

Research paper

Oligomeric proanthocyanidins attenuate airway inflammation in asthma by inhibiting dendritic cells maturation



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ABSTRACT

To date, although a promising anti-inflammatory activity of oligomeric proanthocyanidins (OPCs) has been observed in asthma, the mechanism responsible for these immunomodulatory properties remains obscure. Dendritic cells (DCs) that reside in the airway have been widely perceived as an important contributor to asthma. Our study was to demonstrate OPCs' effects on maturation and immunoregulation of pulmonary CD11c⁺ dendritic cells (DCs). BALB/c mice were exposed to ovalbumin (OVA) to induce murine model of asthma. In addition, pulmonary DCs and bone marrow-derived DCs (BMDCs) cultures were used to evaluate impacts of OPCs on DCs function. The results obtained here indicated that OPCs treatment dramatically reduced airway inflammation, such as the infiltration of inflammatory cells and the levels of allergen-specific serum IgE and Th2 cytokines. The expression of co-stimulatory molecules especially CD86 distributed on pulmonary DCs and bone marrow-derived DCs (BMDCs) also markedly declined. The phosphorylation of cAMP responsive element-binding protein (CREB) was significantly inhibited while no changes were observed in the expression of cAMP responsive element modulator (CREM). By transferring BMDCs into the airways of naïve mice, we found that OPCs-treated DCs (DC + OVA + OPC) were much less potent in promoting CD4⁺ T cells proliferation than OVA-pulsed DCs (DC + OVA), followed by the ameliorated eosinophilic inflammation in airway. Our findings tailor a novel profile of OPCs in the regulation of DCs function, shedding new light on the therapeutic potential of OPCs in asthma management.

1. Introduction

Allergic asthma is an atopic airway disease characterized by airway inflammation, hyperresponsiveness (AHR), remodeling and mucus hypersecretion. Although Th2 cells have been widely confirmed to contribute most to the cytokines production in asthma (Cho et al., 2016; Leigh et al., 2004), the vital role of dendritic cells (DCs) in the initiation and extension of allergic inflammation might not be underestimated (van Helden and Lambrecht, 2013; Hammad et al., 2010; Phytian-Adams et al., 2010). Serving as a sentinel unit to incoming antigens, DCs are the most efficient antigen-presenting cells. Upon exposure, DCs undergo a maturation process involving the secretion of assorted cytokines as well as the transformation of co-stimulatory molecules

(Lombardi et al., 2010). Actually, DCs maturation is so pivotal for uncommitted Th0 to differentiate to specific effector T subsets, promoting the development of consequent airway inflammation (Cochand et al., 1999; Vermaelen et al., 2001). Thus, pulmonary DCs have taken the center stage as cellular therapeutic targets in asthma.

Comprising the mainstay of asthma therapy, corticosteroids are known as non-specific agents and have presented eminent systemic and topical side effects. Since the poor performance of corticosteroids in curing asthma radically or even retarding the disease progression, recent studies have paid sufficient attention to natural plant products as a complementary or an alternative treatment.

Oligomeric proanthocyanidins (OPCs) are polyphenolic compounds found in vegetables, flowers, fruits and grape seeds. OPCs bind less

Abbreviations: OPCs, oligomeric proanthocyanidins; DCs, dendritic cells; OVA, ovalbumin; BALF, bronchiolar lavage fluid; AHR, airway hyperresponsiveness; CREB, cAMP responsive element-binding protein; CREM, cAMP responsive element modulator; IL, interleukin; Mch, methacholine; MFI, median fluorescence intensity; RL, resistance of lung; CD, cluster of differentiation; BMDCs, bone marrow derived dendritic cells

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strongly to proteins, and are more soluble and biologically active (Fine, 2000). More specific pharmacological actions of OPCs have been recently reported, such as scavenging free radical, anti-oxidative damage and regulating immune response. Previous studies have shown that OPCs exhibited an anti-inflammatory activity in some disorders such as inflammatory bowel disease (Wang et al., 2011) and arthritis (Park et al., 2011). It has also been demonstrated that OPCs effectively suppressed airway inflammation, AHR and remodeling in asthma (Lee et al., 2012). However, the underlying mechanism still remains obscure.

Our preliminary study has revealed that OPCs exhibited a potent potential in asthma management (data not published). As the major components of inflammatory network, DCs are involved in the pathogenesis of asthma. In the present study, by using pulmonary DCs and bone marrow-derived DCs (BMDCs), we investigated the impacts of OPCs on DCs function both *in vivo* and *in vitro*.

2. Materials and methods

2.1. Animals

Specific pathogen-free female BALB/c mice (18–22 g), 6–8 weeks of age, were obtained from Vital River Laboratory (Beijing, China). The mice were kept in a temperature-controlled room under a 12 h dark/light cycle and were provided with food and water *ad libitum*. All experiments that involved animals and tissue samples were performed in accordance with the guidelines of the National Institutes of Health and Nanjing Medical University and all procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University (Nanjing, China).

