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Lactobacillus casei HY2782 and *Pueraria lobata* Root Extract Complex Ameliorates Particulate Matter-Induced Airway Inflammation in Mice by Inhibiting Th2 and Th17 Immune Responses

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ABSTRACT: This study aimed to investigate the effects of *Lactobacillus casei* HY2782 and *Pueraria lobata* root extract complex (HY2782 complex) in mitigating airway inflammation resulting from exposure to particulate matter ≤2.5 μm in diameter (PM2.5) in an animal model. Chronic inflammatory airway disease is associated with Th2-related cytokines interleukin (IL)-4, IL-5, and IL-13 and Th17-related cytokine IL-17A, which are the major contributors to allergy and asthma. Results indicated that PM2.5 elevates allergen-related airway inflammation and respiratory hyperresponsiveness in C57BL/6 mice. The HY2782 complex significantly reduced Th2/Th17-derived cytokines IL-4, IL5, IL-13, and IL-17A; immunoglobulin E; and leukotriene C_4 in bronchoalveolar lavage fluid (BALF) and serum. Furthermore, the HY2782 complex was associated with the modulation of oxidative stress-related genes. Administration of the HY2782 complex resulted in a markedly reduced number of neutrophils and eosinophil infiltration in BALF. Histopathological observation of lung tissue also showed reduced inflammatory cell infiltration into airways and surrounding tissue. The HY2782 complex may be a promising candidate for the preventive therapy of allergic diseases and airway inflammation caused by PM2.5 inhalation.

Keywords: airway inflammation, *Lactobacillus*, oxidative stress, *Pueraria lobata*, particulate matter

INTRODUCTION

Air pollution comprises a complex mixture of organic and inorganic substances as particles, such as fine dust, pollen, soot, smoke, liquids, and particulate matter (PM). In developing countries, hazardous air pollutants result from rapid industrial development and economic growth. Recent studies have documented chronic and acute exposure to PM, specifically fine particles with aerodynamic particle diameters of ≤2.5 μm (PM2.5) in the atmosphere. The toxicity caused by PM2.5 is a combined effect of particles and toxic pollutants adsorbed to the particles, such as biological components (bacteria, fungi, and viruses and their metabolites), volatile organic compounds [benzene, toluene, and polycyclic aromatic hydrocarbons (PAHs)], and heavy metals (Shen et al., 2018). PM2.5 is one of the most harmful pollutants to human health because it can even penetrate the alveoli and bronchi and cause airway inflammation and respiratory diseases (Liu et al., 2021a). Inhalation of PM2.5 is associated with many respiratory tract diseases, such as allergic rhinitis, airway hyperresponsiveness (AHR), asthma, and chronic obstructive pulmonary disease (COPD; Li and Liu, 2021). In a mouse model, PM2.5 could induce allergic airway inflammation in mice potentially hypersensitive to mite allergens (Ogino et al., 2014).

The classic view of allergic airway inflammation is that of Th2 cell-related airway inflammation, Th2 cell-related interleukin (IL)-4, IL-5, IL-13, and total immunoglobulin E (IgE), as high levels of eosinophil are observed. However, PM induces new molecular and cellular mediators, such as Th17 cells and its signature cytokine IL-17A, in airway inflammation and pulmonary inflammation of PM-induced mice (Li et al., 2010; Cong et al., 2020). A subset of $CD4^+$ Th17 cells that produce IL-17A plays an important role in the aggravation of asthma symptoms. Importantly, asthmatic patients with overexpression of neutrophils and IL-17A have the worst asthma and the lowest lung function control compared to other inflammatory endotypes (Hirose et al., 2017; Crawford et al., 2020).

Bronchodilators and anti-inflammatory drugs, such as

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dexamethasone, are currently used in treating respiratory diseases, but they are accompanied by serious side effects, such as renal dysfunction and gastrointestinal disorders. Therefore, alternative medicines that use natural products with few side effects are needed (Mahemuti et al., 2018; Kim et al., 2021). Natural products are important sources of new drugs against various pathological conditions. Almost every part of *Pueraria lobata* has been used by Asians for various purposes. For example, the leaves, buds, and sprouts have been consumed as tea and juice in Korea. Its tuberous roots have been utilized as a source of starch, which is an essential material for cooking, and used as food in Japan. To date, *P. lobata* is one of the most popular herbal plants in traditional oriental medicine (Tungmunnithum et al., 2020). *P. lobata* root extract (PRE) has a variety of pharmacological properties. Puerarin is a natural compound isolated from PRE and is used as an antioxidant for treating apoptosis, hyperglycemia, ischemia, and vasodilation of blood vessels; puerarin also acts as a platelet coagulation inhibitor (Zhou et al., 2014).

Lactobacillus species may be helpful probiotics. Several studies have concluded that *Lactobacillus paracasei* may prevent or reduce asthmatic symptoms (Wang et al., 2017b; Lin et al., 2020). The oral administration of *Lactobacillus gasseri* can attenuate allergen-induced airway inflammation in mice by inhibiting the Th17 proinflammatory response (Jan et al., 2012). A previous study confirmed that *Lactobacillus casei* HY2782 is effective against PM toxicity in human intestinal cells and a *Caenorhabditis elegans* model (Kim et al., 2020) and could improve lung inflammation in a PM2.5-induced COPD mouse model (Nam et al., 2020). In addition, the HY2782 complex ameliorates ovalbumin (OVA)-induced AHR in mice (Nam et al., 2021).

The most popular animal model for airway inflammation is created via sensitization with OVA (Ballantyne et al., 2007; Yao et al., 2014). However, the use of OVA as an allergen is less suitable for investigating pollution and PM-induced airway inflammation (Lee et al., 2021). This study aimed to explore the synergistic effects of the HY2782 complex against airway inflammation and AHR using a PM2.5-induced mouse model and gain insights into the mechanism by which the HY2782 complex modulates the immune system.

