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Interleukin-33/ST2 signaling promotes production of interleukin-6 and interleukin-8 in systemic inflammation in cigarette smoke-induced chronic obstructive pulmonary disease mice

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ABSTRACT

Interleukin-33 is a newly described member of the interleukin-1 family. Recent research suggests that IL-33 is increased in lungs and plays a critical role in chronic airway inflammation in cigarette smoke-induced chronic obstructive pulmonary disease (COPD) mice. To determine the role of IL-33 in systemic inflammation, we induced COPD mice models by passive cigarette smoking and identified the IL-33 expression in bronchial endothelial cells and peripheral blood mononuclear cells (PBMCs) of them. After isolation, PBMCs were cultured and stimulated in vitro. We measured expressions of interleukin-6 and interleukin-8 in PBMCs in different groups. The expression of IL-33 in bronchial endothelial cells and PBMCs of COPD mice were highly expressed. Stimulated by cigarette smoke extract (CSE), the expression of IL-6 and IL-8 were induced and enhanced by IL-33. PBMCs of COPD mice produced more IL-6 and IL-8 stimulated by CSE and IL-33. Expression of IL-6 and IL-8 were decreased when stimulated by IL-33 together with soluble ST2. The mRNA production of ST2 in IL-33 stimulated PBMCs was increased. Being pretreated with several kinds of MAPK inhibitors, the secretions of IL-6 and IL-8 in PBMCs did not decrease except for the p38 MAPK inhibitor. We found that IL-33 could induce and enhance the expression of IL-6 and IL-8 in PBMCs of COPD mice via p38 MAPK pathway, and it is a promoter of the IL-6 and IL-8 production in systemic inflammation in COPD mice.

1. Introduction

Chronic obstructive pulmonary disease is a major worldwide and still increasing health problem [1], and it is characterized by incomplete reversible airflow obstruction associated with pulmonary inflammation involved in several kinds of inflammatory cells [2]. There are many kinds of inflammatory cells, cytokines and chemokines influencing injury and remodeling lungs and airways which are the characteristic changes of COPD [3]. Although the chronic irritants that trigger the inflammatory response of the airways in COPD were ceased, the inflammation still persists and the levels of cytokines in lungs and bloodstream remain abnormal in COPD patients even when they are in a stable phase of the disease. The persistence of systemic inflammation contributes to the development of systemic complications of COPD, including weight loss, skeletal muscle dysfunction, osteoporosis and atherosclerosis [4–6]. COPD patients have persisting chronic inflammation in their airways, and several studies have demonstrated that the systemic levels of inflammatory markers are much higher [7,8]. In other words, the chronic inflammation exhibiting in COPD patients is not only in lungs and airways, but also exists and influences other parts of the body.

Interleukin-33 (IL-33) is a cytokine of the interleukin-1 family, which also includes IL-1α/β and IL-18 [9], and singles via ST2 [10]. Most of the IL-1 family members play important roles in Th1 immune responses, but IL-33 is described as a promoter of Th2 immunity and systemic inflammation in vivo models and in vitro experiments [10]. In human and mice, IL-33 is expressed by innate cells, primarily epithelium and endothelium, and is released when they are stimulated by inflammation or necrosis [10,11]. Recent research suggests that IL-33 is a multi-effective

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cytokine that could induce complex immune responses in immunity and in diseases [12].

IL-33 is significantly increased in lung and plays an important role in respiratory disease [10,13]. It could induce airway inflammation, airway hyper-responsiveness and goblet cell metaplasia in allergen-naive mice, and aggravate asthma-like responses in allergen-exposed mice [13]. IL-33 plays a critical role in the airway inflammation in chronic respiratory disease, such as COPD, but whether or not it influences the production of IL-6 and IL-8 in systemic inflammation of COPD is unknown. In this study, we investigated the role of IL-33 in peripheral blood mononuclear cells (PBMCs) of cigarette smoke (CS) induced COPD mice models, and found that it could induce and enhance the expression of IL-6 and IL-8 in PBMCs of mice, especially for the COPD. We hypothesized that IL-33 participated in promoting production of IL-6 and IL-8 in systemic inflammation of COPD.

2. Materials and methods

2.1. Animal studies

All animal experiments were approved by the Huazhong University Animal Experiment Ethics Committee and were performed in accordance with the Regulations for Animal Experiments and Related Activities at Huazhong University.

Male Blab/c mice were obtained from Hubei Provincial Laboratory Animal Public Service Center, Wuhan, Hubei Province, China. Mice were housed in sterilized cages with filter tops in specific pathogen-free conditions at Tongji Medical College, Huazhong University of Science and Technology.

2.2. Cigarette smoke exposure and lung function measurement

The male mice were randomly divided into two groups, CS exposure group and Control group. The CS exposure mice were exposed to cigarette smoke passively in PAB-S200 Animal Passive Smoking Exposure System (BioLab Technology Co. Ltd., Beijing, China) for 8 months. We gave the CS group 10 research cigarettes inhaling for 3 h per day, and 5 continuous days for a week. Simultaneously, the controls were exposed to room air.

