



PM_{2.5} affects establishment of immune tolerance in newborn mice by reducing PD-L1 expression

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This study was conducted to determine whether exposure to particulate matter 2.5 (PM_{2.5}) affects the immune tolerance of neonatal mice via the regulation of PD-L1 expression. One-week-old BALB/c mice were exposed to PM_{2.5} for 8 days. From day 8 to day 18, the mice were treated with 5 µg house dust mite (HDM) (i. n.) every two days. Adenovirus-carried PD-L1 overexpression vectors were infected into mice via nasal inhalation 6 days after exposure to PM_{2.5}. Airway hyper-responsiveness (AHR) was examined in mice 19 days after exposure to PM_{2.5}, and the related parameters of airway inflammation were studied on day 22. Co-exposure to PM_{2.5} and HDM reduced PD-L1 expression but greatly increased infiltration of inflammatory cells, which was reversed by PD-L1 overexpression. Co-exposure to PM_{2.5} and HDM also elevated serum IL-4, IL-5 and IL-13 levels and reduced TGF-β level. Exposure to PM_{2.5} alone slightly increased the numbers of dendritic cells (DCs) but reduced the numbers of antigen-presenting cells expressing PD-L1 and Treg cells. Therefore, early exposure to PM_{2.5} reduced PD-L1 expression in the lungs of neonatal mice, which interfered with immune tolerance establishment and subsequently resulted in allergic airway inflammation.

Keywords. Airway inflammation; immune tolerance; PD-L1; PM_{2.5}

1. Introduction

Particulate matter 2.5 (PM_{2.5}) refers to airborne particles with an aerodynamic diameter less than or equal to 2.5 µm which threaten human health, causing conditions including cardiovascular and respiratory morbidity and mortality (Madrigano *et al.* 2013; Habre *et al.* 2014; Neophytou *et al.* 2014). PM_{2.5} is a serious air pollutant that consists of sulfate, nitrate, nitrogen salt and heavy metals. Due to its small size, PM_{2.5} can reach the deep respiratory tract and affect blood circulation by penetrating lung cells (Perrone *et al.* 2013; Bono *et al.* 2015). Several research studies have demonstrated that exposure to PM_{2.5} may lead to acute and chronic respiratory disease including chronic bronchitis (Abbey *et al.* 1995), asthma in children (Mar *et al.* 2005; Nikasinovic *et al.* 2006; Tecer *et al.* 2008), and lung cancer (Loomis *et al.* 2013). Exposure to PM_{2.5} also increases the risk of developing cardiovascular disease (Dockery 2001; Brunekreef *et al.* 2005) and even liver cancer (Zhang *et al.*

2017). With the rapid economic development in China, air pollution due to PM_{2.5} is growing more and more serious and is becoming one of the leading causes of death. It is urgent to understand how and by which mechanism PM_{2.5} affects human health, especially the respiratory system.

Previous study showed that allergic airway inflammation was aggravated upon four-time co-exposure to ovalbumin and Asian sand dust, while eight-time co-exposure attenuates inflammation with significantly increased transforming growth factor-beta 1 (TGF-β) in bronchoalveolar lavage fluid (BALF) (He *et al.* 2013), suggesting that the responses are correlated with TGF-β-related immune tolerance. We hypothesized that long-term exposure to PM_{2.5} affects immune tolerance. The underlying mechanism needs to be further elucidated. TGF-β is a key regulator of immune regulation and has been shown to regulate T-cell differentiation and function. Loss of TGF-β results in systemic inflammation (Shull *et al.* 1992). TGF-β plays fundamentally important roles in maintaining immune tolerance and is

a well-known promoter of CD4⁺ regulatory T (Treg) cell differentiation (Fantini *et al.* 2004; Mucida *et al.* 2007). Treg cells play a vital role in the maintenance of immune tolerance to self and innocuous non-self. Treg cells develop as an independent T-cell lineage from common T-cell precursors in the thymus, in which Treg cell differentiation requires signals from the T-cell receptor (TCR) for antigen, from costimulatory molecules, and from cytokine receptors (Apert *et al.* 2018). Treg can be divided into two subpopulations: naturally occurring (nTreg) and adaptive (iTreg) which develop in the thymus and the periphery from CD4⁺FoxP3⁻ naive T cells, respectively. In the context of IL-2 stimulation, the *Foxp3* gene was induced to express TGF- β in mouse peripheral CD4⁺CD25⁻ naive T cells, converting them into Foxp3⁺ Treg cells (iTreg cells) capable of suppressing T-cell proliferation (Chen *et al.* 2003). The surface receptor programmed death-1 (PD-1) is critical for regulating T-cell function during immunity and tolerance. The interaction of PD-1 with its ligands PD-L1 and PD-L2 inhibits the functioning of the T-cell effector in an antigen-specific manner (Fife and Pauken 2011). It is noteworthy that PD-1 and PD-L1 were detected on Treg and were linked to Treg development and activation (Francisco *et al.* 2010). It was demonstrated that TGF- β upregulated the expression of PD-L1 on dendritic cells (DCs) with the induction of immune tolerance (Song *et al.* 2014). The binding of PD-L1 with PD-1 expressed on T cells is responsible for T-cell immune activity suppression. TGF- β is required for Treg cell production and co-regulation of Foxp3⁺iTregs development with PD-L1, indicating that PM may regulate immune tolerance through PD-L1.

This study demonstrated that exposure to PM_{2.5} caused defects in the establishment of immune tolerance and led to allergic lung disease. We found that exposure to PM changed PD-L1 expression levels in newborn mice. There is a correlation between PD-L1 and airway sensitivity in mice. Exposure to PM affects the acquired immune tolerance of neonatal mice.