MATERIALS AND METHODS

Reagents and equipment

Diesel PM (#NIST1650B) and dexamethasone (#D2915) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Mouse IL-4 (#M4000B) and IL-5 (#M5000) Quantikine™ enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems (Minneapolis, MN, USA). Mouse IL-13 (#BMS6015) and IgE (#88-50460-88) ELISA kits were purchased from Invitrogen (Waltham, MA, USA). Mouse IL-17A (#ab199081) ELISA kits were purchased from Abcam (Cambridge, UK). Mouse leukotriene C_4 (LTC_4; #mbs731784) ELISA kits were purchased from MyBioSource (San Diego, CA, USA). Easy-spin RNA kits (#17221) were obtained from iNtRON Biotechnology (Seongnam, Korea). Lysing Matrix D (#6913-500) was purchased from MP Biomedicals (Santa Ana, CA, USA). Fastprep-24 (MP Biomedicals) was used for the experiments. Omniscript reverse transcriptase (RT) kits were obtained from Qiagen (Hilden, Germany). Gene Expression Master Mix (#4639016) was purchased from Applied Biosystems (Foster City, CA, USA). QuantStudio™ 6 Flex Real-time Instrument (Thermo Fisher Scientific, Waltham, MA, USA) was used for the experiments.

Preparation of PRE

P. lobata root was purchased from Humanherb Co., Ltd. (Daegu, Korea). Dried *P. lobata* root was mixed by weight with water at a ratio of 1:15 and extracted for 16 h at 95°C. After filtration, the filtrate was concentrated to 15±2°Bx. PRE was made into a powder using a spray dryer. The prepared PRE was stored at −20°C until use.

Preparation of *L. casei* HY2782

L. casei strain HY2782 was obtained from hy Co., Ltd. (Seoul, Korea). *L. casei* HY2782 was inoculated into a sterilized medium at 37°C for 24 h. After incubation, the collected cells of *L. casei* HY2782 were made into a powder using a freeze dryer, and the number of bacteria was calculated by plating a dilution series and counting the colonies. The prepared *L. casei* HY2782 was stored at −20°C until use.

Animals

C57BL/6 mice were purchased from DooYeol Biotech (Seoul, Korea) at age 6 weeks and housed in the animal laboratory (22±2°C, relative humidity of 50±20%, 12-h light/dark cycle, nonspecific pathogen-free, and fed a standard diet with ultraviolet sterilizer). The experimental procedures were approved by the Ethics Review Committee of hy Co., Ltd. (Yongin, Korea; approval no. AEC-2021-0008-Y).

Administration to animals

The AHR mouse model was generated according to the method described by Nam et al. (2020). Briefly, male C57BL/6 mice were randomly divided into five groups of 8 mice each: (1) control group (Con), (2) PM2.5-induced group (PM), (3) *L. casei* HY2782-administered group (HY2782), (4) combination of *L. casei* HY2782 and PRE-

Table 1. Gene names and symbols used in gene expression analysis

Gene symbol	Gene name	Catalog no.	Reference sequence
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Mm99999915_g1	NM_021283.2
SOD1	Superoxide dismutase 1	Mm01344233_g1	NM_011434.1
CAT	Catalase	Mm00437992_m1	NM_009804.2
AhR	Aryl hydrocarbon receptor	Mm00478932_m1	NM_013464.4
Nrf2	Nuclear factor erythroid 2-related factor 2	Mm00477784_m1	NM_010902.3

administered group (HY2782 complex), and (5) dexamethasone-treated group (Dex). During the 7-day acclimation period, mice were fed the AIN-93G diet. All groups, except the Con group, were intranasally instilled with PM2.5. The test samples were obtained from all groups fed by the diet. PM was administered intranasally at 200 μg/20 μL for 28 days to induce lung disease in mice. PM was suspended in phosphate-buffered saline (PBS) at 10 mg/mL and sonicated at 60°C for 12 h. The experimental group was fed the AIN-93G diet containing *L. casei* HY2782 [1×10^8 colony forming unit (CFU)/kg/d], and a combination of HY2782 (1×10^8 CFU/kg/d) and PRE (10 mg/kg/d). Dexamethasone was administered via oral gavage (0.5 mg/kg/d). After 28 days, the mice were sacrificed, and bronchoalveolar lavage fluid (BALF), blood, and lungs were extracted. The organs and blood were stored at −80°C until used for cytokine and gene expression analyses.

Analysis of cell composition in BALF

BALF was collected from mice immediately after euthanasia, as described previously (Nam et al., 2021). Bronchial sections of the sacrificed mice were cut vertically in half. A feeding needle was inserted for oral administration, and 1 mL PBS was added and collected again. The collected BALF was centrifuged at 1,500 *g* for 10 min at 4°C, and the supernatants were used for cytokine analysis. The sediments suspended in PBS were used to assess immune cell composition. The resuspended cells were analyzed using a Mindray BC-5000 Vet (Shenzhen Mindray Animal Medical Technology Co., LTD., Shenzhen, China). The number of cells was expressed as 1×10^3 cells/mL.

Analysis of cytokines by ELISA

The cytokines secreted in BALF were analyzed using an ELISA kit. To separate serum, blood from mice was kept at 25°C for 30 min and centrifuged at 2,000 *g* for 10 min at 4°C. The subsequent steps to detect IL-4, IL-5, IL-13, IL-17A, IgE, and LTC_4 were performed according to the ELISA kit instructions.

Analysis of gene expression in lung tissue

Total RNA was extracted using an Easy-spin RNA kit. Lung tissue and lysis buffer were added to Matrix D tubes and pulverized with a FastPrep-24 instrument. After grinding the tissue, the Easy-spin RNA kit protocol was followed. Total RNA was stored at −20°C until gene expression analysis. The extracted RNA was reverse-transcribed into cDNA using the Omniscript RT Kit. cDNA was amplified with Gene Expression Master Mix and TaqMan Probe in a QuantStudio™ 6 Flex Real-time Instrument. Table 1 lists the names and catalog numbers of these genes.

