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Effects of PM2.5 exposure on the Notch signaling pathway and immune imbalance in chronic obstructive pulmonary disease☆

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ABSTRACT

Chronic Obstructive Pulmonary Disease (COPD) is associated with T lymphocytes subset (Th1/Th2, Th17/Treg) imbalance. Notch signaling pathway plays a key role in the development of the adaptive immunity. The immune disorder induced by fine particulate matter (PM2.5) is related to COPD. The aim of this study was to investigate the mechanism by which PM2.5 influences the Notch signaling pathway leading to worsening immune disorder and accelerating COPD development. A COPD mouse model was established by cigarette smoke exposure. PM2.5 exposure was performed by aerosol inhalation. γ-secretase inhibitor (GSI) was given using intraperitoneal injection. Splenic T lymphocytes were purified using a density gradient centrifugation method. $CD4^+$ T lymphocyte subsets (Th1/Th2, Th17/Treg) were detected using flow cytometry. mRNA and proteins of Notch1/2/3/4, Hes1/5, and Hey1 were detected using RT-PCR and Western blot. Serum INF-γ, IL-4, IL-17 and IL-10 concentrations were measured using ELISA. The results showed that in COPD mice Th1% and Th17%, Th1/Th2 and Th17/Treg were increased, and the levels of mRNA and protein in Notch1/2/3/4, Hes1/5, and Hey1 and serum INF-γ and IL-17 concentrations were significantly increased, and Th2%, Treg%, and serum IL-4 and IL-10 concentrations were significantly decreased. COPD Mice have Th1- and Th17-mediated immune disorder, and the Notch signaling pathway is in an overactivated state. PM2.5 promotes the overactivation of the Notch signaling pathway and aggravates the immune disorder of COPD. GSI can partially inhibit the activation of the Notch signaling pathway and alleviate the immune disorder under basal state and the immune disorder of COPD caused by PM2.5. This result suggests that PM2.5 is involved in the immune disorder of mice with COPD by affecting the Notch signaling pathway and that PM2.5 aggravates COPD.

1. Introduction

Recent studies have found that patients with chronic obstructive pulmonary disease (COPD) have an immune disorder involving an imbalance of T cell subsets, which perform the acquired immune response dominated by Th1 and Th17. This immune response can lead to the persistence and expansion of the chronic inflammation of COPD and accelerate airway remodeling, which is involved in the pathogenesis and the aggravation of COPD (Jin et al., 2014). Wang et al. (2015) found that patients with COPD had an imbalance between Th17 and regulatory T cells (Treg) and a significant increase in serum interleukin (IL)-6 and IL-17 levels, which were closely associated with forced vital capacity (FVC), first second of forced expiratory volume (FEV1), and a decrease in the FEV1/FVC value, and these changes were associated with the severity of the disease in patients with COPD. Bhat et al. (2015) found a significant increase in the levels of Th1 cell-associated proinflammatory cytokine interferon (IFN)-γ and IL-12 in patients with COPD, which was negatively correlated with the FEV1.

Fine particulate matter (PM2.5) is the atmospheric particles with an aerodynamic equivalent diameter less than or equal to 2.5 μm in ambient air, and PM2.5 is an important component of air pollution. The main constituents are heavy metal particles, acidic oxides, organic pollutants, bacteria, fungi, and viruses, which deposit in the airway and lung tissue after being inhaled and frequently trigger abnormal immune inflammatory responses (Bowers et al., 2013; Rogula-Kozlowska et al., 2016; Shi et al., 2016). Epidemiological studies have shown that the atmospheric PM2.5 concentration increases by 10 $\mu g/m^3$, the mortality of respiratory diseases is increased by 2%, and the hospitalization rate in patients

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with COPD is increased by 1.72–6.87% (Tsai et al., 2013; Zanobetti and Schwartz, 2009). Lagorio et al. (2006) reported that PM2.5 could stimulate the release of proinflammatory cytokines in the respiratory tract, which could aggravate oxidative damage, increase mucosal permeability and reduce mucin activity, thereby exacerbating COPD. Studies found that PM2.5 could aggravate the imbalances of Th1/Th2 and Th17/Treg of COPD and could induce a significant increase in the secretion of the mouse serum Th1-associated factors IFN-γ and IL-12, an increase in the Th17-associated factor IL-17 and a decrease in the Treg-associated cytokine IL-10, resulting in immune imbalance and immune injury (He et al., 2016). Tong et al. (2015) reported that PM2.5 could stimulate Jurkat T cells to increase their cytoplasmic synthesis of tumor necrosis factor (TNF)-α and IL-2. Zhao et al. (2012) reported that PM2.5 upregulated the Toll-like receptor 2 and 4 (TLR2 and TLR4, respectively) activity in mouse alveolar macrophages, inducing a Th1/Th2 cell imbalance. PM2.5 could also induce an increase in the secretion of the Th17-associated factor IL-17A in mice with asthma (Zhang et al., 2015). In addition, adolescents who were exposed to PM2.5-based air pollutants showed a suppression of the Th1-mediated immune response and an exacerbation of the Th2-mediated humoral immune response, which are the basis of the pathogenesis of allergic and autoimmune diseases (Dobreva et al., 2015). In 2016, Pope et al. (2016) reported that PM2.5 could induce increased levels of TNF-α, IL-8, and IL-6 in the peripheral blood and lungs of non-smoker adolescents, causing mild inflammation in the entire body and immune disorder.

