consent form. Our study was approved by the Research Ethics Committee of Huazhong University of Science and Technology.

Cytokine ELISAs. ELISA was used to measure the protein concentrations of IL-33, ST2, and IL-1RAcP in serum or cell culture supernatants. First, an ELISA plate was coated with capture antibody (R&D Systems, Minneapolis, MN). The serum or sample supernatants were then added to the plate, and horseradish peroxidase-conjugated secondary monoclonal antibody was added. After a washing step, tetramethylbenzidine was added in the dark. To stop the reaction, 2 mol/l H2SO4 was added to each well. Absorbance was determined at 490 nm and a reference wavelength of 570 nm using a spectrophotometer. Concentrations were calculated based on a standard curve. The limits of detection for the IL-33, ST2, and IL-1RAcP ELISA kits were 1.65, 13.50, and 31.20 pg/ml, respectively.

Flow cytometry. Flow cytometry was used to investigate the cellular source of IL-33 in peripheral blood. An aliquot of 100 μl whole blood was added to a six-well plate containing 800 μl RPMI-1640 Medium (GIBCO Laboratories, Grand Island, NY). Biologend Brefeldin A solution (Becton Dickinson, Franklin Lakes, NJ) was added to the mixed cell cultures, which were incubated for 4 h. After red blood cells were lysed using lysis solution (Becton Dickinson), cytokine/ cytokperm solution (Becton Dickinson) was added to the tube for incubation for 20 min. The cell suspension was then divided into two tubes. In the dark, monoclonal anti-human IL-33 phycoerythrin (IL-33-PE; R&D Systems) and IgG2B isotype control-PE (R&D Systems) were added to the cells separately. After one final wash, cells were resuspended in 2% paraformaldehyde and assessed using a Becton Dickinson LSR flow cytometer. Data were analyzed using CellQuest software.

Immunofluorescence. Formalin-fixed and paraffin-embedded lung tissue was obtained from six patients with COPD and six healthy control subjects. Then, 5-μm sections mounted onto slides were used for immunofluorescence detection of IL-33, ST2, and IL-1RAcP. After deparaffinization in methanol and rehydration in a graded alcohol series, the sections were washed in TBS and boiled in sodium citrate buffer for 20 min in a microwave oven for epitope retrieval. Slides were incubated with 10% donkey serum for 1 h at room temperature and then were incubated overnight with mouse anti-human IL-33 antibody (1:100; Alexis Biochemicals, Lausen, Switzerland), mouse anti-human ST2 antibody (1:100, Alexis Biochemicals), rabbit anti-human IL-1RAcP antibody (1:200; GeneTex, San Antonio, TX), or matched isotype controls (Becton Dickinson) at 4°C. Sections were incubated with Alexa Fluor 488 donkey anti-mouse, Alexa Fluor 594 goat anti-mouse, and Alexa Fluor 488 donkey anti-rabbit secondary antibody. Finally, slides were visualized using laser-scanning confocal microscopy (Zeiss, Oberkochen, Germany).

Total RNA extraction, reverse transcription, and quantitative real-time PCR. Total RNA from cells and tissue was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). The cDNA was prepared from 500 ng total RNA using Prime Script RT Master Mix (Takara, Dalian, China) following the manufacturer’s instructions. Real-time PCR was performed to quantify mRNA levels using an ABI Prism 7500 sequence detection system with SYBR Premix Ex Taq (Takara). The PCR parameters were 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, and 60°C for 30 s. Results were expressed as 2 \(-\Delta\Delta CT\), normalized to levels of GAPDH in untreated cells or control subjects. Specific primers used in this study were designed using the program BatchPrimer v1.0 and are as follows: IL-33, GAAGAACACAG-CAAGCAAGAGC and TACCAAAGGCAAGACCTC; GADPH, ACCACATCTGGCATCAC and TCCACCACCTTGGCT-GTA.