Histopathological analysis

Briefly, lung tissue was fixed in 10% formalin solution and used to prepare hematoxylin and eosin (H&E)-stained slides by Korea Productivity Center (Gwangju, Korea). The epithelial thickness of the bronchial tube was measured using an Olympus CK2 microscope (Olympus Corporation, Tokyo, Japan) at 400× magnification. The thickness of the respiratory epithelium was analyzed using ImageJ (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

Data are presented as the mean±standard deviation of independent experiments. Statistically significant differences between groups were determined using the unpaired Student's *t*-test in SPSS (version 26.0, IBM Corp., Armonk, NY, USA). Comparisons were considered statistically significant at $P<0.05$ or $P<0.01$ compared to the PM group and $P<0.05$ or $P<0.01$ compared to the Con group.

RESULTS

Administration of the HY2782 complex inhibits increased inflammatory cytokine levels in BALF and serum of PM2.5-induced mice

Stimulation with PM2.5 promoted the production of inflammatory factors in the respiratory tract. The secretion of IL-4, a key Th2-related cytokine associated with airway inflammation, increased to 39.17±7.83 pg/mL in BALF of the PM-exposed group, whereas its levels were 18.59±5.90 pg/mL in the Con group. However, HY2782 intake decreased IL-4 levels to 27.17±12.43 pg/mL, and HY2782 complex treatment significantly decreased IL-4 levels to 17.43±7.27 pg/mL (Fig. 1A). IL-5 secretion increased to 21.90±4.15 pg/mL in BALF of the PM-exposed group,

whereas its levels were 7.40±2.62 pg/mL in the Con group. However, HY2782 intake decreased IL-5 levels to 13.21±4.68 pg/mL, and HY2782 complex treatment significantly decreased IL-5 levels to 8.75±4.19 pg/mL (Fig. 1B). IL-13 secretion increased to 43.82±11.68 pg/mL in BALF of the PM-exposed group, whereas its levels were 21.42±7.48 pg/mL in the Con group. However, HY2782 intake decreased IL-13 levels to 33.71±7.62 pg/mL, and HY2782 complex treatment significantly decreased IL-13 levels to 22.42±6.92 pg/mL (Fig. 1C). The secretion of IL-17A, a key Th17-related cytokine in airway inflammation, increased to 9.60±3.10 pg/mL in BALF of the PM-exposed group, whereas its levels were 3.96±0.36 pg/mL in the Con group. However, HY2782 intake decreased IL-17A levels to 4.71±2.91 pg/mL, and HY2782 complex treatment significantly decreased IL-17A levels to 3.36± 1.97 pg/mL, but the decrease due to dexamethasone treatment was not significant (P=0.6) at 7.97±3.04 pg/mL (Fig. 1D). This result was confirmed through the analysis of serum levels.

IL-4, IL-5, IL-13, and IL-17A secretion (Fig. 2) in serum showed the same pattern. IL-4, IL-5, IL-13, and IL-17A cytokine expression levels in BALF and serum of the HY2782 complex group were significantly decreased compared to those in PM2.5-induced mice. Results suggested that the HY2782 complex decreased the inflammatory response by modulating the Th2 and Th17 immune responses in PM2.5-exposed mice, whereas treatment with dexamethasone failed to decrease IL-17A levels. Thus, administration of the HY2782 complex attenuated systemic Th2 and IL-17A inflammatory responses and AHR induced by PM2.5 exposure.

Administration of the HY2782 complex inhibits increased IgE and LTC_4 levels in PM2.5-induced mice

Systemic allergic responses were also attenuated after HY2782 intake. In Fig. 3A and 3B, IgE secretion after HY2782 intake significantly decreased to 168.76±45.52 ng/mL in BALF and 565.59±156.16 ng/mL in serum compared to levels in the PM group (295.69±81.46 ng/mL in BALF and 853.59±258.13 ng/mL in serum). Moreover, HY2782 complex treatment significantly decreased