2.2. Ovalbumin (OVA) sensitization and challenge

Fig. 1B schematically depicts the protocols used in this study. In total, 49 specific pathogen-free female BALB/c mice were randomly divided into 7 groups ($n = 7$ per group) as follows: control; ovalbumin (OVA) (Grade V, Sigma-Aldrich, St. Louis, MO, USA), OVA + OL (OPC 7.5 mg/kg OPC, Sigma-Aldrich); OVA + OM (OPC 15 mg/kg OPC); OVA + OH (OPC 30 mg/kg OPC) (OL, OM and OH represent low, medium and high dose of OPCs, respectively); OVA + dexamethasone (DEX, 1 mg/kg); and OVA + dimethylsulfoxide (DMSO). Dexamethasone is known for its potent anti-inflammatory effect and used as the positive-control. DMSO is the solvent for OPCs and used as the

vehicle-control. The acute asthma model was established by OVA sensitization. Specifically, all of the mice in the OVA, OVA + OL, OVA + OM, OVA + OH, OVA + DEX and OVA + DMSO groups were sensitized on days 0, 7 and 14 by the intraperitoneal injection of 50 μ g of OVA emulsified in 2 mg of aluminum hydroxide gel (Invivo-Gen, San Diego, CA, USA) in a total volume of 200 μ L. Mice were anesthetized using inhaled sevoflurane and instilled with an OVA solution (4 mg/mL) alone by administering 12.5 μ L per nostril on days 20, 21 and 22. OPCs and DMSO (0.4 μ L in a total of 200 μ L of saline) were administered by intraperitoneal injection at 30 min prior to each OVA challenge (Daubeuf and Frossard, 2013). The control subjects were sensitized and challenged using the same protocol with saline alone. All mice were sacrificed 24 h after the last OVA challenge. Bronchoalveolar lavage fluid (BALF) and lung tissues were collected for analysis.

2.3. Measurement and analysis of airway responsiveness

Lung function was assessed by direct measurement of lung resistance. Mice underwent tracheostomies and were mechanically ventilated using the FinePointe RC system (Buxco Research Systems, Wilmington, NC, USA) under general anesthesia as previously described (Kerzerho et al., 2013).

2.4. BALF collection and differential cell counts

After measurement of AHR, the tracheae were exposed and BALF was collected by lavage with ice-cold phosphate-buffered saline via a tracheal catheter (PBS, 400 μ L $\times$ 3; 85%–90% of the lavage volume was recovered). The lavage samples from each mouse were centrifuged at 1000 rpm for 10 min at 4 °C. The total number of cells in the BALF was counted on a hemocytometer. Differential cell counts were performed using Wright's staining on the basis of morphological criteria by two independent investigators in a single-blind study and at least 200 cells each were analyzed from three different random locations using a microscope. Then, the supernatant was collected, divided into 5 equal portions and frozen at -80 °C for use in an enzyme-linked immunosorbent assay (ELISA).

2.5. Lung histologic analysis

Lungs were inflated with 4% paraformaldehyde at a standard pressure of 20 cm H₂O. Tissues were then paraffin embedded and cut at a thickness of 5 μ m. Sections were stained with hematoxylin and eosin

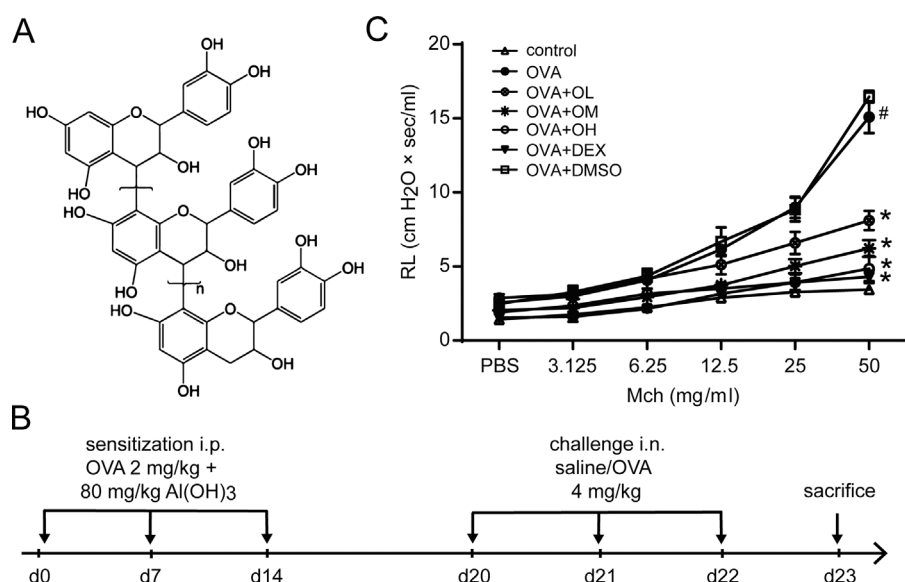


Fig. 1. Chemical structure of OPCs and experimental protocol for the acute asthma model. Treatment with OPCs decreased AHR. (A) General chemical structure of OPCs. (B) Brief flowchart of animal sensitization and challenge. (C) Mice inhaled increasing doses of methacholine (3.125–50 mg/mL). AHR was measured and shown as RL (cm H₂O × s/mL). Data represent the mean + SEM. ($n = 7$ per group). # $P < 0.05$ compared to the control group, and * $P < 0.05$ compared to the OVA group.

