Letter to the Editor

Pollen Extract of *Populus deltoides* Stimulates Lung Inflammation in a Mouse Model*

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Allergic asthma is an inflammatory disease of the airways characterized by recurrent episodes of wheezing, bronchoconstriction, and airway hyperresponsiveness (AHR)[1]. In recent years, allergic asthma has become a heated public health problem because of the highly increased incidence and prevalence[2]. The main pathogenesis of asthma is airway inflammation and AHR. AHR is caused by an imbalance of Th1/Th2 cells with predominance to Th2, whereas airway inflammation is caused by increased levels of Th2 cytokine[3]. Major effects of pathological changes in the respiratory include eosinophil infiltration, bronchial epithelial cell damage, obstruction of the mucus plug, and thickening of the submucosal muscle layer[4,5].

Allergic diseases can be caused by pollen, which is an important source of airborne allergens[6]. *Populus deltoides* is widely cultivated in most parts of China because of its urban greening, windbreak, and sand-fixing berm. In April and May of each year, mature pollen of *P. deltoides* is densely suspended in the air, which not only causes public hazards, such as fire, but also frequently contact people's eyes, nostrils, mouth, and skin, which leads to tearing, sneezing, itching, and other uncomfortable symptoms. Previous studies have proved that allergens may exist in the pollen of *P. deltoides*[7]. However, the biological activity of this pollen in sensitization remains largely unknown.

During sample collection, the maximum pollen of *P. deltoides* concentration under local natural conditions was 0.5 μg/mL. Pre-experiments could mean that the minimum- and half-effective concentrations (EC50) of pollen of *P. deltoides* extract (PDPE) were 0.3976 and 0.42048 μg/mL, respectively. Moreover, PDPE was used to sensitize mice and challenge them with different concentrations (PDPE group 0.5 μg/mL and high-dose group 2 μg/mL). Finally, the biochemical parameters of the mice were detected, including IgE antibodies, Th1/Th2 cells, as well as their cytokine. This could provide theoretical support for the prevention and diagnosis of pollen-induced allergic diseases.

Acquired experimental results showed that the levels of IgE antibodies in the PDPE group (15.98 ± 5.31 ng/mL) and the high-dose (hd) group (26.25 ± 10.12 ng/mL) were significantly increased compared with that in the phosphate buffer saline (PBS) group (7.03 ± 3.03 ng/mL, $P < 0.01$). Meanwhile, compared with the PDPE group (135.37 ± 19.88 pg/mL), PDPE significantly increased the levels of IL-4 in the hd group (264.82 ± 60.53 pg/mL, $P < 0.01$), and the levels in the PBS group (70.97 ± 19.87 pg/mL) were lower than those in the PDPE group ($P < 0.05$). The level of interferon (IFN)-γ in the PDPE group (130.22 ± 17.09 pg/mL) was lower than that in the PBS group (185.71 ± 52.40 pg/mL, $P < 0.05$). In addition, the IL-5 level in the PDPE group (97.59 ± 36.57 pg/mL) increased significantly compared with that in the PBS group (11.54 ± 8.40 pg/mL, $P < 0.01$) but was lower than the level in the hd group (239.28 ± 33.84 pg/mL, $P < 0.01$). The level of IL-13 in the PDPE group (90.34 ± 31.07 pg/mL) was lower than that in the hd group (285.03 ± 71.77 pg/mL, $P < 0.01$), and the level in the PBS group (18.78 ± 13.52 pg/mL) was lower than that in the PDPE group ($P < 0.01$). Compared with the PDPE group (128.06 ± 21.36 pg/mL), the level of tumor necrosis factor (TNF)-α in the hd group (221.24 ± 45.57 pg/mL) was significantly increased ($P < 0.05$, Figure 1). IgE is the main mediator of allergic diseases. Interleukin (IL)-4, 5, and 13 are cytokines secreted by Th2 cells, and as TNF, they are important pro-inflammatory factors. IFN-γ is an immune regulator secreted by Th1 cells. Hence, we perceive that PDPE can stimulate the

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immune system to produce IgE and increase the secretion of Th2 cytokines in the allergic asthma mouse model.