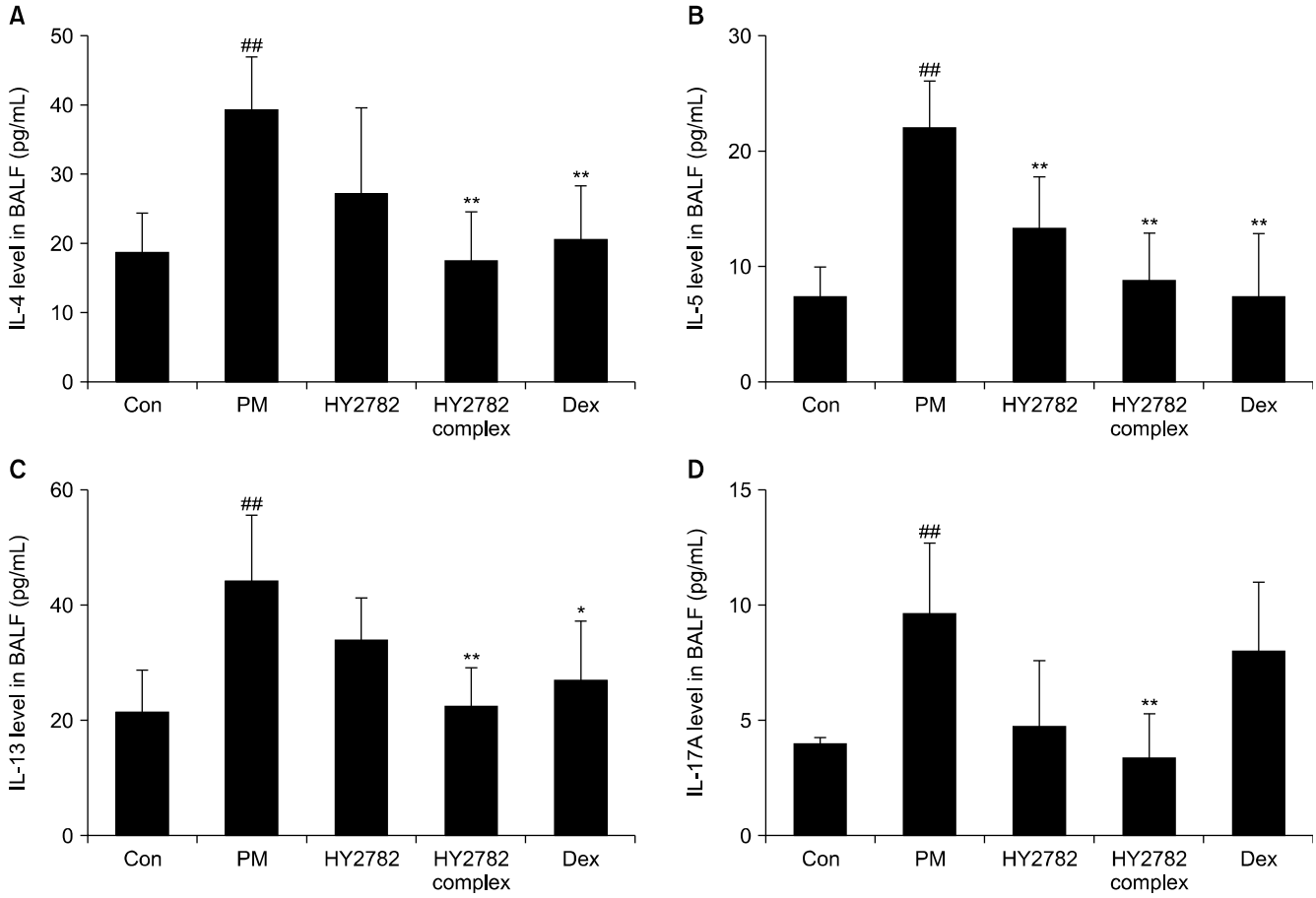

Fig. 1. Particulate matter (PM) 2.5 exposure exacerbated the airway inflammation response and increased cytokine levels in bronchoalveolar lavage fluid (BALF). BALF was collected and analyzed to determine interleukin (IL)-4, IL-5, IL-13, and IL-17A secretion. Increased secretion of IL-4 (A), IL-5 (B), IL-13 (C), and IL-17A (D) in BALF of mice after 28 days of PM2.5 exposure. Data are represented as the mean±SD (n=6 mice per group). $^{\#\#}P<0.01$ compared to the Con group; $^{*}P<0.05$, $^{**}P<0.01$ compared to the PM group. Con, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus casei* HY2782-fed PM group; HY2782 complex, *L. casei* HY2782 and PRE-fed PM group; Dex, dexamethasone-treated PM group.