2. Materials and methods

2.1 Preparation of aPM_{2.5}

Mesoporous silica nanoparticles (MSNs) were synthesized according to the published protocol (Wu *et al.* 2013) using tetraethyl orthosilicate (J&K Chemical Ltd., China) as a silicon source and cetyltrimethylammonium bromide (J&K Chemical Ltd., China) as the template. The following steps were adopted according to the protocol published by Yan (Yan *et al.* 2017). Three chemical species, H₂SO₄, NH₄NO₃, and K₂Cr₂O₇, were loaded into the pores of MSNs using the impregnation method. MSN (100 g each) was added to 1 L of ethanol solution containing 0.04 mg/mL of H₂SO₄, 0.04 mg/mL of NH₄NO₃, and 1.6 × 10⁻⁶ mg/mL of K₂Cr₂O₇. Magnetic stirring was done at room temperature for 24 h to

maximize the loading efficiency. The chemical species-loaded MSNs (aPM_{2.5}) were collected via centrifugation, washed twice with ethanol and dried under vacuum. To determine the size of the aPM_{2.5}, a particle powder (2.0 mg) was resuspended and ultrasonicated in 10 mL of water and then measured with a NICOMP 380 ZLS Particle Sizer (USA).

2.2 Mice and treatment

BALB/c mice were maintained in specific pathogen-free conditions. One-week-old BALB/c mice were exposed to PM_{2.5} for 8 days (1.13 g/m³, 60 min each time, 1 × per day). From day 8 to day 18, the mice were treated with 5 μg house dust mite (HDM) (i. n.) once every two days. Airway hyper-responsiveness (AHR) was examined in mice 19 days after exposure to PM_{2.5}, and the related parameters of airway inflammation were studied on day 22. aPM_{2.5} was administrated using a nose-only inhalation system with a particle flow of 1 mL/min and an internal flow of 0.8 m³/h. The animals were fixed on the aerosol generator to ensure the balance and consistency of the inhaled PM aerosol. The exposure procedure lasted 60 min/day, 7 days/week using a Hope-med 8052 automatic inhalation device (Hope-med Industry and Trade Co., Ltd., Tianjin, China). This study was approved by the ethics committee of our hospital (approval No. KJ1600206, 2016).

2.3 Construction of PD-L1 overexpression vector and adenovirus transfection

The adenovirus-carried PD-L1 overexpression vector was constructed and produced using the AdEasy Adenoviral Vector System kit (Stratagene, USA) according to the manufacturer's protocol. For the overexpression of PD-L1, the open reading frame (ORF) of the mouse *PD-L1* gene was amplified and cloned into the backbone vector pShuttle-IRES-hrGFP. Adenoviruses carrying PD-L1 or Ad-PD-L1 were amplified in 293AD cells. Viral particles were injected into the mice. According to the previous reports (Peng *et al.* 2011), adenoviruses carrying PD-L1 (Ad-PD-L1) was constructed. Briefly, the desired replication-deficient adenovirus containing the PD-L1 ORF was generated by homologous recombination through co-transfection of plasmids pShuttle-IRES-hrGFP-PD-L1 and adenoviral skeletal plasmid pBHG1oXE1, 3Cre in AD-293 cells in the presence of DOTAP liposome (Roche, Germany). After plaque purification of several rounds, ad-PD-L1 was amplified and purified from cell lysates. Viral products were desalted and stored at 80°C in phosphate-buffered saline (PBS) containing 10% glycerol (v/v). A recombinant adenovirus carrying the *LacZ* gene under the control of CMV (Ad-LacZ) served

as the control. Two days before the HDM treatment, the Ad-LacZ and Ad-PD-L1 viral particles were separately intravenously injected at 1×10^9 PFU (plaque-forming unit) per mouse per week for 2 weeks (Cheong *et al.* 2014).

2.4 Sample preparation

After mice were euthanized with methoxyflurane, en-bloc lungs were collected. Half of the right lobe was ground in a mortar with liquid nitrogen for total RNA extraction, and the other half was homogenized and lysed within RIPA buffer and used for Western blot analysis. The left lung lobe was fixed in 4% paraformaldehyde for PAS and HE staining.

2.5 RNA extraction and quantitative PCR

Total RNA extraction from lung tissue was performed using Trizol reagent (Invitrogen). The total RNA (1 μ g) obtained was reversely transcribed into cDNA using M-MLV Reverse Transcriptase (Promega) following the manufacturer's protocol. qPCR was performed on the ABI 7500 fast real-time PCR system (Applied Biosystems) using SYBR Premix Ex Taq (Takara). The endogenous control was GAPDH mRNA.

2.6 Western blot analysis

Protein samples from lung tissue were prepared using RIPA buffer (Beyotime, China) containing protease inhibitor cocktail (Sigma). After determination of protein concentration using bicinchoninic acid protein assay kit (Beyotime, Shanghai, China), SDS-PAGE electrophoresis was performed. The separated protein blots were then transfected onto a PVDF membrane (Millipore). After blocking with 5% (w/v) nonfat milk, the membrane was incubated with primary antibodies (against PD-L1 [Abcam, ab213480, 1:1000], PD-L2 [Abcam, ab187662, 1:2000], PD-1 [Abcam, ab187662, 1:1500] and β -actin [Abcam, ab8226, 1:2000]) overnight and secondary antibody for about 4 h. After being visualized using ClarityTM Western ECL substrate (Bio-Rad), the protein bands were quantified using Image J software.

2.7 Histological analysis

Tissues were fixed with 4% paraformaldehyde. Tissue sections were stained with hematoxylin and eosin for pathological evaluation. Periodic acid-Schiff (PAS) staining was performed to analyze changes in mucus secretion. The PAS staining procedure was the same as described in the previous literature (Cheong *et al.* 2014).