(H & E) to assess inflammatory cell infiltration. The inflammation score was determined as follows: grade 0: no inflammation; grade 1: occasional cuffing with inflammatory cells; grades 2, 3 and 4: most bronchi or vessels which were surrounded by a thin layer (1–2 cells: grade 2), a moderate layer (3–5 cells: grade 3) or a thick layer (> 5 cells: grade 4) of inflammatory cells, respectively. The total inflammation score was calculated by the addition of the peribronchial (PB) and perivascular (PV) inflammation scores.

2.6. Measurement of ovalbumin-specific IgE and cytokine expression

The levels of IL-4, IL-5, IL-13, IFN- γ and IL-12p70 were measured in the BALF using commercially available ELISA kits (R & D Systems, Minneapolis, MN, USA). Serum anti-ovalbumin IgE was detected using an ELISA kit (Yifeixue, Nanjing, China). All steps were carried out in accordance with the manufacturer's instructions.

2.7. Evaluation of co-stimulatory molecule expression on pulmonary DCs

Lung single-cell suspensions were prepared as described before (Jungblut et al., 2009). Flow cytometry was used to evaluate the expression of surface co-stimulatory markers using eFluor[®] 450-CD45, APC-CD11c, PE-MHC-II, FITC-CD80, PE-CD86 and FITC-CD40 monoclonal antibodies, respectively (eBioscience, Inc., San Diego, CA, USA). At least 1×10^5 cells were analyzed using a FACScan (Becton Dickinson, Franklin Lakes, NJ, USA) and CellQuest software (Becton Dickinson). The expression of CD80, CD86, CD40 and MHC-II on CD45⁺, CD11c⁺ and low autofluorescence pulmonary DCs were detected by flow cytometry (Vermaelen and Pauwels, 2004). The expression of activation markers was presented as the median fluorescence intensity (MFI).

2.8. Generation of bone marrow-derived dendritic cells (BMDCs)

BMDCs were cultured and isolated as described previously (Inaba et al., 1992). On day 6, non-adherent and loosely adherent DCs aggregates were harvested and sub-cultured overnight. On day 7, > 80% of the CD11c⁺ non-adherent cells were used.

2.9. Induction of the mouse asthma model by adoptive transfer of BMDCs

BMDCs were pulsed for 24 h with 100 μ g/mL of OVA dissolved in cell culture medium. Control DCs were incubated in OVA-free culture medium. After OVA pulsing, the cells were washed to remove free OVA, resuspended at 1×10^6 cells/mL in PBS and adoptively transferred into the trachea of naive mice. The animals received 4×10^4 DCs in 40 μ L of PBS (Sung et al., 2001). For intratracheal injection, mice were under sevoflurane anesthetization. DCs were injected under direct vision through the opening vocal cords using a 25 gauge metal catheter connected to the outlet of a micropipette. After 10 days, the mice were exposed to OVA aerosols (1%) for 30 min on 3 consecutive days and sacrificed in 24 h after the last dose (Lambrecht et al., 2000a).

2.10. Measurement of CD4⁺ T cells proliferation in the lung

On day 7 after adoptive intratracheal transfer of BMDCs, mice were killed by CO₂ asphyxiation, lungs were resected and individual cell suspensions were prepared as described (Jungblut et al., 2009). Flow cytometry was used to measure the number of CD4⁺ T cells in the lung (FITC-CD4, eBioscience, Inc).

2.11. Analysis of co-stimulatory molecules expression on BMDCs in vitro

On day 6 of culture, BMDCs were collected and added to 12-well cell culture plates at 6×10^5 cells/mL and pulsed over night with 100 μ g/mL of OVA in the presence or absence of OPCs (5 μ M) (Lambrecht et al.,

2000b). Harvested cells were washed three times with PBS. DCs were incubated with APC-CD11c, FITC-CD80 and PE-CD86 or APC-CD11c, FITC-CD40 and PE-MHC-II at 4 °C for 30 min as per the manufacturer's instructions. Stained cells were analyzed by flow cytometry.

2.12. Western blotting

BMDCs were pretreated with OPCs (5 μ M) or PBS for 30 min prior to OVA stimulation. On the 2nd day, PBS-treated DCs (PBS + DC), OVA-pulsed DCs (OVA + DC), OPC-treated OVA-pulsed DCs (OPC + OVA + DC) and DMSO + OVA + DC were harvested respectively. Whole cell extractions from BMDCs were obtained using RIPA lysis buffer in the presence of protease inhibitors and phosphatase inhibitor. Equal amounts of total protein samples were separated by SDS-PAGE and blotted with antibodies against cAMP responsive element-binding protein (CREB), phosphorylated-CREB (Abcam, Cambridge, UK), cAMP responsive element modulator (CREM; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and β -actin, as previously described (Ma et al., 2016).

2.13. Statistical analysis

The results are presented as the means $\pm$ SEM. Differences between groups were determined with Prism 6, using one-way ANOVA tests. Differences were considered to be statistically significant at a $P < 0.05$.

3. Results

3.1. Effects of OPCs on AHR in an acute murine asthma model

The general structure of OPCs was shown in Fig. 1A. Data obtained from AHR analysis showed that the resistance of lung (RL) values dose-dependently increased with increasing methacholine (Mch) doses ranging from 3.125 to 50 mg/mL in the OVA-challenged mice (Fig. 1C). Treatment with OPCs (7.5 mg/kg, 15 mg/kg and 30 mg/kg) or DEX (1 mg/kg) led to a decrease in airway resistance compared to the OVA group, implying a relief of airway inflammation in OVA-challenged mice. However, the lower dose of OPCs did not profoundly reduce RL as did the higher dose. Anyway, these data indicated that OPCs treatment improves airway function of the murine asthma model following exposure to OVA.