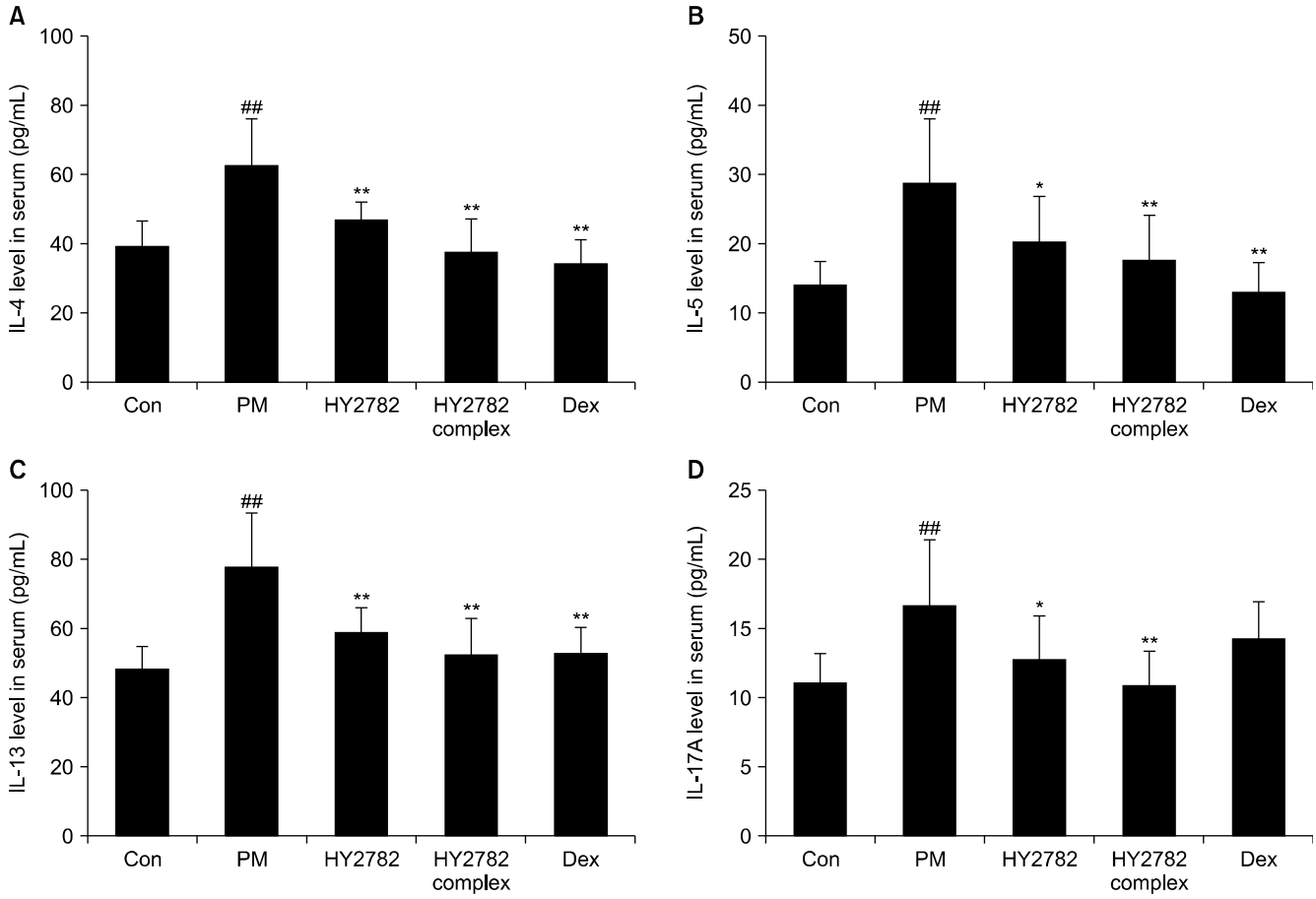

Fig. 2. Particulate matter (PM) 2.5 exposure exacerbated the systemic and airway inflammation response and increased cytokine levels in serum. Serum was collected and analyzed to determine interleukin (IL)-4, IL-5, IL-13, and IL-17A secretion. Increased secretion of IL-4 (A), IL-5 (B), IL-13 (C), and IL-17A (D) in the serum of mice after 28 days of PM2.5 exposure. Data are represented as the mean±SD (n=6 mice per group). $^{\#\#}P<0.01$ compared to the Con group; $^{*}P<0.05$, $^{**}P<0.01$ compared to the PM group. Con, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus casei* HY2782-fed PM group; HY2782 complex, *L. casei* HY2782 and PRE-fed PM group; Dex, dexamethasone-treated PM group.

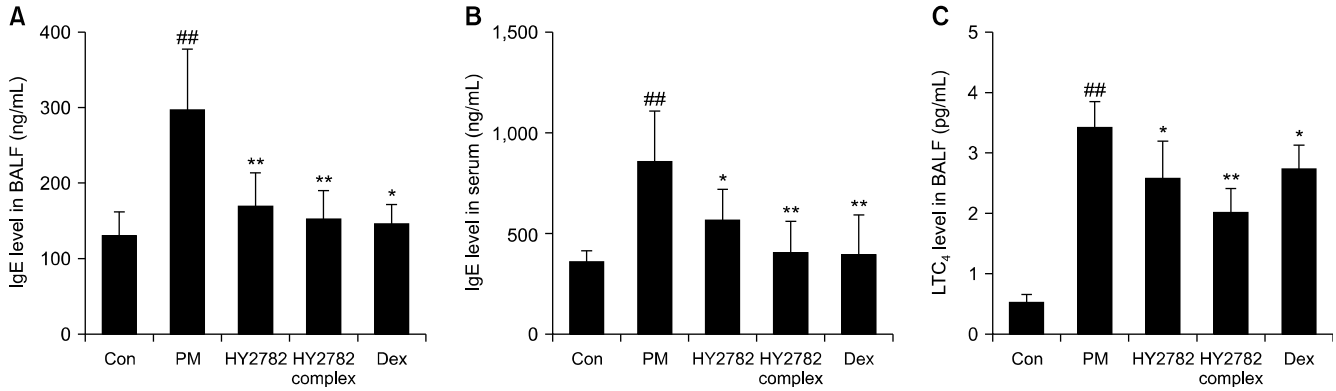

Fig. 3. Airway inflammation response related to immunoglobulin E (IgE) secretion in bronchoalveolar lavage fluid (BALF) and serum and leukotriene C_4 (LTC_4) secretion in BALF. IgE secretion in BALF (A) and serum (B) and LTC_4 secretion in BALF (C). Data are represented as the mean±SD (n=6 mice per group). $^{\#\#}P<0.01$ compared to the Con group; $^{*}P<0.05$, $^{**}P<0.01$ compared to the PM group. Con, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus casei* HY2782-fed PM group; HY2782 complex, *L. casei* HY2782 and PRE-fed PM group; Dex, dexamethasone-treated PM group.

IgE levels to 151.40±39.23 ng/mL in BALF and 404.65±162.31 ng/mL in serum. In immune cells stimulated by IL-4, LTC_4 secretion increased. LTC_4 is a representative AHR trigger. LTC_4 secretion in BALF of PM-exposed mice increased (3.39±0.47 pg/mL) compared to that in the Con group (0.52±0.16 pg/mL). However, HY2782 intake decreased LTC_4 levels to 2.57±0.64 pg/mL, and HY2782 complex treatment significantly decreased LTC_4 levels to 2.01±0.41 pg/mL (Fig. 3C).

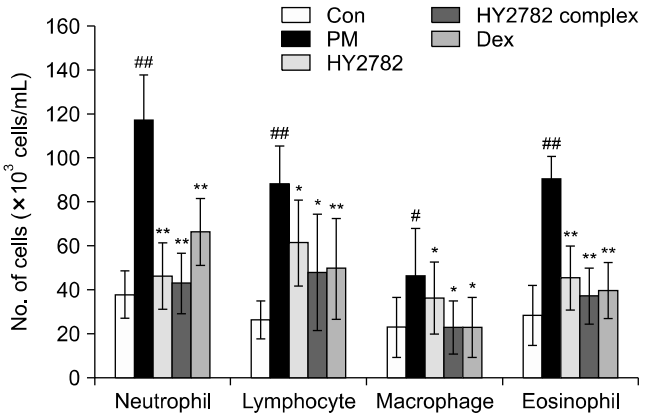

Fig. 4. Immune cell composition in bronchoalveolar lavage fluid. Immune cell count as influenced by particulate matter (PM) 2.5 exposure in control and experimental groups. Data are represented as the mean±SD (n=6 mice per group). $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared to the Con group; $^{*}P<0.05$, $^{**}P<0.01$ compared to the PM group. Con, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus casei* HY2782-fed PM group; HY2782 complex, *L. casei* HY2782 and PRE-fed PM group; Dex, dexamethasone-treated PM group.