To determine whether PDPE stimulates the proliferation of $CD4^+$ T cells and causes Th2 immune response, flow cytometry was used to detect the number of $CD4^+$ T cells and subsets in spleen cells. Compared with the PBS group (12.54% ± 0.76%), PDPE significantly promoted the proliferation of $CD4^+$ T cells ($P < 0.01$). Regarding the Th1/Th2 ratio of $CD4^+$ T subgroups, compared with the PBS group (2.26 ± 0.14), the PDPE group (2.01 ± 0.28) and the group (1.06 ± 0.32) ratios were significantly decreased ($P < 0.01$, Supplementary Figure S1 available in www.besjournal.com). From these results, PDPE was able to produce Th2-sensitized lymphocyte proliferation, which was consistent with the typical cytological characteristics of allergic inflammation in the lung. Meanwhile, PDPE was suggested to possess immunogenicity. These results were consistent with those obtained by enzyme-linked immunosorbent assay. Moreover, western blot analysis revealed that IgE antibodies can be specifically bound by PDPE between 40 and 100 kD (Figure 2). Therefore, PDPE has immunoreactivity, which could provide a reference for the diagnosis of allergic diseases caused by the pollen of *P. deltoides*.

To observe the pathological features of PDPE-sensitized lung, mouse lung tissue was removed and fixed with formalin. From a macro perspective, the PDPE group had obvious pulmonary hyperemia, which is dark red, with hyperplasia of the hilar connective tissue and enlarged lymph nodes. In addition, the lungs of the hd group had more distinct pulmonary hyperemia and many hemorrhagic spots in the pleura (Supplementary Figure S2 available in www.besjournal.com). The pathological section of the lung tissue showed that in the PBS group, the capillary endothelium of mouse lung tissue was intact, and there was no obvious edema in the vascular muscle layer, the alveolar structure was intact, the bronchiolar ciliated columnar epithelial cells were intact, and there was no obvious proliferation of goblet cells. However, PDPE significantly damaged the capillary endothelium in the lungs of mice, and edema occurred in vascular myometrial hyperplasia, destruction of the alveolar septum led to an irregular alveolar cavity, ciliated

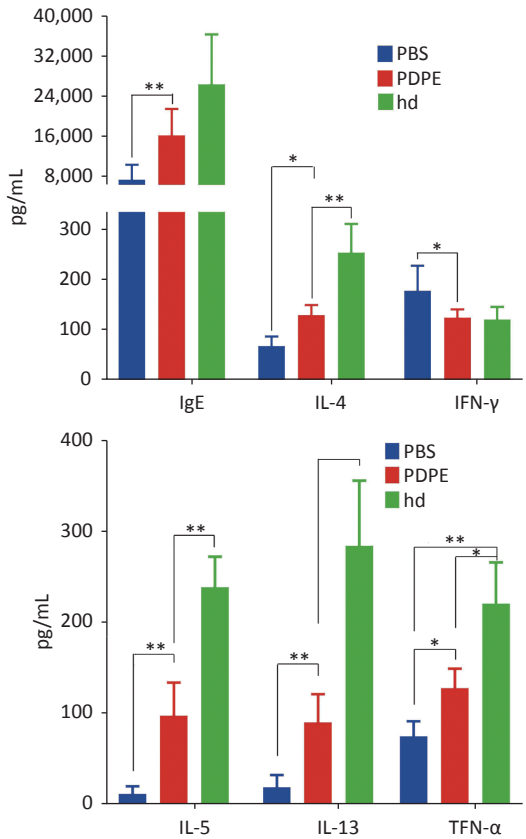

Figure 1. Effect of PDPE on the expression of antibodies and cytokines. PDPE significantly increased the IgE, IL-4, IL-5, IL-13, and TNF-α and decreased the expression of IFN-γ. The vertical axis represents the concentration of antibodies or cytokines. Values are presented as the mean ± SD. $^*P < 0.05$; $^{**}P < 0.01$. PDPE, pollen of *P. deltoides* extract; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