3.2. Effects of OPCs on airway inflammation in a murine asthma model

24 h after the last OVA challenge through intranasal instillation, BALF and lung tissues were collected. Total and differential cell counts in the BALF were performed. Compared to the control group, the OVA challenge led to increases of total cells, as well as eosinophils, macrophages and lymphocytes in the BALF (Fig. 2A). Notably, treatment with a high dose of OPCs (30 mg/kg) or DEX reduced the counts of these cells ($P < 0.05$), especially the counts of eosinophils ($P < 0.05$) (Fig. 2A). Lung tissue sections stained by H & E were evaluated for the development of airway inflammation as well. It was demonstrated that OVA and OVA + DMSO groups developed severe airway inflammatory responses compared to control. Extensive infiltration of inflammatory cells around the respiratory tracts and vessels were observed, most of which were eosinophils (Fig. 2B, 2C). On the contrary, pretreatment with different doses of OPCs (7.5, 15 and 30 mg/kg) inhibited OVA-induced eosinophilic inflammation to varying degrees (Fig. 2B and C).

3.3. Effects of OPCs on OVA-specific IgE and Th2 cytokine

Compared to the control, OVA-specific IgE, IL-4, IL-5 and IL-13 were markedly increased in the OVA mice. Administration of OPCs significantly decreased IgE level (Fig. 2E) and reduced IL-4 secretion in a

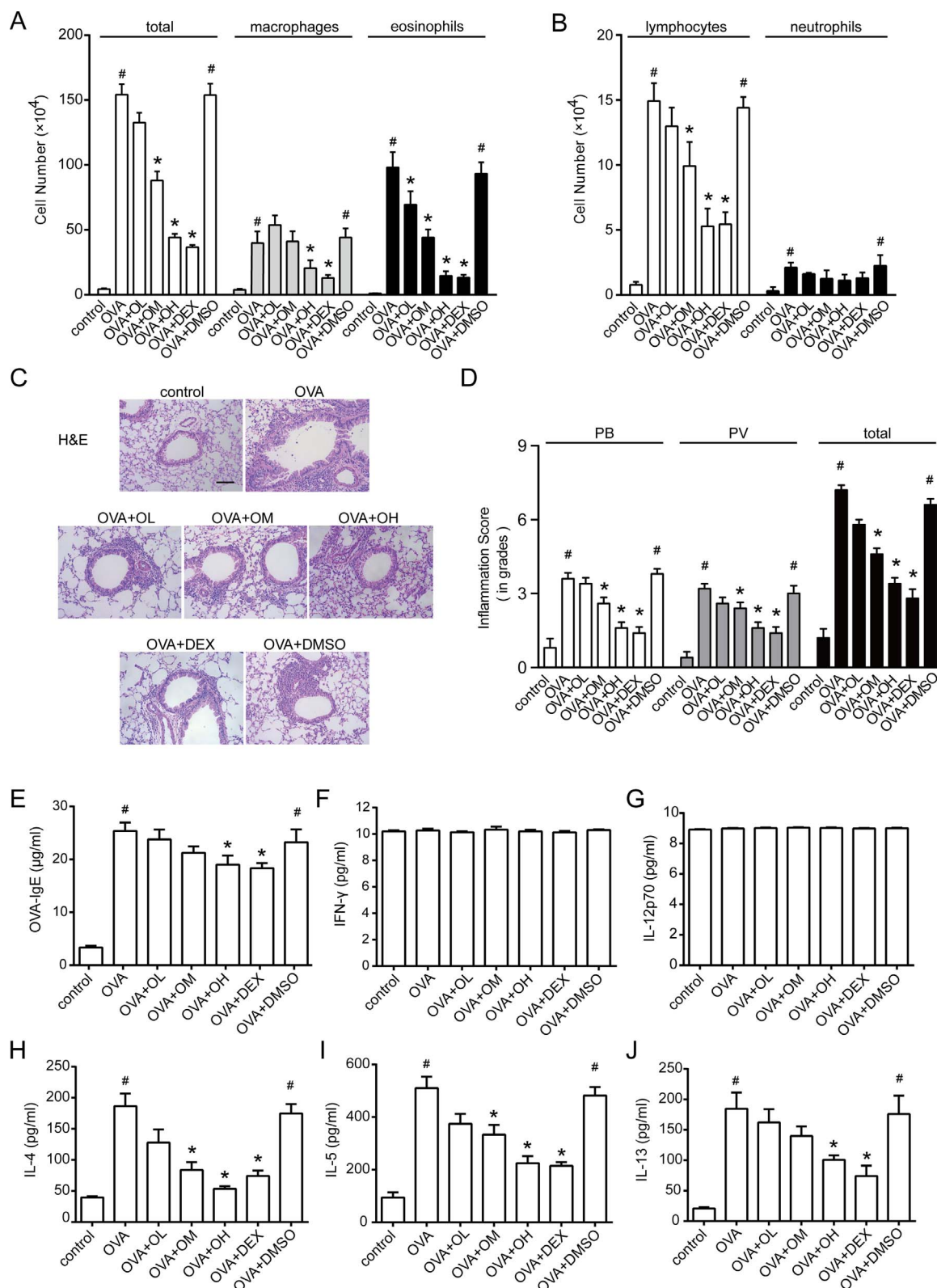
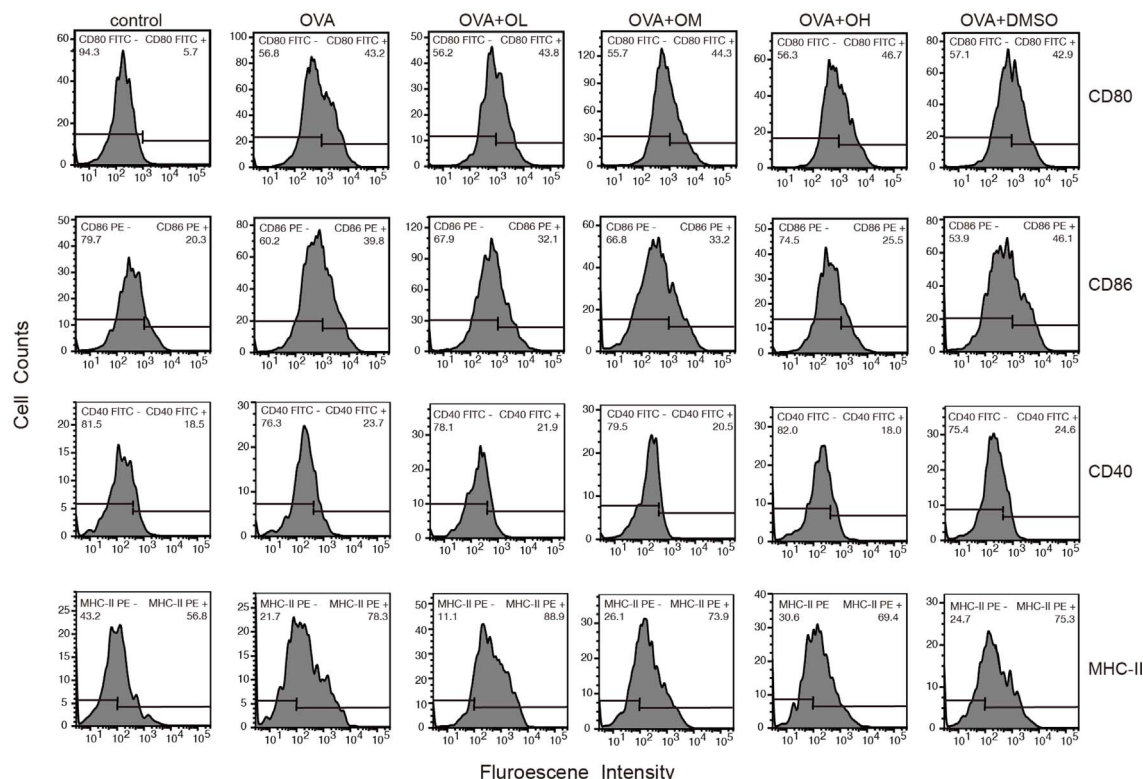