Administration of the HY2782 complex reduces immune cell composition in BALF after PM2.5 exposure

To investigate the anti-inflammatory effects of the HY2782 complex on the lungs, mice were induced with PM2.5, and immune cells in BALF were counted. The number of neutrophils, lymphocytes, macrophages, and eosinophils in BALF significantly increased in the PM2.5 group compared to that in the Con group (Fig. 4). The number of neutrophils and eosinophils in BALF was $117.33\pm20.47\times10^3$ and $90.62\pm10.33\times10^3$ cells/mL in the PM group, which decreased to $46.67\pm15.06\times10^3$ and $45.83\pm14.55\times10^3$ cells/mL, respectively, after HY2782 intake ($P<0.01$). The number of neutrophils and eosinophils significantly decreased to $43.33\pm13.66\times10^3$ and $37.670\pm12.75\times10^3$ cells/mL, respectively, in the HY2782 complex group ($P<0.01$) compared to that in the PM group. The ingestion of the HY2782 complex reduced immune cell recruitment in the respiratory tract (Fig. 4).

Administration of the HY2782 complex ameliorates oxidative stress in lung tissue induced by PM2.5 exposure

The nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant response pathway plays an important role in responding to PM-induced oxidative stress. The relative gene expression of *Nrf2* significantly decreased (0.54±0.12) in PM2.5-exposed mice. *Nrf2* expression increased (0.81±0.06) after HY2782 ingestion and significantly increased (1.35±0.21) after ingestion of the HY2782 complex (Fig. 5A). Furthermore, aryl hydrocarbon receptor (AhR), which is related to the AhR-Nrf2 pathway, is involved in antioxidant response. In PM2.5-exposed mice, the *AhR* expression levels significantly increased (1.75±0.21) but decreased after HY2782 ingestion (1.27±0.10) and significantly decreased (0.88±0.16) after ingestion of the HY2782 complex (Fig. 5B). Another key antioxidant enzyme gene is *Cu/Zn superoxide dismutase 1* (*SOD1*), the expression levels of which decreased (0.71±0.14) in PM2.5-exposed mice but increased after HY2782 ingestion (1.19±0.15) and significantly increased (1.45±0.17) after ingestion of the HY2782 complex (Fig. 5C). *Catalase* (*CAT*) showed the same pattern as *SOD1* (Fig. 5D).

Overall, the consumption of the HY2782 complex increased the levels of antioxidant factors, such as SOD1 and CAT, for the regulation of oxidative stress after PM2.5 exposure. Furthermore, increased levels of Nrf2 have been shown to reduce oxidative stress-related factors such as the AhR response.

Administration of the HY2782 complex alleviates PM2.5-induced lung inflammation

Through histopathology, intranasal inoculation with PM 2.5 alone induced a severe inflammatory response in lung tissue, as represented by inflammatory cell infiltration into the bronchiole epithelium and surrounding tissue (Fig. 6A). The thickness of the respiratory epithelium and inflammatory cell infiltration in lung tissue were exacerbated in PM2.5-exposed mice. In the PM group, the thickness increased to 36.10±10.75 μm but decreased to 27.97±6.77 μm after HY2782 intake ($P<0.05$), and it significantly decreased to 21.61±4.10 μm after intake of the HY2782 complex ($P<0.01$; Fig. 6B). These results suggest that the HY2782 complex has a positive effect on reducing the PM2.5-exposed airway inflammatory response in that the HY2782 complex alleviates PM2.5-induced signs of bronchiolitis.

DISCUSSION

Many studies have reported that PM is associated with negative effects on human health and has a relationship with respiratory disease. Because of the small size of PM 2.5, it can penetrate deep into lung alveoli and potentially into the bloodstream (Zhao et al., 2020). In addition, representative components of PM, such as heavy metals and PAHs, trigger the production of reactive oxygen species (ROS), an important inflammatory mediator in various inflammatory responses (Robinson et al., 2018). Most allergic disorders disrupt the Th1/Th2 balance, resulting in a more active Th2 immune response and high levels of Th2 cytokines, such as IL-4, IL-5, and IL-13, thus increasing IgE production (Gavett et al., 2003). For example, Th2 cells produce cytokines that play important roles downstream in the pathogenesis of asthma (Herath et al., 2020). Airway epithelial cells play a pivotal role in causing asthma symptoms and impaired epithelial barrier function in relation to Th2/Th17 responses (Lambrecht