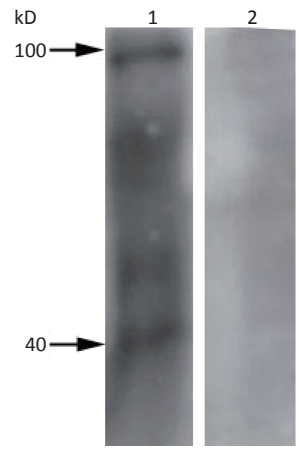

Figure 2. PDPE binds to serum IgE antibodies from PDPE-sensitized mice. Lane 1, incubation with PDPE mouse serum. Lane 2, incubation with the PBS mouse serum. PDPE, pollen of *P. deltoides* extract; PBS, phosphate buffer saline.

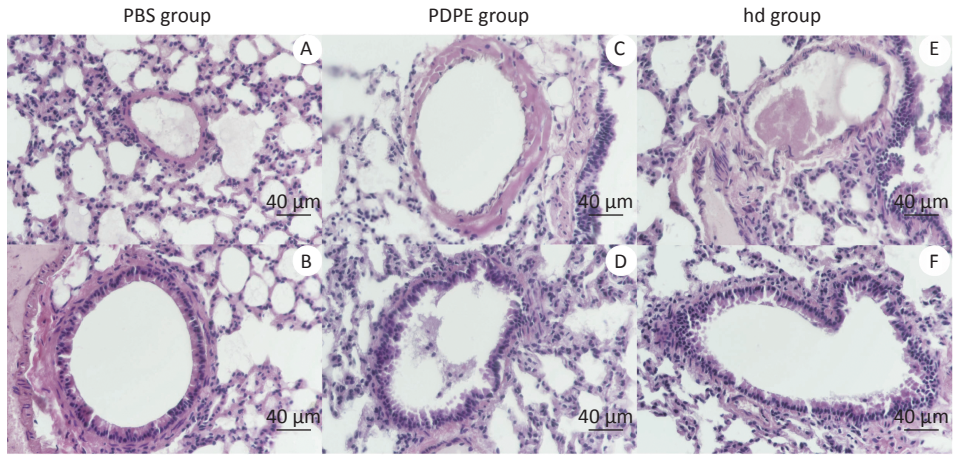

Figure 3. PDPE induces inflammation and allergic pathological features in lung tissue. (A, B) PBS group. (C, D) PDPE group. (E, F) hd group. PDPE, pollen of *P. deltoides* extract; PBS, phosphate buffer saline; Hd, high-dose group.

columnar epithelial cells showed disordered arrangement, and some goblet cells fell off from the basement membrane, accompanied by mucus exudation and inflammatory cells. In the hd group, obvious capillary congestion and thrombosis were observed. The terminal bronchus was collapsed with mucous exudation, and the thickening of the alveolar septum was more evident (Figure 3). Further examination of bronchoalveolar lavage fluid showed that the inflammatory cells included lymphocytes, neutrophils, eosinophils (Supplementary Figure S3 available in www.besjournal.com), and monocytes. Eosinophils are the main effector cells of airway inflammation[8], suggesting the existence of allergic inflammation in mice lung tissue. After cell counting, the number of inflammatory cells in the hd group was significantly higher than that in the other groups ($P < 0.05$, Supplementary Figure S4 available in www.besjournal.com).

The incidence and prevalence of allergic diseases depend on the concentration of allergens and the duration of exposure. In north China, the incidence of atopic diseases caused by pollen of *P. deltoides* is high, ranging from 21.3% to 37.5%; allergic rhinitis was the main allergic disease, followed by asthma[9,10]. From this study, the biological activity of PDPE and the pathological characteristics of lung tissues in sensitized mice could be systematically obtained, which can provide a reference for the prevention and treatment of allergic diseases.