Fig. 2. Treatment with OPCs decreased airway inflammation and reduced the levels of Th2 cytokines in the BALF and OVA-specific IgE in serum. (A, B) The cell numbers and differentiation in the BALF were determined by counting on a hemocytometer; at least 200 cells were counted. (C) Lung sections were stained with H & E to analyze the infiltration of inflammatory cells (magnification, 200 × ; scale bar, 100 μm). (D) The layers of inflammation cells were counted and the total inflammation score was summed up with peribronchial (PB) and perivascular (PV) inflammation scores. (E–J) The concentrations of OVA-IgE, IFN-γ, IL-12p70, IL-4, IL-5 and IL-13 were measured by ELISA. Data represent the mean + SEM (n = 7 per group). [#]P < 0.05 compared to the control group, and ^{*}P < 0.05 compared to the OVA group.

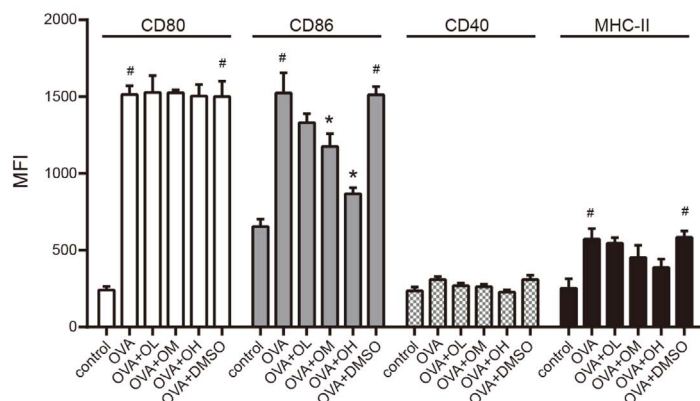
dose-dependent manner compared to the OVA-group (Fig. 2H). No significant reduction in IL-5 was observed in OVA + OL group (Fig. 2I), while the IL-13 level was significantly decreased in OVA + OH group

alone (Fig. 2J). A previous study reported that the critical role of IL-12p70 in DC-induced Th1 responses (Chang et al., 2011). In the present study, the production of IFN-γ and IL-12p70 was not significantly