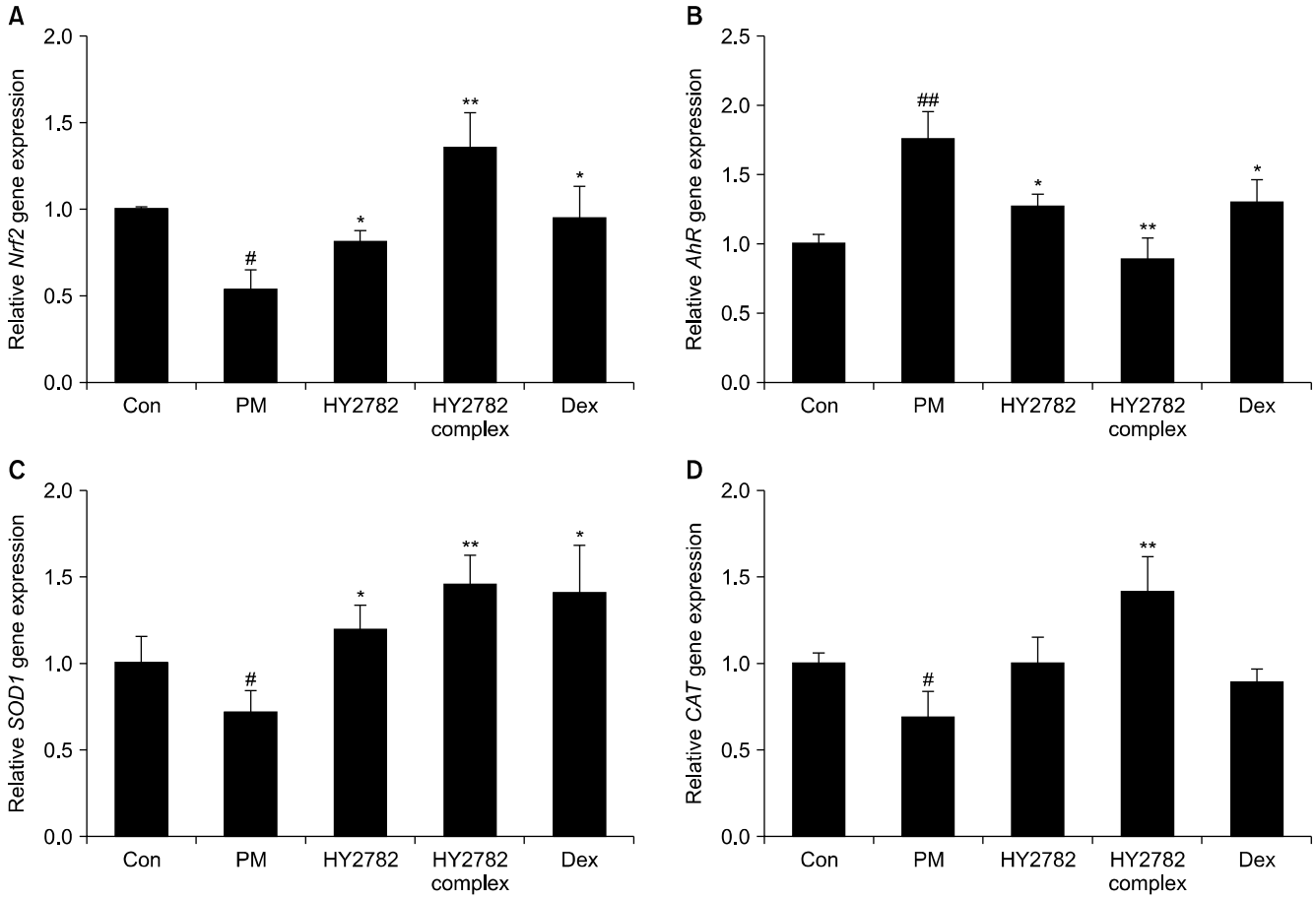

Fig. 5. Oxidative stress-related gene expression levels in lung tissue. *Nrf2* (A), *AhR* (B), *SOD1* (C), and *CAT* (D) expression levels in particulate matter (PM) and experimental groups. Data are represented as the mean±SD (n=6 mice per group). $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared to the Con group; $^{*}P<0.05$, $^{**}P<0.01$ compared to the PM group. Con, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus casei* HY2782-fed PM group; HY2782 complex, *L. casei* HY2782 and PRE-fed PM group; Dex, dexamethasone-treated PM group.

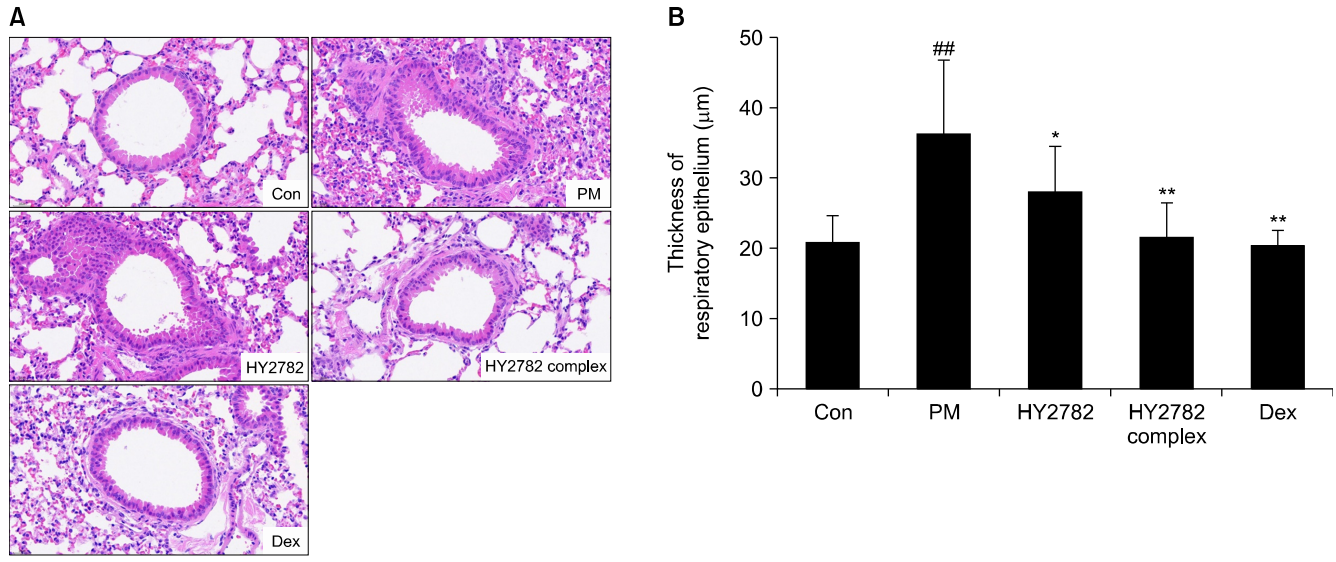

Fig. 6. Histopathology of lung tissue. (A) Inflammatory cell infiltration in the peribronchiolar region and bronchiole epithelium thickness as evaluated by H&E staining (magnification, 400×). (B) Thickness of respiratory epithelium as measured using ImageJ. Data are represented as the mean±SD (n=4 mice per group). $^{\#\#}P<0.01$ compared to the Con group; $^{*}P<0.05$, $^{**}P<0.01$ compared to the particulate matter (PM) group. Con, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus casei* HY2782-fed PM group; HY2782 complex, *L. casei* HY2782 and PRE-fed PM group; Dex, dexamethasone-treated PM group.

et al., 2019). The heterogeneity of memory $CD4^+$ T-cell responses can mediate multiple phenotypes, such as Th2 cell-mediated eosinophilic asthma and Th17 cell-mediated neutrophilic asthma (Wakashin et al., 2008; Pappu et al., 2012). These data suggest that severe asthma is associated with Th2 and Th17 responses when exposed to PM2.5-induced immune reaction and AHR. In this study, the HY2782 complex treatment downregulated IL-4, IL-5, IL-13, and IgE levels in BALF and serum. Furthermore, it decreased the expression level of IL-17A. These results suggest that the HY2782 complex effectively decreases Th2 and Th17 inflammation induced in PM2.5-exposed mice. Previous studies have shown that *L. casei* HY2782 has various health benefits, such as preventing intestinal disease by tight-junction proteins (Jung et al., 2021), increasing IL-12 expression in dendritic cells (Park et al., 2020), and preventing toxic effects of PM (Kim et al., 2020; Nam et al., 2020).