The Notch signaling pathway is a highly conserved signal transduction pathway that includes the 4 homologous receptors Notch1/2/3/4 and the 5 ligands Delta1/3/4 and Jagged1/2, which are expressed on the surface of T cells and multiple mature immune cells and participate in the differentiation of T cells (Mochizuki et al., 2011; Shi et al., 2009). After the binding of the Notch receptor and the ligand, a downstream target gene is activated to suppress the expression of differentiation regulatory factors, which eventually affects cell differentiation, proliferation, and apoptosis (Tilley et al., 2009). Laky et al. (2015) found that Delta-like 4 (DLL4) on the surface of antigen-presenting cells bound to the Notch receptor on the surface of $CD4^+$ T cells to increase the secretion of IL-2 and regulate adaptive immune responses. Activation of the Notch1 receptor can regulate Th17 cell differentiation (Keerthivasan et al., 2011). Gamma-secretase is a catalytic enzyme necessary for the transmembrane activation of the Notch signaling pathway (Espinoza and Miele, 2013), and γ-secretase inhibitors (GSIs) are the specific inhibitors of γ-secretase, which can competitively inhibit Notch receptor activity (Espinoza and Miele, 2013; Takebe et al., 2014). The Notch signaling pathway is activated in asthma patients. Blocking the Notch signaling pathway can reduce the differentiation of naïve T cells into Th2 cells, reduce the Th2-mediated immune response in asthma patients, and slightly increase Th1 cell differentiation, which can regulate the immune disorder of asthma and other diseases (Gu et al., 2012; Kang et al., 2009). Yang et al. (2016) reported that the Notch signaling pathway can regulate the immune imbalance of Th1/Th2 and Th17/Treg of COPD and participates in the pathogenesis of COPD.

PM2.5 can aggravate the poor ability of alveolar macrophages to engulf *E. coli* in COPD. However, the impact of PM2.5 on the immune imbalance in COPD and its relationship with the Notch signaling pathway have been rarely reported. This study aimed to observe the impact of PM2.5 on the immune imbalance in mice with COPD and its relationship with the Notch signaling pathway to further investigate the mechanism of the impact of PM2.5 on immune imbalance in COPD.

2. Materials and methods

2.1. Collection and preparation of PM2.5

As described by Chu et al. (2016), an Air Intelligent Total Suspended Particulate Sampler (Laoying 2050D, Laoshan Institute of Application Technology, Qingdao, China) was used to collect PM2.5 from an area next to the main traffic route, Tianshui Road, of Lanzhou. The PM2.5 sampling head was used to filter the total suspended particulate matter and PM10, the sampling flow rate was 100 L/min and 22 h/day, and the interval was 2 h. The collected PM2.5 was frozen, dried, and then stored at 4 °C in the dark for future use.

2.2. Experimental animals and grouping

Eighty specific pathogen free (SPF) grade 6 - 8-week-old male BALB/c mice with an average body weight of (20 ± 2) g were used in this study. Animals were purchased from the Experimental Animal Center of Lanzhou University, China (batch number: SCXK (Gan) 2013-0002+). The animals were randomly divided into 8 groups: healthy control group, healthy + PM2.5 group, healthy + GSI group, healthy + PM2.5 + GSI group, COPD group, COPD + PM2.5 group, COPD + GSI group, and COPD + PM2.5 + GSI group, with 10 animals per group. This study was approved by the Animal Experiment Ethics Committee of the First Hospital of Lanzhou University (license number:LDYYKY20130226-24).

2.3. Preparation of the COPD model

According to the method by Chu et al. (2016), mice were placed in a 50 cm × 30 cm × 35 cm plexiglass box and were exposed to cigarette (amount of tar 8 mg, amount of nicotine of flue gas 0.7 mg, amount of carbon monoxide of flue gas 14 mg, from Gansu Tobacco Industry Co., Ltd., China) smoke 4 times per day (4 cigarettes × 45 min), with a 1-h interval for 90 consecutive days. All PM2.5 groups inhaled the PM2.5 aerosol at a concentration of approximately 770 μg/m^3 for 90 consecutive days. According to the method by Jiao et al. (2014), from the 61st day of the modeling, the GSI group and the control group were intraperitoneally injected with GSI (Sigma-Aldrich, USA)every other day at a dose of 1 mg/kg until the 90^{th} day.

2.4. Pulmonary function test

After the modeling was completed, the mice were placed in a noninvasive plethysmography box, according the method by Chu et al. (2016), in the sober and freely moving condition. A noninvasive mouse pulmonary function measurement system (GYD-003 Noninvasive Mouse Lung Function telemetry device, Emka, France) was used to examine the peak inspiratory flow (PIF) and peak expiratory flow (PEF).

2.5. Pathomorphological observation of lung tissue

After the modeling was completed, according to the method by Chu et al. (2016), the mice were sacrificed by the cervical dislocation method. Mouse lung tissue was removed and placed in a 10% neutral formalin solution for fixation. The tissue was embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). The section was dehydrated in ethanol, dried and sealed with neutral resin. A pathological image acquisition and analysis system was used to observe and record the pathologic changes of the mouse lung tissue.