A



B



C

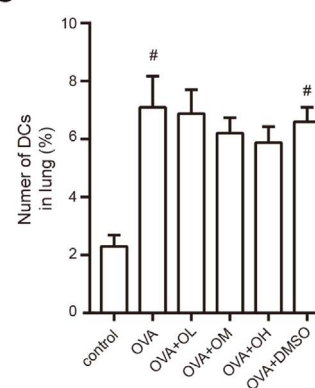


Fig. 3. Treatment with OPCs inhibited the expression of CD86 on pulmonary DCs. (A, B) The expression of CD80, CD86, CD40, and MHC-II on pulmonary DCs was analyzed by flow cytometry. The expression of activation markers is presented as MFI. (C) The number of pulmonary DCs was analyzed by flow cytometry. Data represent the mean + SEM (n = 7 per group). #P < 0.05 compared to the control group, and *P < 0.05 compared to the OVA group.

different among these groups (Fig. 2F and G). These data indicated that OPCs might reconcile the Th2-predominant immune imbalance in an OVA-challenged model.

3.4. Effects of OPCs on the expression of pulmonary DCs CD86

Given the vital role of pulmonary DCs in Th2 cell differentiation and sensitization, we determined the expression of co-stimulatory molecules on CD11c⁺ pulmonary DCs, which were indispensable for DC-induced Th2 cell differentiation. As shown in Fig. 3, the levels of CD80, CD86 and MHC-II were significantly up-regulated by OVA challenge. With OPCs treatment, the high level of CD86 on pulmonary DCs was notably decreased (Fig. 3B) (P < 0.05) while no significant reduction in CD80, CD40 and MHC-II was observed. As shown in Fig. 4B, OPCs did not reduce the amount of pulmonary DCs, hinting a retardation of maturation but not the number of pulmonary DCs by OPC in OVA model

(Fig. 3C).

3.5. Effects of OPCs on CD86 and MHC-II expression on BMDCs

After the determination of pulmonary DCs function *in vivo*, the direct effects of OPCs on DCs *in vitro* were explored. As shown in Fig. 4A, BMDCs viability was 84% + 5% at 5 μ M after 24 h incubation. DCs treated with OPC + OVA presented decreases of CD86 (predominant) and MHC-II at a dose of 5 μ M (Fig. 4B and C) compared to OVA-pulsed DCs.

3.6. Effects of OPCs on mouse BMDC p-CREB expression

To investigate the impacts of OPCs on the signaling involved in DCs maturation, CREB and CREM were investigated by using primary BMDCs cultures. It was found that OVA stimulation promoted the