P. lobata root contains three prominent isoflavones: daidzein, daidzin, and puerarin. Puerarin, the most abundant isoflavone in *P. lobata* roots, is a chemotaxonomic marker of *Pueraria* and is not found in soy products (Lippert et al., 2022). Puerarin has shown antioxidant effects by regulating the expression of the Nrf2 pathway and antioxidant enzymes (Wang et al., 2020). It attenuated OVA-induced lung inflammation in an asthma model (Dong et al., 2014). Puerarin alleviated inflammatory cytokines by modulating the Th1/Th2 balance and has been investigated for its effects on delayed-type hypersensitivity induced by OVA (Tong et al., 2018). A study on the HY2782 complex demonstrated amelioration of OVA-induced AHR in mice. Dexamethasone suppressed levels of cytokines, such as IL-4, and immunocytes, such as eosinophils (Nam et al., 2021). Dexamethasone has also been used as a positive control agent. In this study, dexamethasone alleviated inflammation induced by PM2.5. However, dexamethasone exhibited lesser effects on decreasing neutrophils and IL-17A levels in AHR in PM2.5-exposed mice. In this study, Th17 cells were involved in steroid-resistant neutrophilic asthma and airway inflammation (Yu and Chen, 2018; Pang et al., 2019). Results showed that the HY2782 complex decreased neutrophil infiltration and IL-17A expression, suggesting an influence on Th17 cell differentiation.

Leukotrienes are lipid mediators of inflammation and chemotaxis in allergic diseases and chronic and acute inflammation (Jo-Watanabe et al., 2019). Leukotrienes are chemicals that the body releases after encountering an allergen or allergy trigger. Leukotrienes cause breathing difficulties due to excessive production of fluid and mucus and tightness of airway muscle. These chemicals play a key role in allergic rhinitis and asthma. LTC_4 is an important regulator that mediates eosinophil migration from the lungs to paratracheal lymph nodes in experimental allergic asthma (Wang et al., 2017a). In this study, the HY2782 complex reduced the respiratory inflammatory response by decreasing LTC_4 expression in BALF. This result suggests that the HY2782 complex can effectively respond to an immune hypersensitivity reaction in the bronchus and trachea.

Respiratory hyperresponsiveness is more susceptible to oxidative stress caused by ROS production in patients with illnesses, such as asthma, compared to healthy individuals (Misso and Thomson, 2005). Therefore, prevention of oxidative stress could be advantageous for inhibiting progression to a sustained inflammation state. The Nrf2 antioxidant response pathway plays a crucial role in countering PM-induced oxidative stress (Li et al., 2013; Chen et al., 2021). Recent findings regarding PM-related PAHs and interactions with AhR and their effects on immune responses emphasize the potential significance of the AhR-PAH axis in the progress of allergic inflammatory diseases (Dietrich, 2016; Sun et al., 2020). Many Nrf2-mediated antioxidant products can modulate AhR signaling (Furue et al., 2017). Redox circulatory enzymes, such as glutathione peroxidase (GPX), several glutathione *S*-transferases, SOD, and CAT, which are enzymes that mediate ROS removal, are all Nrf2 targets (He et al., 2020). GPX, SOD, and CAT activities constitute a first-line antioxidant defense system that plays a key role in entire defense strategies and mechanisms in biological systems (Ighodaro and Akinloye, 2019). This study also confirmed that the ingestion of the HY2782 complex increased the levels of antioxidant-related factors, such as Nrf2, SOD1, and CAT, which inhibit ROS production. In a previous study, diesel PM2.5 significantly altered the structure and composition of mice gut microbiota, in which a decrease in *Lactobacillus* abundance could suppress butyrate production and thus could exacerbate the immune defense of the gut (Liu et al., 2021b).

In this study, HY2782 probiotic alone did not effectively lower the increased Th2/Th17 response due to PM 2.5 exposure. In contrast, ingestion of the HY2782 complex resulted in a more effective anti-inflammation and antioxidant effect than dexamethasone. It was predicted that this is probably due to a large amount of puerarin in PRE. This result suggested that the HY2782 complex modulates immune hypersensitivity reactions more effectively than probiotics alone. However, the details of the mechanisms remain to be explored.

In conclusion, data showed that administration of the HY2782 complex concurrently with PM2.5 exposure significantly attenuated Th2/Th17-related cytokines IL-4, IL-5, IL-13, and IL-17A and IgE in mice. The HY2782 complex also significantly attenuated LTC_4-induced airway inflammation resulting from PM2.5 exposure. In accordance with the changes in the percentage of inflammatory cells, such as neutrophils and eosinophils, the

HY2782 complex also acted as a protective agent to ameliorate oxidative stress. Furthermore, histopathological observation of lung tissue showed reduced inflammatory cell infiltration into airways and surrounding tissue. By most measures, the HY2782 complex was as effective as dexamethasone.

In summary, the HY2782 complex showed significant efficacy in preventing exacerbation of the airway inflammatory response and respiratory hyperresponsiveness in mice. Thus, the HY2782 complex may be a promising candidate for preventive therapy of allergic diseases and airway inflammation caused by PM2.5 inhalation, especially for individuals suffering from air pollutant exposure-related respiratory diseases.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: SHJ, CHB, JHK. Analysis and interpretation: SHJ, CHB, JHK. Data collection: SHJ. Writing the article: SHJ. Critical revision of the article: SDP, JJS, JLL. Statistical analysis: SHJ. Overall responsibility: JLL. Final approval of the article: all authors.

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