At the end, the lung function of all mice, including CS exposure and controls, were measured in the AniRes 2005 Lung Function system (BoiLab Technology Co. Ltd, Beijing, China). The FVC, $FEV_{0.1}$ and $FEV_{0.1}$/FVC were recorded. The CS exposure mice, in which $FEV_{0.1}$/FVC were below 0.7, were picked out as COPD group. Their blood were drawn off from inferior vena cava and lung were fixed with paraformaldehyde for Hematoxylin–Eosin and immunohistofluorescence for IL-33 staining, accompanied with controls.

2.3. PBMCs culture and stimulation

PBMCs were isolated from blood of all mice, including 6 COPD mice and 6 controls. After counted, PBMCs were seeded at a density of 10^5 cells/cm^2 and grown at 37 °C with 5% CO2 in 1640 medium with 10% heat-inactivated FBS. Then the stimulant was added to the well. The stimulus, including IL-33, soluble ST2 (R&D systems, USA), CSE (Murty Pharmaceuticals, Inc, Lexington, Kentucky, USA), and pharmacological inhibitors, were made up to different combinations in different groups. PBMCs were cultured with stimulus for 24 h. Then supernatants were collected and stored at −80 °C and PBMCs were gathered for RNA extraction. In some experiments, PBMCs were pretreated for 1 h with U-0126 (10 μM), SP-600125 (10 μM), SB-203580 (10 μM) before the stimulation of IL-33 (10 ng/ml), CSE (10 μg/ml), or both. The pharmacological inhibitors SB-203580 (a p38 MAPK inhibitor), U-0126 (a p42/p44 ERK inhibitor), SP-600125 (a JNK inhibitor) were purchased from Santa Cruz Biotechnology, TX, USA and dissolved in dimethyl sulfoxide (DMSO).

2.4. RNA extraction and real-time quantitative RT-PCR

Total cellular RNA was isolated from PBMCs after stimulation by using Trizol (Invitrogen, Carlsbad, CA) and reverse transcription was performed by using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany). Quantitative real-time PCR was performed by using the SYBR Green Real-time PCR Master Mix (Roche, Mannheim, Germany) with the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Primers for Q-PCR were synthesized by Invitrogen and as follows: GADPH (forward, 5′-aactttggcattgtggaagg-3′; reverse, 5′-ggatgcagggatgatgttct-3′), IL-6 (forward, 5′-ccggagaggagacttcacag-3′; reverse, 5′-tccacgatttcccagagaac-3′), CXCL1 (forward, 5′-tgcacccaaaccgaagtc-3′; reverse, 5′-gtcagaagccagcgttcacc-3′), CXCL2 (forward, 5′-aaagtttgccttgaccctgaa-3′; reverse, 5′-ctcagacagcgaggcacatc-3′), ST2 (forward, 5′-aggggaatgttgaactcacg-3′; reverse, 5′-caacccagcctaagggtaca-3′). The cDNA fragments were denatured at 95 °C for 15 s, annealed and extended at 60 °C for 60 s for 40 cycles. Each sample was examined in triplicate and the amounts of the PCR products were normalized to that of GADPH which served as internal control.

2.5. IL-33, IL-6 and IL-8 cytokines measured by ELISA

ELISA experiments were conducted on serum and PBMCs culture medium supernatants, which were collected when the stimulation was completed. Supernatants were spun down for 10 min to ensure the absence of cells in suspension and stored as aliquots at −80 °C until use. The mouse IL-33 CytoSet (Invitrogen) was used to quantify IL-33 as the manufacturer's instructions. And the mouse IL-6 and IL-8 CytoSet (Invitrogen) were also used to quantify IL-6 and IL-8. Each sample was examined in triplicate.

2.6. Statistical analysis

The quantitative RT-PCR data and part of the ELISA are expressed as the medians, whereas data from experiments done on IL-33 serum levels and IL-6, IL-8 protein levels in supernatants are expressed as means ± SEMs. Unless specified, data are representative of the indicated number of subjects or independent experiments. Student's t tests were performed to analyze the differences between data obtained in different groups of subjects. Parts of the paired data were analyzed by using paired Student's t test. Values of $p < 0.05$ were considered as statistical significant difference.

3. Results

3.1. Expression of IL-33 is increased in bronchial endothelial cells and PBMCs of COPD mice

Recent studies by Qiu et al. have demonstrated that the expression levels of IL-33 were markedly enhanced in the lung tissue of mice inhaling CS [14]. In our study, we measured the IL-33 expression in bronchial endothelial cells and PBMCs in CS exposed mice and controls. The expression of IL-33 was shown in immunohistofluorescence staining and noted in the nucleus of endothelial cells. IL-33 staining intensity of COPD was obviously higher than that of controls, and a quantitative analysis of the IL-33 staining in bronchial endothelial cells area showed a 3.9-fold increase in COPD

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