2.8 Cytokine measurements and inflammatory cell count in BALF

After the animals were killed, BALF was collected through lavage of the right lung using 0.6 mL of ice-cold PBS. After centrifugation at 400 g for 15 min, the supernatant was collected for IL-4, IL-5, IL-13 and TGF- β determination using a Milliplex MAP assay kit (Millipore, Billerica, MA). IL-33, thymic stromal lymphopoietin (TSLP), and IL-25 production were determined using commercial ELISA kits (R&D systems), according to the manufacturers' protocol. However, the pellet was used for inflammatory cell counts and classification using a hematology analyzer (Nihon Kohden, Tokyo, Japan) after resuspension in PBS.

2.9 Serum immunoglobulins assay

The serum concentration of IgE and HDM-specific IgG1 was determined using ELISA, as described elsewhere (Trompette *et al.* 2014).

2.10 Flow cytometry

To analyze antigen-presenting cell (APC) subsets, lung tissue was digested by collagenase IV (Sigma, USA) in Iscove's modified Dulbecco's medium at 37 °C for 30 min. A 70- μ m cell strainer was used to filter samples that were then washed in PBS containing 0.2% bovine serum albumin and counted with a Coulter counter. Cells were then stained with PerCP/Cy5.5 anti-mouse CD103 antibody (clone: 2E7, 121416, 1:400), Pacific BlueTM anti-mouse/human CD11b antibody (clone: M1/70, 101224, 1:600), APC/Cy7 anti-mouse CD11c antibody (clone: N418, 117324, 1:600), Alexa Fluor[®] 700 anti-mouse I-A/I-E antibody (clone: M5/114.15.2, 107622, 1:800), and Alexa Fluor[®] 647 anti-mouse F4/80 antibody (clone: BM8, 123122, 1:500). The percentage of regulatory T cells of the whole CD4⁺ T-cell compartment in the lung was determined by staining with anti-CD4-PerCP, anti-CD3, anti-CD25-PE and intracellularly with anti-FoxP3. All antibodies were purchased from Biolegend (San Diego, USA). Cells were measured on LSRII (BD Biosciences, USA). Results were analyzed using FlowJo 9.4.11 software (Tree Star Inc.).

2.11 AHR measurement

One day after the last challenge, mice were anesthetized using i.p. injection of ketamine (10 mg/mL) and xylazine (1 mg/mL). Measurements of airway resistance and dynamic compliance were conducted with the Fine Pointe RC System (Buxco Research Systems, Wilmington, NC), in which mice were mechanically ventilated as described previously in detail (Lombardi *et al.* 2010; Galle-Treger *et al.* 2016). Mice

were sequentially challenged with aerosolized PBS (baseline), followed by increasing doses of acetylcholine. Maximum lung resistance (R_L) and minimum compliance (C_{dyn}) values were recorded during a 3-min period after each methacholine challenge.

2.12 Statistical analysis

Results were analyzed using SPSS 18.0 software (SPSS, Chicago, IL) and presented as mean \pm SD values. One-way ANOVA and Student's *t*-test were employed to analyze the difference in data among various groups and between two groups, respectively. A *p*-value less than 0.05 was considered statistically significant.

3. Results

3.1 Exposure to $PM_{2.5}$ reduced PD-L1 expression in the lung of neonatal mice

To study the effect of $PM_{2.5}$ on PD-L1 expression, we constructed artificial $PM_{2.5}$ (a $PM_{2.5}$) particles and exposed the mice for 15 days (1.13 g/m³, 60 min each time, 1 \times per day), followed by HDM treatment (5 μ g, i.n.) every two days. The schematic working model is shown in figure 1A. We detected

PD-L1/L2 and PD-1 expression after exposure to $PM_{2.5}$. The results showed that HDM exposure had little influence on PD-L1/L2 and PD-1 expression, while simultaneous exposure to HDM and $PM_{2.5}$ significantly reduced PD-L1 expression in lung (figure 1B and C). The reduced expression of PD-L1 can be rescued by PD-L1 overexpression vector (figure 1B and C). PD-L1 mRNA level was also attenuated upon exposure to $PM_{2.5}$ and HDM, as detected by qRT-PCR (figure 1D). Importantly, HDM+ $PM_{2.5}$ only affected PD-L1 expression but had no effect on PD-L2 and PD-1 (figure 1B–D).

3.2 PD-L1 expression level was highly correlated with allergic airway inflammation

Since exposure to $PM_{2.5}$ reduced PD-L1 expression, we further studied the role of $PM_{2.5}$ in immune response. We exposed mice to HDM to induce allergic airway inflammation. We found that only when mice were co-exposed to $PM_{2.5}$ and HDM, the numbers of inflammatory cells including lymphocyte, eosinophils, and neutrophil in BALF increased significantly (figure 2A). This infiltration of inflammatory cells was related to PD-L1 expression. PD-L1 overexpression reduced infiltrated inflammatory cell numbers (figure 2A). HE staining confirmed the infiltration of inflammatory cells in lung tissue treated with exposure to $PM_{2.5}$ and HDM (figure 2B). Higher mucus production was