Cell culture and treatments. HBEs were purchased from ATCC (Manassas, VA), and PBLs were isolated from peripheral blood samples obtained from study subjects. The cell culture medium consisted of DMEM (GIBCO) or RPMI 1640 medium, 10% fetal bovine serum (GIBCO), penicillin, and 100 μg/ml streptomycin (KeyGEN, Nanjing, China). When cells reached 80% confluence, HBEs were digested with 0.25% trypsin, and the cell concentration was adjusted to 107/ml. Meanwhile, PBLs were also suspended in culture medium at a concentration of 106/ml. Cells and medium with or without irritant (as a control) were then added to the six-well tissue culture plate. The final concentrations of CSE (Murty Pharmaceuticals, Lexington, KY) and LPS (Sigma-Aldrich, St. Louis, MO) were 10 μg/ml and 100 ng/ml, respectively. After incubation in a humidified incubator (95% air-5% CO2, 37°C) for 24 h, supernatants were stored at −20°C for ELISA analysis, and cells were collected and stored at −80°C in TRizol for mRNA extraction. All experiments on HBEs were performed in triplicate and were repeated at least three times.

Cell apoptosis assay. Cell apoptosis was quantified using annexin V/propidium iodide (PI) apoptosis detection kit (KeyGen). After treatment, HBEs and PBLs were collected and resuspended in 500 μl binding buffer and then incubated with a mixture of 5 μl FITC-labeled annexin V and 5 μl PI for 15 min at room temperature in the dark. Samples were analyzed by flow cytometry using a Becton Dickinson LSR flow cytometer. The data were analyzed with CellQuest software. Annexin V labeled the apoptotic cells, whereas PI labeled the late apoptotic and necrotic cells with membrane damage. Undamaged cells remained negative for both parameters.

DNA extraction and genotyping. Genomic DNA was extracted from 5 ml whole peripheral blood using a DNA extraction kit (Qiagen, Hilden, Germany) according to the standard protocol. PCR primers were designed using Primer 3 input (version 0.4.0) and were as follows: rs4986790 and rs4986791, TGTATTCAGGCTCTGGCTGTG

Table 1. The characteristics of patients with COPD and controls

<table>
<thead>
<tr>
<th>COPD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>112</td>
</tr>
<tr>
<td>Age, yr</td>
<td>65.27 ± 13.84</td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>95:17</td>
</tr>
<tr>
<td>Smoking, pack year</td>
<td>34.16 ± 17.96</td>
</tr>
<tr>
<td>Serum cotinine, ng/ml</td>
<td>151.50 ± 138.47</td>
</tr>
<tr>
<td>FEV1, l</td>
<td>1.12 ± 0.47</td>
</tr>
<tr>
<td>FEV1 % pred</td>
<td>55.71 ± 13.33</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>53.18 ± 7.55</td>
</tr>
</tbody>
</table>

Applicable data are shown as means ± SD. COPD, chronic obstructive pulmonary disease; FEV1 % pred, FEV1% predicted.

L620 IL-33 AND COPD
and TGTTCaAAT TGGATTGTGGGA; rs11536889. CTGGGATC-
CCTCCCTGTGA and TTTCCCTG ATGACATCCTGA. PCR was
performed using the mix of 100 ng genomic DNA, 1.5 mM MgCl2, 0.2 mM
deoxyribonucleotide triphosphates, 1× PCR buffer, 0.5 μl Taq polymerase,
and 1 μl of the forward and reverse primers in a total of 50 μl. The PCR
conditions were as follows: 94°C for 1 min, 58°C for 50 s, 72°C for 90
s for 40 cycles, and 72°C for 10 min then followed by 4°C until the
reaction mixtures were removed from the cycler. PCR products were
sequenced using an ABI3730xl sequencer (Applied Biosystems, Foster
City, CA).

Statistical analyses. Data are presented as means ± SD. Statistical
analyses were performed using SPSS 19.0 (SPSS, Chicago, IL) and
Prism 5 software (GraphPad Software, San Diego, CA). Student’s
\( t \)-test, Mann-Whitney test, one-way ANOVA test, and Kruskal-Wallis
test or Friedman’s test, followed by Dunn’s test and \( \chi^2 \) test were used
as appropriate. All statistical tests were two-tailed, and \( P < 0.05 \) was
considered a statistically significant difference.