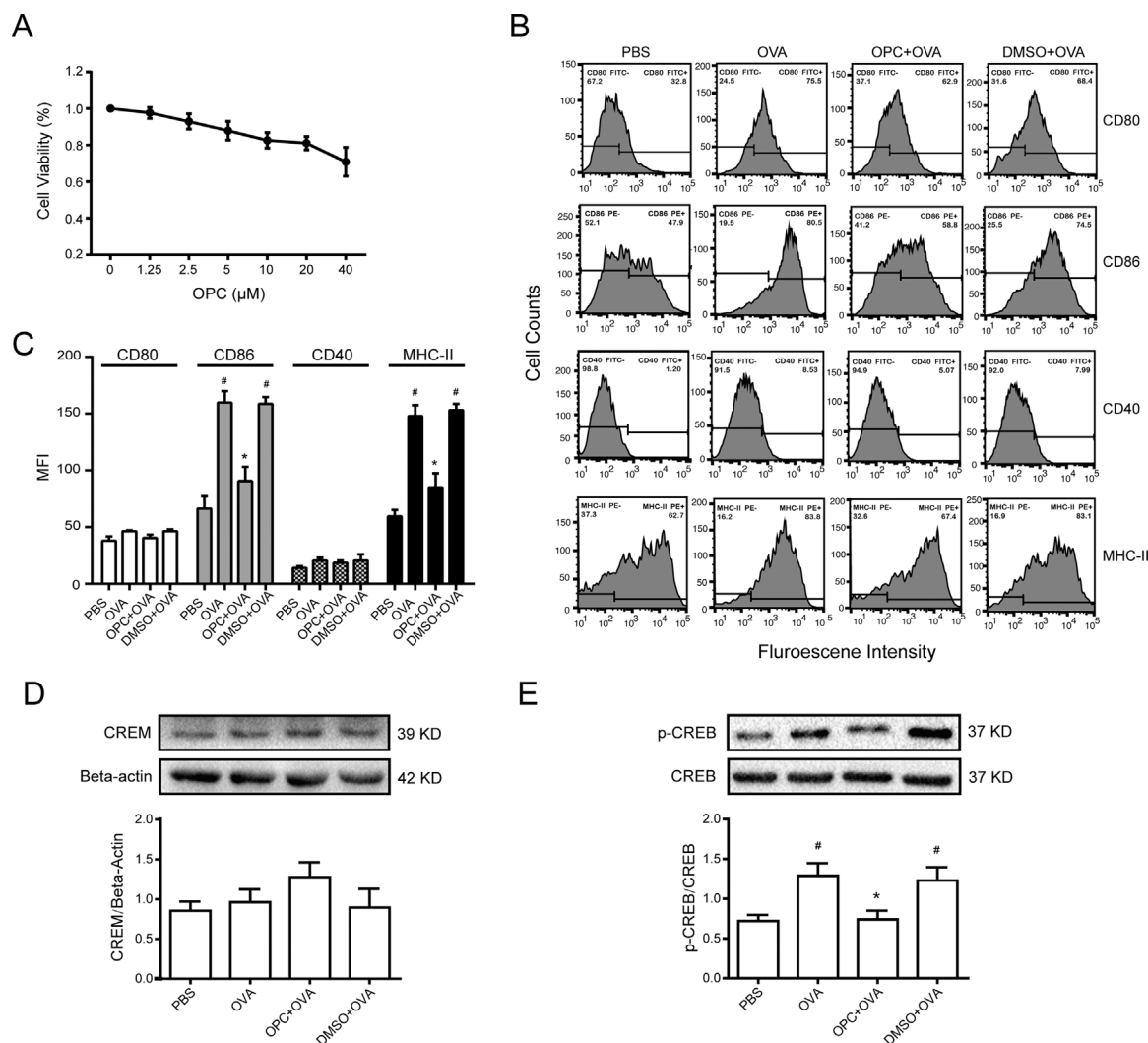


Fig. 4. Treatment with OPCs inhibited the expression of CD86, MHC-II and phosphorylation of CREB on BMDCs. (A) Effect of OPCs on the viability of BMDCs was assessed by the CCK-8 assay. (B, C) The expression of CD80, CD86, CD40, and MHC-II on BMDCs was analyzed by flow cytometry. The expression of activation markers is presented as MFI. (D, E) The expression of CREM, CREB and p-CREB was measured by western blotting. Data represent the mean + SEM from three independent experiments. #P < 0.05 compared to the PBS group, and *P < 0.05 compared to the OVA group.

phosphorylation of CREB. In contrast, OPCs treatment strikingly inhibited the phosphorylation of CREB (Fig. 4E) but not the expression of CREM (Fig. 4D).

3.7. Effects of OPCs on the ability of adoptive DCs to induce airway inflammation

To clarify the impact of OPCs on DCs potential in promoting airway inflammation, we adoptively transferred DCs from OVA + DC or OPC + OVA + DC group into the airways of mice. As expected, mice given OVA + DC exhibited marked allergic inflammation, which was characterized by the infiltration of lymphocytes and eosinophils into the BALF compared to those given non-pulsed DCs. As shown in Fig. 5A, OPCs-pretreated DCs transferring significantly reduced the amount of total cells, lymphocytes, eosinophils and neutrophils in the BALF. Similarly, this transferring also ameliorated the inflammatory responses in the airway of mice compared to the OVA + DC group (Fig. 5B).

3.8. Effects of OPCs on the proliferation of CD4⁺ T cells in the lung by adoptive transfer of DCs

DCs are essential for the proliferation of T lymphocytes *in vivo*. To investigate whether OPCs were able to impact DCs potential to activate

naive T cells, we transferred OVA + DC or OPC + OVA + DC adoptively into the airways of mice, and the CD4⁺ T cells were harvested from lung by flow cytometry. As shown in Fig. 5D, OPC treatment inhibited the proliferation of CD4⁺ T cells compared to OVA + DC.

4. Discussion

As promising agents among polyphenols, OPCs have gained much attention for the potent biological properties such as antibacterial, antiviral and anticarcinogenic effects. Their impacts on inflammatory disorders including inflammatory bowel disease (Wang et al., 2011), collagen-induced arthritis (Ahmad et al., 2013) and allergic asthma (Lee et al., 2012; Zhou et al., 2011) have been recently identified. Our previous study has revealed that OPCs exhibited a potent potential in asthma management (data not shown). However, the underlying mechanism is still obscure and needs to be clarified.

In the present study, we established a murine model of acute asthma to investigate the effects of OPCs on allergic inflammation. Our results suggested that OPCs alleviated OVA-induced disturbance of Th1/Th2, probably due to the modulation of DCs function. Given the strategic location of DCs in Th2-dependent eosinophilic inflammation in asthma (Hammad and Lambrecht, 2008), we put the focus on OPCs in the modulation of DCs maturation.