The authors declare no conflict of interest.

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GUO Wei and XI Yi Long conceived the idea and wrote the manuscript. JIANG Yu Xin and ZHAN Xiao Dong depicted figures and analyzed data. JIANG Feng contributed to the revision. XI Yi Long contributed to overall editing and supervision. All authors approved its submission.

Statement of Ethics This study was conducted in strict accordance with the proposals of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and the mice were reviewed and approved by the Institutional Animal Care and Use Committee of Hangzhou Hibio Technology Co.Ltd. (IACUC protocol no. HBFM3.68-2015). The pollen of *P. deltoides* used in this study was collected at the Yangtze River embankment and conducted in strict accordance with the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

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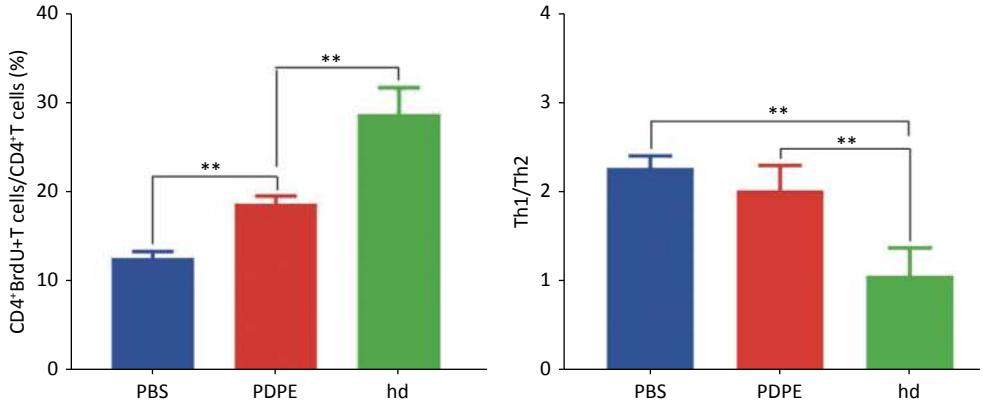
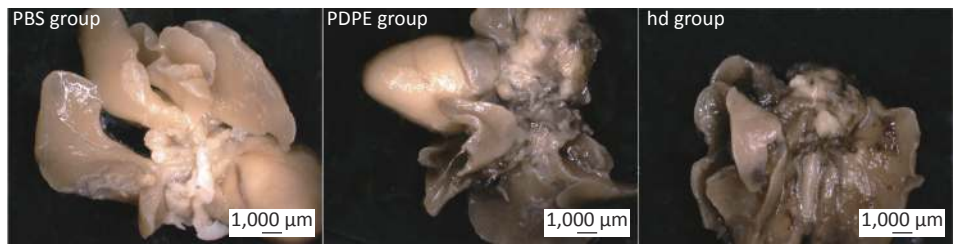
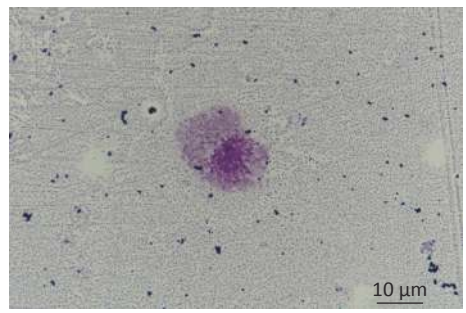


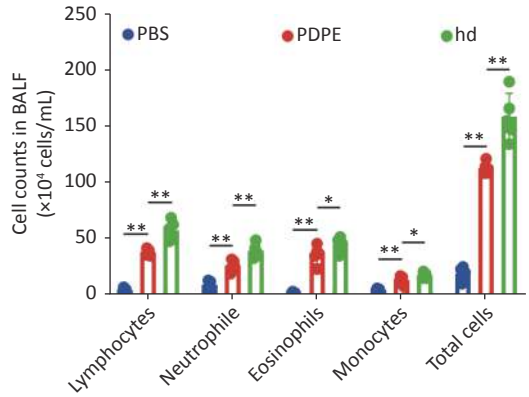
Supplementary Figure S1. The proportion of $CD4^+$ T cells and their subsets. $^{**}P < 0.01$.