RESULTS

Levels of IL-33 and its receptors are elevated in serum from
patients with COPD. Protein levels of IL-33, sST2, and IL-
1RAcP in the serum were measured in patients with COPD and
controls. Significantly higher levels of IL-33 in serum were
detected in patients with COPD (319.24 ± 77.47 pg/ml) than
control subjects (222.60 ± 26.11 pg/ml, Fig. 1A). ST2 and
IL-1RAcP are receptors for IL-33. Because of different ST2
mRNA splice variants, there are two ST2 protein isoforms
(10). The form that we detected in serum was sST2, which has
been widely studied in allergic and heart disease as an inflam-
atory marker (11, 32, 50). A significant difference in ST2
two levels was observed between patients with COPD (3.13 ± 0.95
ng/ml) and control subjects (0.93 ± 0.66 ng/ml, Fig. 1B).
Additionally, we found that the soluble IL-1RAcP levels in
serum from subjects with COPD (2.85 ± 0.10 ng/ml) were
higher than those in healthy subjects (0.98 ± 0.05 ng/ml, Fig.
1C). Thus our data indicated that patients with COPD showed
increased levels of IL-33 and its soluble receptors in serum
compared with healthy subjects.

IL-33 expression in peripheral blood cells, including PBLs
and neutrophils. Significantly higher levels of serum IL-33
protein could be observed in patients with COPD. To corrob-
orate the ELISA findings and characterize the cellular sources
of IL-33 in peripheral blood, we also measured IL-33 expression
in PBLs and neutrophils from 20 patients with COPD and
20 controls by flow cytometry. We found that IL-33-positive
cells were detectable in the lymphocyte group and that the
frequency of IL-33-positive cells was higher in patients with
COPD than in control patients (11.62 ± 8.848 vs. 2.238 ± 4.490,
respectively, Fig. 2). A low frequency of IL-33-positive
cells was detected in the neutrophil group, and there was no
significant difference between patients with COPD and control
patients.

IL-33 is highly expressed in the lungs of patients with
COPD. We next investigated whether IL-33 expression was
increased in the lungs of patients with COPD. Immunofluores-
cence staining showed expression of IL-33 in HBEs, but there
was little signal detected in ASMCs (Fig. 3, A and B). In
agreement with previous reports that IL-33 expression could be
observed in nuclei of endothelial cells (2, 36, 51), IL-33 was
detected in the nuclei of epithelial cells. Additionally, the IL-33
staining intensity of COPD was obviously higher than that of
the control groups (Fig. 3C). Finally, we discovered that ST2
and IL-1RAcP were expressed in the cytoplasm of HBEs, and
IL-1RAcP was also expressed in ASMCs (Fig. 3D). However,
the expression levels of ST2 and IL-1RAcP were not signifi-
cantly different between patients with COPD and control
patients. The isotype controls were negative in the epithelial
cells.

IL-33 expression is upregulated by CSE and LPS in HBEs
and PBLs. On the basis of our observations that HBEs and
PBLs were the cellular sources of IL-33, we further charac-

![Fig. 1.](http://ajplung.physiology.org/ by 10.1152/ajplung.00305.2014 · www.ajplung.org)
ized the regulatory factors involved in controlling IL-33 expression, including CSE and LPS, which are risk factors for the development of COPD. The protein levels of IL-33 in supernatants of HBEs cultured with or without stimulation were measured by ELISA. IL-33 secretion was markedly increased after CSE stimulation compared with untreated cells (Fig. 4A). We further assessed the effect of LPS treatment on IL-33 expression by HBEs and found that there was also a significant difference between cells treated with or without LPS. A stronger response was induced in cultured HBEs when those cells were treated with a mixture of LPS and CSE. This finding indicated that a mixture of LPS and CSE might synergistically stimulate IL-33 expression in HBEs.