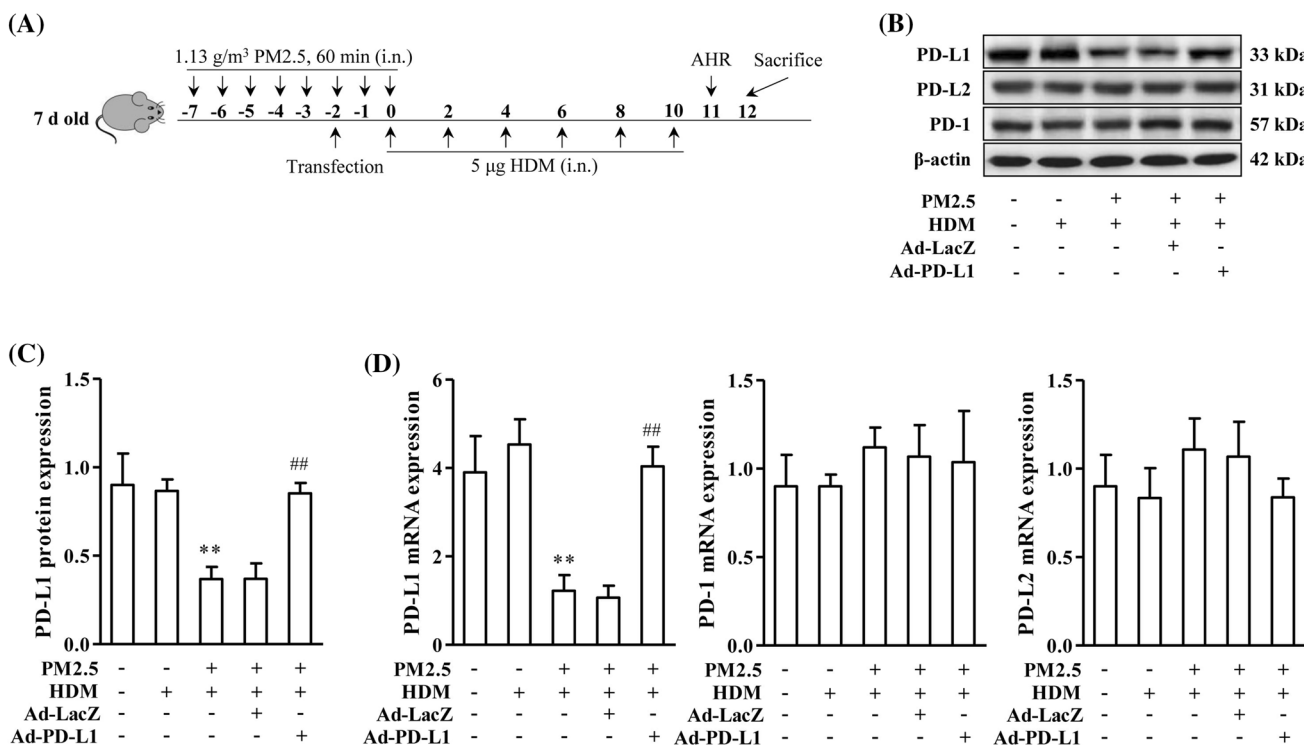


Figure 1. Exposure to $PM_{2.5}$ reduced PD-L1 expression in the lung of neonatal mice. (A) The schematic working model of the experimental design. (B) PD-1, PD-L1, and PD-L2 protein expression measured using Western blotting. (C) Protein quantification results of PD-L1. (D) PD-1, PD-L1, and PD-L2 mRNA expression determined by RT-qPCR assay. Error bars indicate SD. ***P*<0.01 vs $PM_{2.5}$; ##*P*<0.01 vs $PM_{2.5}$ plus Ad-LacZ.