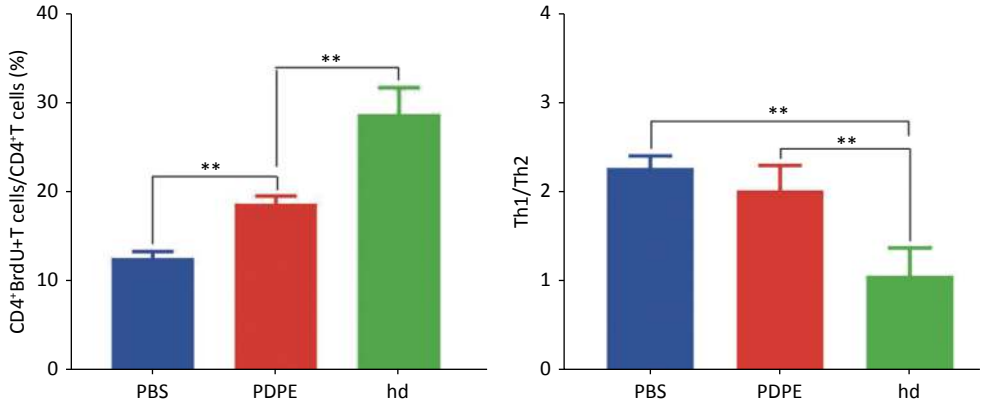
Supplementary Figure S2. The appearance of lung tissue in each experimental group.



Supplementary Figure S3. Eosinophils in bronchoalveolar lavage fluid in HD group.



Supplementary Figure S4. Count of inflammatory cells in bronchoalveolar lavage fluid in each experimental group. $^{*}P < 0.05$; $^{**}P < 0.01$.



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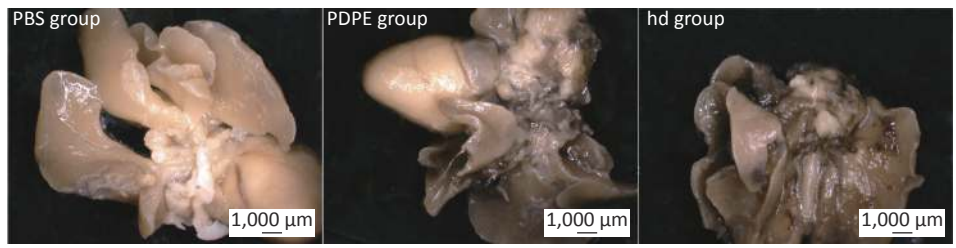

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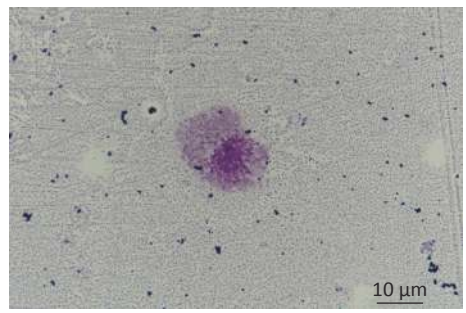

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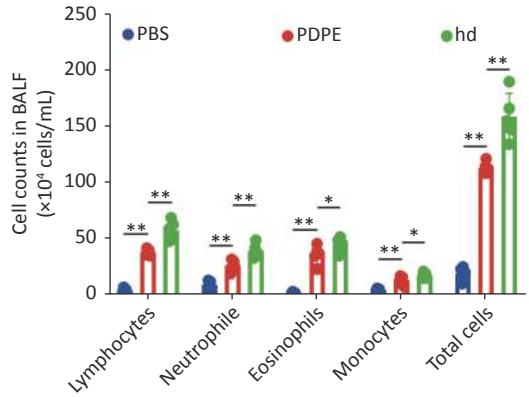

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