Expression of IL-33 in cultured PBLs was examined in cells from 20 patients with COPD and 20 healthy controls with or without stimulation. After treatment with CSE, LPS alone, or both, the expression level of IL-33 in PBLs from patients with COPD was significantly increased compared with that of untreated cells (Fig. 4B). Notably, a stronger response was induced in cultured PBLs from patients with COPD than those cells were stimulated with the mixture of CSE and LPS. These data were similar to those obtained using CSE- and LPS-treated HBEs. However, there were no statistical differences in IL-33 gene expression levels between treated and untreated PBLs from healthy controls (P = 0.174, Friedman’s test). Importantly, after stimulation with the mixture, IL-33 gene expression levels in PBLs from patients with COPD were significantly higher than those from healthy controls. These findings indicated that the responses to these stimuli of cells from patients with COPD and healthy controls were different.

In addition, when cells were double stained with annexin V and PI, no apparent differences were observed in the cell death (apoptosis and necrosis) rates between the cells incubated with CSE and LPS and those incubated with media alone (Fig. 4C), which suggested that IL-33 was actively secreted from HBEs and PBLs without apparent cell damage.

**TLR4 polymorphism analysis in patients with COPD and controls.** rs4986790, rs4986791, and rs11536889 polymorphisms of Toll-like receptor 4 (TLR4) gene have been detected in 20 patients with COPD and 20 controls. The genotype distribution of TLR4 single-nucleotide polymorphisms (SNPs) in these subjects was summarized in Table 2. In the 40 samples, no heterozygous or homozygous variant genotypes of the rs4986790 and rs49867901 polymorphisms were detected. Our data showed that there were no significant differences in the rs11536889 genotype distribution between patients with COPD and controls (P > 0.05).

**DISCUSSION**

There is increasing evidence implicating IL-33 in various inflammatory diseases, such as rheumatoid arthritis (33), atopic dermatitis (21), ulcerative colitis (39), and asthma (30, 42, 46); however, no study to date has investigated the role of IL-33 in COPD. Herein, we show that levels of IL-33 expression are elevated in COPD and confirm that the HBEs in the airway and PBLs in the peripheral blood were the cellular sources of IL-33. IL-33 could be induced by CSE and LPS, which are risk factors for COPD, and we found that the sensitivity of PBLs from COPD subjects to stimuli was much higher than that of healthy subjects.

Our study is the first to provide evidence that the expression of IL-33 and its receptors is increased in the serum of patients with COPD. A recent study showed that the concentrations of serum IL-33 in patients with COPD during
acute episodes were significantly lower than those in individuals with healthy controls and patients at the stable phase of COPD (53). However, as an alarmin, it has been demonstrated that IL-33 could be released when cells sense inflammatory signals or undergo necrosis (36). Increased serum levels of IL-33 were found to be associated with numerous inflammatory diseases, including systemic lupus erythematosus (34), systemic sclerosis (59), inflammatory skin disorders (59), and Crohn’s disease (9). Moreover, a previous study in an animal model showed that the serum level of IL-33 was elevated in COPD (43), which is consistent with the detection of increased serum IL-33 levels in patients with COPD in our study. Additionally, immunofluorescence staining showed that the expression of IL-33 was also increased in lung tissues of patients with COPD, indicating that IL-33 upregulation was probably related to systemic and airway inflammation in COPD. Furthermore, both serum sST2 and IL-1RAcP levels showed a striking increase in patients with COPD compared with those in healthy subjects. Because a beneficial effect of the high levels of soluble IL-1RAcP and ST2 is to antagonize the proinflammatory function of IL-33 (58), the enhanced levels of these molecules demonstrated increased IL-33 expression and release in COPD.