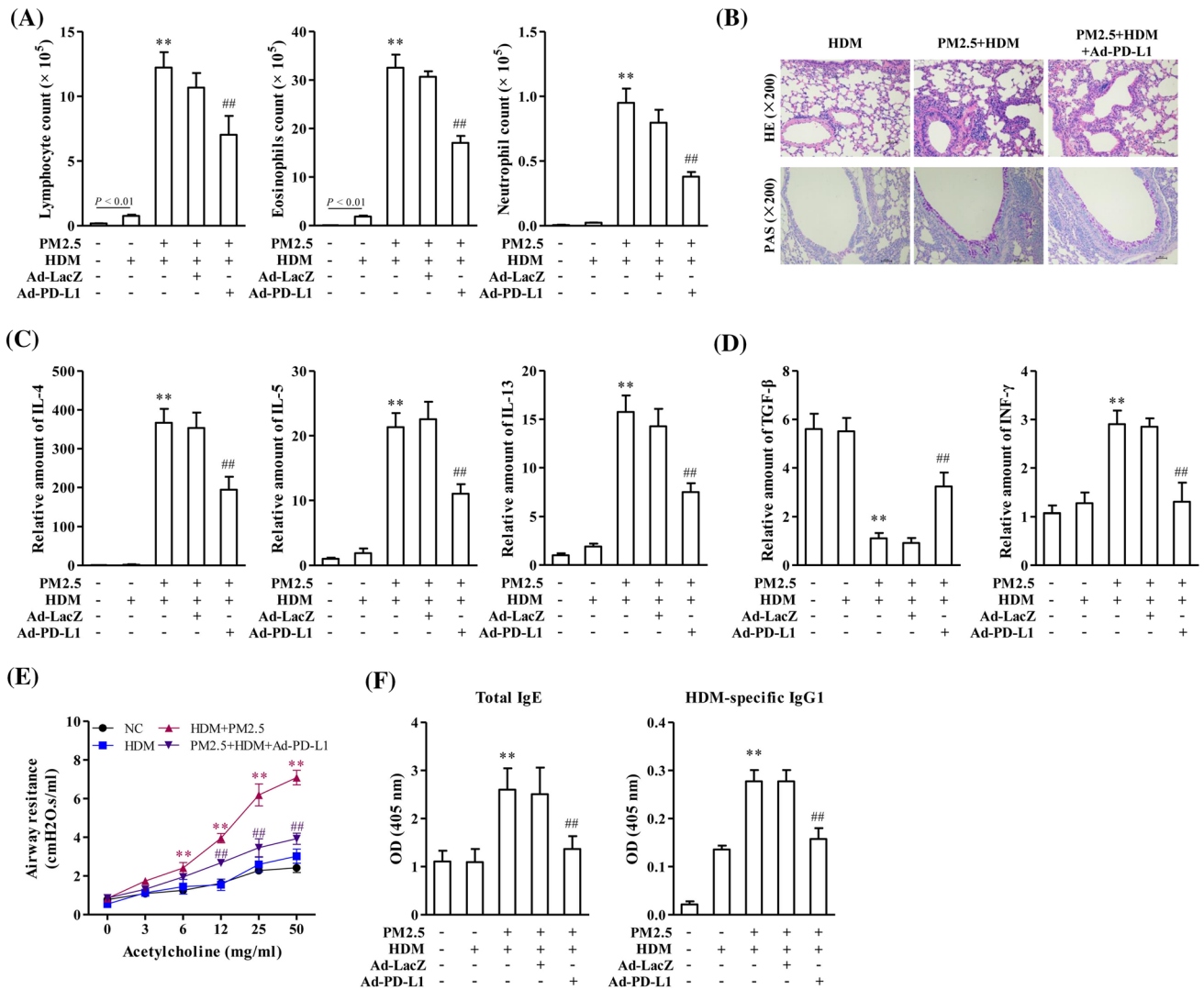


Figure 2. PD-L1 expression level was highly correlated with allergic airway inflammation. (A) Frequencies and total numbers of lymphocytes, eosinophils, and neutrophils infiltrating the BALF. (B) HE and PAS staining of histological sections. (C) Concentrations of interleukin-4 (IL-4), IL-5 and IL-13 in BALF. (D) Concentrations of TGF- β and INF- γ in BALF. (E) AHR assessment by measuring lung resistance (R_L) and dynamic compliance (C_{dyn}). (F) Concentrations of total IgE and HDM-specific IgG1 in serum. Error bars indicate SD. ** $P < 0.01$ vs HDM; ### $P < 0.01$ vs PM_{2.5} plus HDM plus Ad-LacZ.

also observed upon exposure to PM_{2.5} and HDM, indicated by PAS staining (figure 2B). Both the infiltration of inflammatory cells and mucus production were reduced when PD-L1 was overexpressed (figure 2B), indicating a PD-L1-mediated immune response. Exposure to PM_{2.5} together with HDM also increased IL-4, IL-5 and IL-13 levels in the BALF, which were downregulated upon PD-L1 overexpression (figure 2C). PM_{2.5} largely reduced HDM-induced TGF- β upregulation, which was blocked by PD-L1 (figure 2D). In addition, we found that PM_{2.5}- and HDM-exposed mice developed severe AHR (figure 2E). PD-L1 overexpression reduced HDM-induced AHR development (figure 2E). Exposure to PM_{2.5} and HDM increased serum IgE and HDM-specific IgG1 levels (figure 2F). These results together proved that PM_{2.5}-induced immune response was related to PD-L1. PD-L1 expression level was highly

correlated with the infiltration of inflammatory cells in the BALF of HDM-allergic mice.

3.3 Exposure to PM_{2.5} induced downregulation of PD-L1 altered inflammatory cells numbers in the lung tissue

We sorted and quantified the inflammatory cells in the lung tissue. There were no significant changes in macrophages and mDCs, but exposure to HDM+PM_{2.5} significantly decreased the numbers of pDCs and APCs expressing PD-L1 and Treg cells, which returned to normal levels by PD-L1 overexpression (figure 3A and B). These data showed that PM_{2.5} played a role in the formation of APC and Treg cells. Meanwhile, co-exposure to PM_{2.5} and HDM largely increased activated CD8⁺ and CD4⁺ cells and Th2 effector

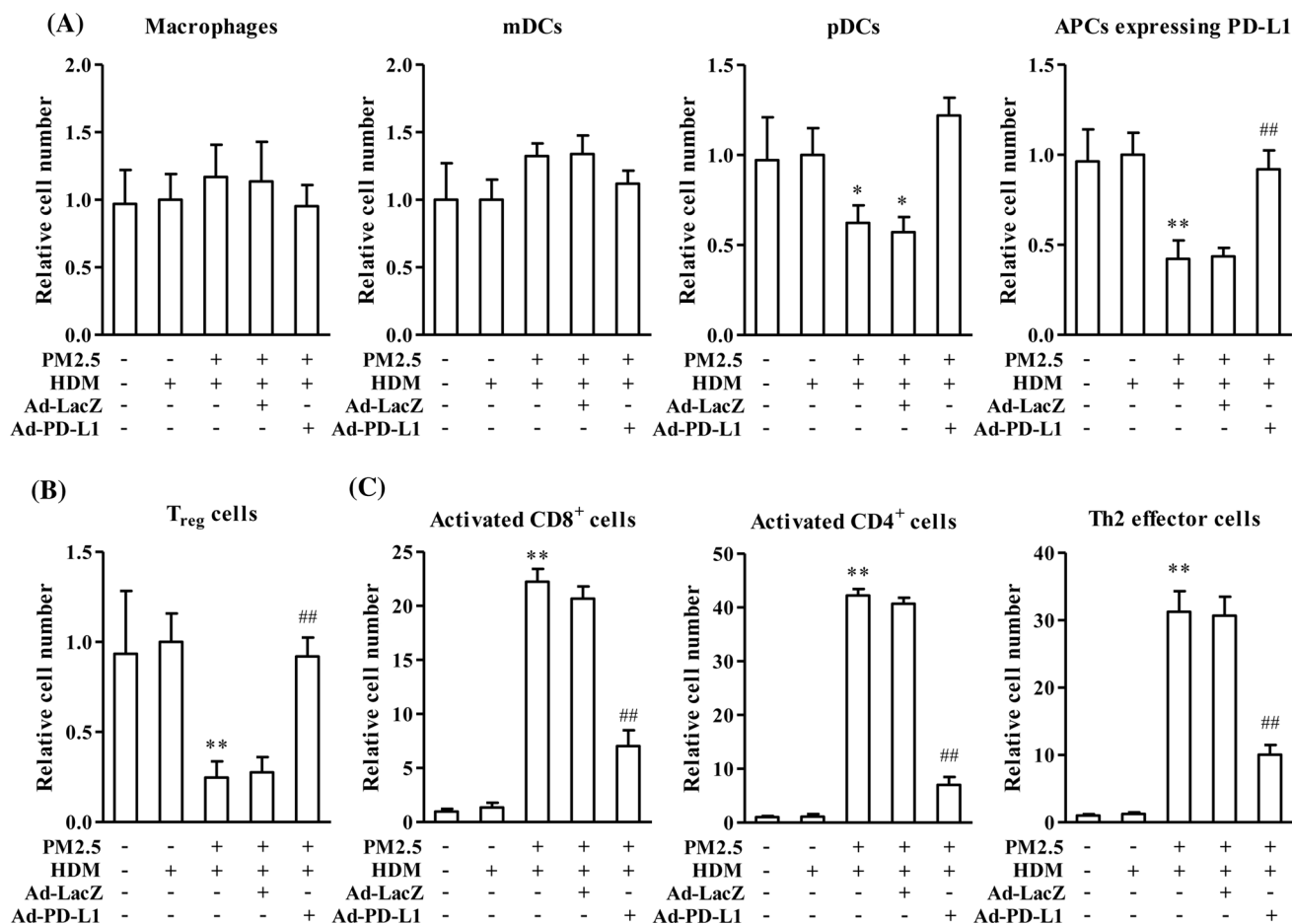


Figure 3. Exposure to PM_{2.5}-induced downregulation of PD-L1 altered inflammatory cells numbers in the lung tissue. **(A)** Relative numbers of macrophages, DCs, and APCs expressing PD-L1. **(B)** Relative numbers of Treg cells. **(C)** Relative numbers of activated CD4⁺, CD8⁺, and Th2 effector cells. Error bars indicate SD. ***P*<0.01 vs control in **A** and **B** or HDM in **C**; ###*P*<0.01 vs PM_{2.5} plus Ad-LacZ in **A** and **B** or PM_{2.5} plus HDM plus Ad-LacZ in **C**.

cells (figure 3C). PD-L1 overexpression abolished HDM+PM_{2.5}-induced immune cell activation (figure 3C).

3.4 Exposure to PM_{2.5} had little influence on the increased production of IL-25, IL-33 and TSLP induced by HDM in the lung tissue

We then determined the effects of HDM and/or PM_{2.5} treatment on the epithelial cell-derived cytokines such as IL-25, IL-33 and TSLP in lung tissue. As shown in figure 4, HDM alone induced upregulation of IL-25, IL-33 and TSLP. Neither PM_{2.5} nor PD-L1 had any effect on these cytokines.

4. Discussion

PM_{2.5} has become the major air pollutant in China over the past years and due to its possible adverse effects on human health, more and more researchers have begun to study the contribution of PM_{2.5} to various diseases (Wang and Hao

2012; Han et al. 2014). The aim of this study was to determine the role of PM_{2.5} in immune tolerance regulation and it was found that exposure to PM_{2.5} affects immune tolerance establishment of neonatal mice by reducing PD-L1 expression. We used artificial PM_{2.5} particles to study its influence on immune response in lung tissue. Previous study demonstrated that in HDM-induced eosinophilia in the airways of newborn mice model, defects in the establishment of immune tolerance may lead to the occurrence of allergic lung disease. Many epidemiological studies demonstrated that exposure to PM_{2.5} may lead to acute and chronic respiratory disease. Because the lungs are the main respiratory organs and also the major target of ambient air contaminants, several studies have shown that PM_{2.5} may cause lung disease (Hamra et al. 2014; Kim et al. 2015).

Interestingly, we found co-exposure to PM_{2.5} and HDM specifically reduced the expression of PD-L1 but not PD-L2 or PD-1. Co-exposure to PM_{2.5} and HDM highly increased infiltration of inflammatory cells, which was reversed by PD-L1 overexpression. PD-L1 and PD-L2 are expressed in many tissues and are highly expressed in the

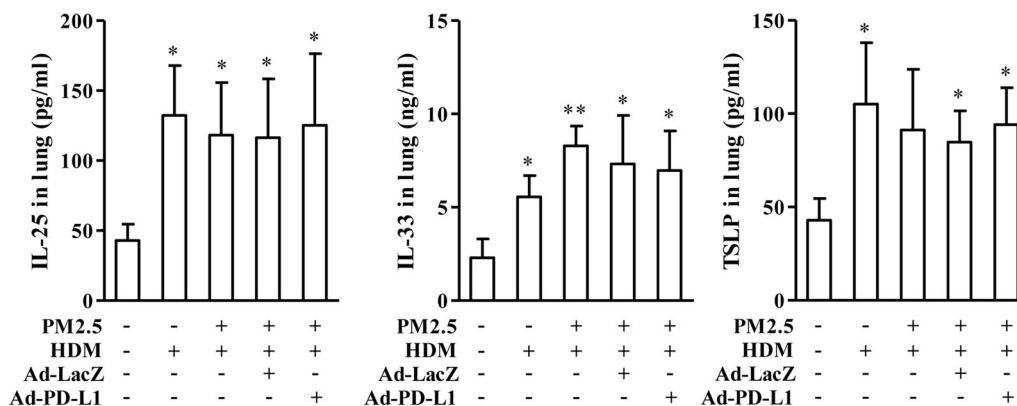


Figure 4. Exposure to PM_{2.5} had a little influence on the increased production of IL-25, IL-33 and TSLP induced by HDM in the lung tissue. Production of IL-25, IL-33 and TSLP in lung tissue was determined using ELISA kits. Error bars indicate SD. * $P < 0.05$ vs normal control; ** $P < 0.01$ vs normal control.