Fig. 3. The high expression levels of IL-33 in nuclei of human bronchial epithelial cells (HBEs) from human lung tissues are related to COPD. IL-33 is stained in green, and cell nuclei are visualized by staining with DAPI dye (blue). A representative immunofluorescence image of IL-33 expression in lung tissue showing staining of cell nuclei of HBEs (red arrow) is shown; we failed to observe immunostaining of airway smooth muscle cells (white arrow). Human lung tissue samples from subjects with COPD (A) and controls (B) were processed by immunofluorescence. C: semiquantitative analysis of IL-33 expression in HBEs from patients with or without COPD. The mean densities were measured using ImageProPlus software and are expressed in histograms as above. Data are presented as means ± SD. ****P < 0.0001, represents significant differences compared with the control group. D: expression of ST2 and IL-1RAcP in human HBEs was examined by immunofluorescence. HBEs were stained for ST2 (red) and IL-1RAcP (green). DAPI dye (blue) was used to visualize cell nuclei.
Recent studies have implicated IL-33 in the pathogenesis of asthma. In asthma, IL-33 can significantly enhance inflammation and cause pathological changes in the airways by recruiting inflammatory cells and inducing the release of inflammatory cytokines (40, 49, 52). Interestingly, a similar pattern of inflammation also exists in COPD. In IL-33-treated mice, the most obvious histopathological changes in the lungs were epithelial lining hypertrophy, goblet cell hypertrophy, and mucus hypersecretion (46), which are the prominent pathophysiological features of patients with COPD. By activating macrophages to secrete cytokines or by direct stimulation, IL-33 can promote neutrophil maturation (56). The activation and recruitment of neutrophils to the airway has been strongly linked to the inflammatory response in COPD. In IL-33 transgenic mice, increased IL-8 was detected in the BALF (61), which is a potent chemotactic cytokine for neutrophils and is thought to play a central role in COPD (8). Combined with the finding of higher IL-33 expression in peripheral blood and airways from patients with COPD in our study, we speculate that IL-33 may play an important role in the pathogenesis of COPD.

Furthermore, we characterized the sources of IL-33 in COPD. We made the novel observation that PBLs and HBEs in the airways produced IL-33 cytokine. We detected IL-33 protein immunoreactivity in HBEs from lung tissue sections from patients with COPD and control subjects, and the expression of IL-33 was significantly higher in patients with COPD than in controls. However, we failed to observe immunostaining of ASMCs. Furthermore, our flow cytometry results showed that PBLs were the major source of IL-33 in the serum, particularly in patients with COPD. By contrast, IL-33-positive neutrophils were scarcely detectable in either patients or healthy controls.

Although IL-33 was initially found in the endothelial cells of high endothelial venules (4), previous reports confirmed the expression of IL-33 in various cell types, including epithelial cells, smooth muscle cells, keratinocytes, fibroblasts, dendritic cells, and macrophages (28, 36, 46, 54). IL-33 expression has also been detected in venules from chronically inflamed tissues from patients with Crohn’s disease or rheumatoid arthritis. Moreover, IL-33 expression levels were correlated with inflammation and tissue damage, which can lead to apoptosis and the release of IL-33 protein. Several studies have demonstrated that IL-33 is highly expressed in epithelial cells from many tissues, including the skin (18), gastrointestinal tract (29), and airway (27), which make contact with allergens, pathogens, tobacco smoke, and other common environmental agents (36). Recent studies of epithelial cells from patients with asthma reported significantly increased expression and secretion levels of the Toll-like receptor single-nucleotide polymorphisms in patients with COPD and controls.

Table 2. Distribution of the Toll-like receptor single-nucleotide polymorphisms in patients with COPD and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>COPD, n (%)</th>
<th>Control, n (%)</th>
<th>χ²</th>
<th>P Value*</th>
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<tbody>
<tr>
<td>rs4986790</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>20(100)</td>
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<tr>
<td>AG</td>
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<td>rs49867901</td>
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<td>CC</td>
<td>20(100)</td>
<td>20(100)</td>
<td></td>
<td></td>
</tr>
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<td>CT</td>
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<td>TT</td>
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<td></td>
<td></td>
</tr>
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<td>rs11536889</td>
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*χ² test.
of IL-33 compared with those from controls (42). In our study, IL-33 was mainly derived from airway epithelial cells and PBLs, which may account for the airway and systemic inflammation observed in COPD.