lung, heart and liver. PD-L1 is expressed in lymphoid and non-lymphoid tissues, suggesting that PD-1–PD-L1 interaction may modulate immune responses in secondary lymphoid organs and target organs. It has been documented that PD-1 and its ligand PD-L1 are involved in central and peripheral tolerance modulation, and loss of PD-1 or PD-L1 induced the generation of CD4⁺CD8⁺ thymocytes (Keir *et al.* 2007). We found that exposure to PM_{2.5} increased inflammatory cell numbers in BALF, which was caused by PD-L1 downregulation. Co-exposure to PM_{2.5} and HDM increased serum IgE and HDM-specific IgG1 levels. Importantly, exposure to PM_{2.5} alone slightly increased DC numbers while it reduced numbers of APCs expressing PD-L1 and Treg cells. PD-L1 overexpression upregulated APC and Treg cell numbers. Taken together, our results showed that exposure to PM_{2.5} reduced PD-L1 expression and increased infiltration of inflammatory cells, which further affects immune tolerance.

PM_{2.5} largely reduced HDM-induced TGF- β upregulation, which was blocked by PD-L1 overexpression. TGF- β is well known as a repair and profibrotic cytokine (Bochner *et al.* 1994). Hyperplasia of bronchial structural cells is a typical feature of airway remodeling; hence, TGF- β plays a key role in the progress of airway remodeling (Halwani *et al.* 2011). TGF- β is also known to be a key immunosuppressive cytokine that induced Foxp3⁺Treg cell production, which plays an important role in immunological tolerance (Hammad and Lambrecht 2008; Wing *et al.* 2008). We demonstrated that exposure to PM_{2.5} induced the alteration of both PD-L1 and TGF- β , which together affect immune tolerance.

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References

- Abbey DE, Ostro BE, Petersen F and Burchette RJ 1995 Chronic respiratory symptoms associated with estimated long-term ambient concentrations of fine particulates less than 2.5 microns in aerodynamic diameter (PM_{2.5}) and other air pollutants. *J. Expo. Anal. Environ. Epidemiol.* **5** 137–159
- Apert C, Romagnoli P and van Meerwijk JPM 2018 IL-2 and IL-15 dependent thymic development of Foxp3-expressing regulatory T lymphocytes. *Protein Cell* **9** 322–332
- Bochner BS, Udem BJ and Lichtenstein LM 1994 Immunological aspects of allergic asthma. *Annu. Rev. Immunol.* **12** 295–335
- Bono R, Tassinari R, Bellisario V, Gilli G, Pazzi M, Pirro V, Mengozzi G, Bugiani M and Piccioni P 2015 Urban air and tobacco smoke as conditions that increase the risk of oxidative stress and respiratory response in youth. *Environ. Res.* **137** 141–146
- Brunekreef B, Janssen NA, de Hartog JJ, Oldenwening M, Meliefste K, Hoek G, Lanki T, Timonen KL, Vallius M, Pekkanen J and Van Grieken R 2005 Personal, indoor, and outdoor exposures to PM_{2.5} and its components for groups of cardiovascular patients in Amsterdam and Helsinki. *Res. Rep. Health Eff. Inst.* 1–70; discussion 71–79. <https://www.ncbi.nlm.nih.gov/pubmed/15916017>
- Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G and Wahl SM 2003 Conversion of peripheral CD4⁺CD25⁻ naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF- β induction of transcription factor Foxp3. *J. Exp. Med.* **198** 1875–1886
- Cheong H, Wu J, Gonzales LK, Guttentag SH, Thompson CB and Lindsten T 2014 Analysis of a lung defect in autophagy-deficient mouse strains. *Autophagy* **10** 45–56
- Dockery DW 2001 Epidemiologic evidence of cardiovascular effects of particulate air pollution. *Environ. Health Perspect.* **109** 483–486
- Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR and Neurath MF 2004 Cutting edge: TGF- β induces a regulatory phenotype in CD4⁺CD25⁻ T cells through Foxp3 induction and down-regulation of Smad7. *J. Immunol.* **172** 5149–5153
- Fife BT and Pauken KE 2011 The role of the PD-1 pathway in autoimmunity and peripheral tolerance. *Ann. N. Y. Acad. Sci.* **1217** 45–59

- Francisco LM, Sage PT and Sharpe AH 2010 The PD-1 pathway in tolerance and autoimmunity. *Immunol. Rev.* **236** 219–242
- Galle-Treger L, Suzuki Y, Patel N, Sankaranarayanan I, Aron JL, Maazi H, Chen L and Akbari O 2016 Nicotinic acetylcholine receptor agonist attenuates ILC2-dependent airway hyperactivity. *Nat. Commun.* **7** 13202
- Habre R, Moshier E, Castro W, Nath A, Grunin A, Rohr A, Godbold J, Schachter N, Kattan M, Coull B and Koutrakis P 2014 The effects of PM_{2.5} and its components from indoor and outdoor sources on cough and wheeze symptoms in asthmatic children. *J. Expo. Sci. Environ. Epidemiol.* **24** 380–387
- Halwani R, Al-Muhsen S, Al-Jahdali H and Hamid Q 2011 Role of transforming growth factor-beta in airway remodeling in asthma. *Am. J. Respir. Cell Mol. Biol.* **44** 127–133
- Hammad H and Lambrecht BN 2008 Dendritic cells and epithelial cells: Linking innate and adaptive immunity in asthma. *Nat. Rev. Immunol.* **8** 193–204
- Hamra GB, Guha N, Cohen A, Laden F, Raaschou-Nielsen O, Samet JM, Vineis P, Forastiere F, Saldiva P, Yorifuji T and Loomis D 2014 Outdoor particulate matter exposure and lung cancer: A systematic review and meta-analysis. *Environ. Health Perspect.* **122** 906–911
- Han L, Zhou W, Li W and Li L 2014 Impact of urbanization level on urban air quality: A case of fine particles (PM_{2.5}) in Chinese cities. *Environ. Pollut.* **194** 163–170
- He M, Ichinose T, Yoshida S, Takano H, Nishikawa M, Sun G and Shibamoto T 2013 Induction of immune tolerance and reduction of aggravated lung eosinophilia by co-exposure to Asian sand dust and ovalbumin for 14 weeks in mice. *Allergy Asthma Clin. Immunol.* **9** 19
- Keir ME, Freeman GJ and Sharpe AH 2007 PD-1 regulates self-reactive CD8⁺ T cell responses to antigen in lymph nodes and tissues. *J. Immunol.* **179** 5064–5070
- Kim JY, Lee EY, Choi I, Kim J and Cho KH 2015 Effects of the particulate matter_{2.5} (PM_{2.5}) on lipoprotein metabolism, uptake and degradation, and embryo toxicity. *Mol. Cells* **38** 1096–1104
- Lombardi V, Stock P, Singh AK, Kerzerho J, Yang W, Sullivan BA, Li X, Shiratsuchi T, Hnatiuk NE, Howell AR, Yu KO, Porcelli SA, Tsuji M, Kronenberg M, Wilson SB and Akbari O 2010 A CD1d-dependent antagonist inhibits the activation of invariant NKT cells and prevents development of allergen-induced airway hyperactivity. *J. Immunol.* **184** 2107–2115
- Loomis D, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Baan R, Mattock H, Straif K and International Agency for Research on Cancer Monograph Working Group IARC 2013 The carcinogenicity of outdoor air pollution. *Lancet Oncol.* **14** 1262–1263
- Madrigano J, Kloog I, Goldberg R, Coull BA, Mittleman MA and Schwartz J 2013 Long-term exposure to PM_{2.5} and incidence of acute myocardial infarction. *Environ. Health Perspect.* **121** 192–196
- Mar TF, Jansen K, Shepherd K, Lumley T, Larson TV and Koenig JQ 2005 Exhaled nitric oxide in children with asthma and short-term PM_{2.5} exposure in Seattle. *Environ. Health Perspect.* **113** 1791–1794
- Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M and Cheroutre H 2007 Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* **317** 256–260
- Neophytou AM, Costello S, Brown DM, Picciotto S, Noth EM, Hammond SK, Cullen MR and Eisen EA 2014 Marginal structural models in occupational epidemiology: Application in a study of ischemic heart disease incidence and PM_{2.5} in the US aluminum industry. *Am. J. Epidemiol.* **180** 608–615
- Nikasinovic L, Just J, Sahraoui F, Seta N, Grimfeld A and Momas I 2006 Nasal inflammation and personal exposure to fine particles PM_{2.5} in asthmatic children. *J. Allergy Clin. Immunol.* **117** 1382–1388
- Peng W, Ran B, Ma Y, Huang X, Chang Q and Wang X 2011 Dendritic cells transfected with PD-L1 recombinant adenovirus induces T cell suppression and long-term acceptance of allograft transplantation. *Cell Immunol.* **271** 73–77
- Perrone MG, Gualtieri M, Consonni V, Ferrero L, Sangiorgi G, Longhin E, Ballabio D, Bolzacchini E and Camatini M 2013 Particle size, chemical composition, seasons of the year and urban, rural or remote site origins as determinants of biological effects of particulate matter on pulmonary cells. *Environ. Pollut.* **176** 215–227
- Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvint D, Annunziata N and Doetschman T 1992 Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* **359** 693–699
- Song S, Yuan P, Wu H, Chen J, Fu J, Li P, Lu J and Wei W 2014 Dendritic cells with an increased PD-L1 by TGF-beta induce T cell anergy for the cytotoxicity of hepatocellular carcinoma cells. *Int. Immunopharmacol.* **20** 117–123
- Tecer LH, Alagha O, Karaca F, Tuncel G and Eldes N 2008 Particulate matter (PM_{2.5}), PM_{10-2.5}, and PM₁₀) and children's hospital admissions for asthma and respiratory diseases: A bidirectional case-crossover study. *J. Toxicol Environ. Health A.* **71** 512–520
- Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, Blanchard C, Junt T, Nicod LP, Harris NL and Marsland BJ 2014 Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* **20** 159–166
- Wang S and Hao J 2012 Air quality management in China: Issues, challenges, and options. *J. Environ. Sci. (China)* **24** 2–13
- Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T and Sakaguchi S 2008 CTLA-4 control over Foxp3⁺ regulatory T cell function. *Science* **322** 271–275
- Wu SH, Mou CY and Lin HP 2013 Synthesis of mesoporous silica nanoparticles. *Chem. Soc. Rev.* **42** 3862–3875
- Yan XD, Wang QM, Tie C, Jin HT, Han YX, Zhang JL, Yu XM, Hou Q, Zhang PP, Wang AP, Zhang PC, Gao Z and Jiang JD 2017 Polydatin protects the respiratory system from PM_{2.5} exposure. *Sci. Rep.* **7** 40030
- Zhang Q, Luo Q, Yuan X, Chai L, Li D, Liu J and Lv Z 2017 Atmospheric particulate matter_{2.5} promotes the migration and invasion of hepatocellular carcinoma cells. *Oncol. Lett.* **13** 3445–3450