Another important finding of our study is that CSE and LPS significantly enhanced IL-33 expression in both PBLs and HBEs. It has been reported that IL-33 and ST2 can be induced in cigarette smoke-exposed mice (43). However, our study is the first to provide evidence that IL-33 expression in HBEs and PBLs could be induced by CSE and LPS, which are risk factors for the development of COPD. Moreover, low levels of IL-33 were detected in the supernatants of cultured ASMCs, irrespective of whether they were stimulated, which was consistent with the negative immunostaining of ASMCs in lung tissue. More importantly, we noted a significantly higher release of IL-33 for patients with COPD compared with healthy controls after PBLs were stimulated with the mixture of CSE and LPS.

As an alarm signal, IL-33 is released to activate the immune system in response to inflammatory signals or tissue injury caused by factors such as toxins, high free fatty acids, inflammation, or oxidative stress (4, 31). Increased IL-33 has been reported to be detected in synovial fibroblasts and keratinocytes after activation with IL-1β and TNF-α (26, 47). It has been speculated that IL-33 protein could be released when cell damage occurs to alert the immune system to danger (60). Cigarette smoking is the key cause of the initiation of COPD, and LPS plays an important role in the progression of COPD. Both stimuli can activate inflammatory cells and enhance cytokine release (15, 20). Consequently, we surmised that CSE and LPS are related to the high expression of IL-33 in COPD, and they may play an important role in the progression of COPD through modulating the release of IL-33.

To explore the mechanism of the high expression of IL-33 in COPD after CSE and LPS stimulation, rs4986790, rs4986791, and rs11536889 polymorphisms of TLR4 gene were detected. TLR4 is a transmembrane protein that plays an essential role in detecting LPS. After LPS binding to TLR4, the intracellular signaling events could affect the production of proinflammatory cytokines (1). TLR4 SNPs have been described in association with greater susceptibility to various inflammatory diseases, including rheumatoid arthritis (44), atherosclerosis (24), asthma (12), and COPD (45). The rs4986790 and rs4986791 polymorphisms of TLR4 gene have been reported to be strongly associated with endotoxin hypersensitivity to LPS in Caucasians (3) by affecting the extracellular domain of TLR4. Another study showed that the rs11536889 polymorphisms in the TLR4 gene were likely associated with COPD in Japanese subjects (22). However, in the present study, we observed no rs4986790 and rs4986791 polymorphisms in our samples, which is consistent with previous studies on Chinese Han populations (19), Koreans (25), and the Japanese (38). Moreover, the analysis of rs11536889 polymorphisms showed that there was no significant difference between patients with COPD and controls, which means TLR4 SNPs may not be related to LPS hypersensitivity in our study.

In conclusion, this study provides compelling evidence for elevated IL-33 expression in COPD, including in the airways and peripheral blood. IL-33 is mainly produced by airway epithelial cells in airway inflammation and PBLs in systemic inflammation. Moreover, CSE and LPS, which are risk factors for COPD, could both promote IL-33 secretion. Our study supports the importance of IL-33 signaling in the pathogenesis of COPD, but the target cells and mechanism of action remain to be elucidated. Therefore, more studies should be carried out to achieve a more comprehensive understanding of the role of IL-33 in COPD and to determine the therapeutic value of anti-IL-33 therapy in patients with COPD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: J.X., Junling Zhao, J.S., and M.L. performed experiments; J.X., Junling Zhao, J.S., Jianping Zhao, J.W., Y.X., and J.X. analyzed data; J.X., Z.Z., Jianping Zhao, and J.X. interpreted results of experiments; J.X., Junling Zhao, and J.X. prepared figures; J.X. and J.X. edited and revised manuscript; Y.X. and J.X. approved final version of manuscript; J.X. conceived and designed of research.

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