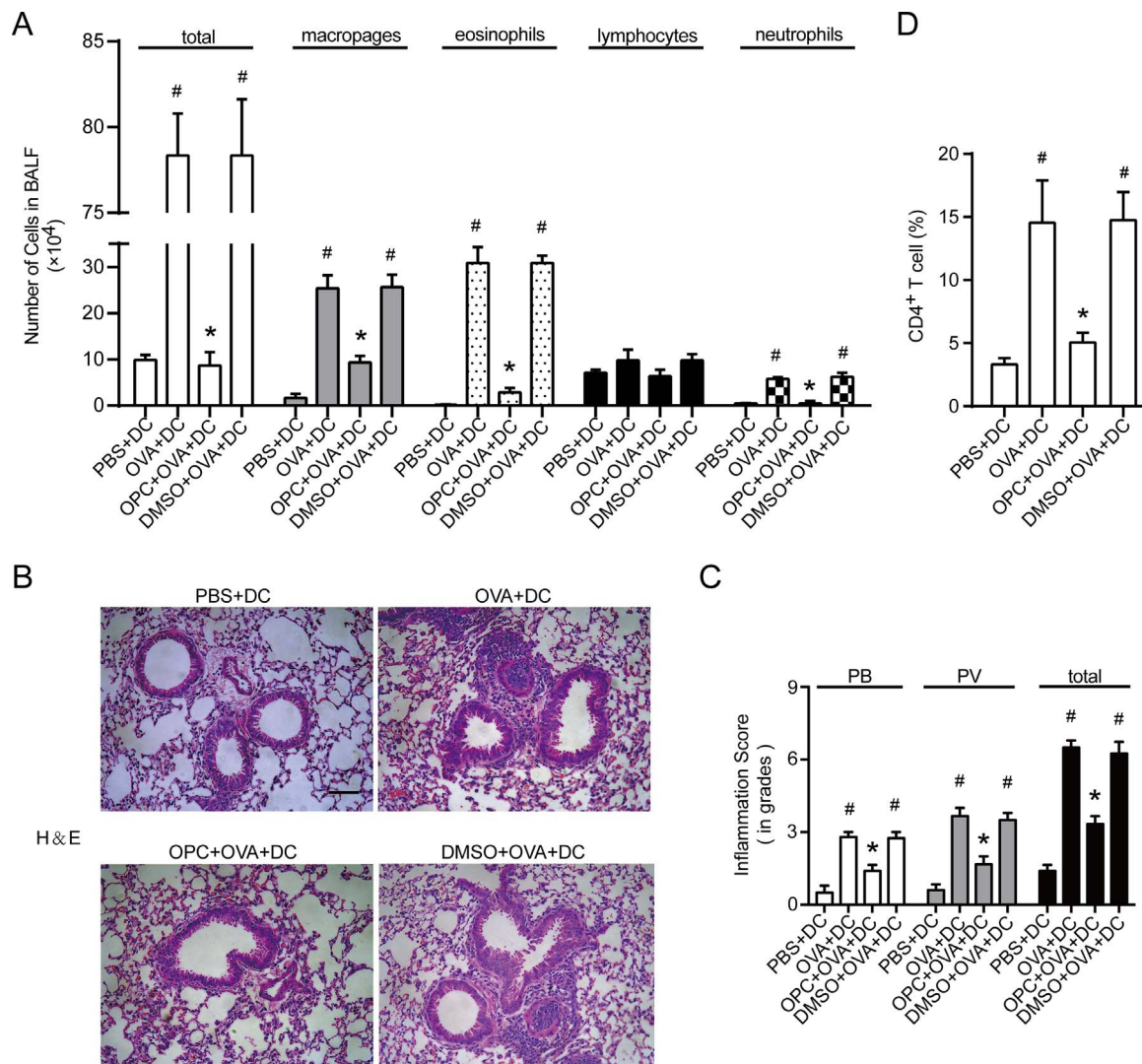


Fig. 5. Treatment with OPCs abolished the ability of adoptive BMDCs to induce airway inflammation and proliferation of CD4⁺ T cells in lung. (A) Cell numbers and differentiation in the BALF were determined by hemocytometer, and at least 200 cells were counted. (B) Lung sections were stained with H & E for measurement of inflammatory cells (magnification, $\times 200$; scale bar, 100 μ m). (C) The layers of inflammation cells were counted and the total inflammation score was summed up with peribronchial (PB) and perivascular (PV) inflammation scores. (D) The number of CD4⁺ T cells in the lung was analyzed by flow cytometry. Data represent the mean $\pm$ SEM ($n = 7$ per group). [#] $P < 0.05$ compared to the PBS + DC group, and ^{*} $P < 0.05$ compared to the OVA + DC group.

As we known, immature DCs are less competent to generate cytokines which are crucial for Th2 response in asthma (Petty and Hunt, 1998). Exposure to exogenous allergens triggers DCs maturation characterized by a provision of co-stimulatory molecules (CD80, CD86, CD40, etc) and MHC-II. Variation of these biomarkers on DCs discloses DCs maturation, and is considered as a key event to T cells activation. Then DCs take up allergens, and migrate to draining lymph nodes where they present allergens to naive T cells (Banchereau and Steinman, 1998). By using flow cytometry, we herein determine the effects of OPCs on co-stimulatory molecules expression. The results showed that the level of CD86 on pulmonary DCs notably declined in OPCs-treated mice compared to those treated with OVA.

Accumulating data have indicated that both CD80 and CD86 on DCs are pivotal for T cells proliferation and proinflammatory cytokines production (Larché et al., 1998; Bashian et al., 1997; Hofer et al., 1998). However, the present points of view remained controversial (Stumbles et al., 1998). CD80 was widely considered to be more implicated in anti-tumor activity, while CD86 was preferentially regarded as a contributor to Th2 responses (Matulonis et al., 1996; Kuchroo et al., 1995). It has been reported that the serum level of soluble CD86 from asthmatics with an acute exacerbation was markedly increased,

and even related to the severity of asthma (Shi et al., 2004). A CD86 antibody was identified to be able to relieve AHR, down-regulate IgE level and alleviate eosinophil inflammation (Haczku et al., 1999). In contrast, CD80 presented complementary roles in allergic responses (Mark et al., 2000). Treatment with CD80 antibody only diminished the extent of eosinophilia in airway (Tsuyuki et al., 1997). Recently, intratracheal administration of small interfering RNA (siRNA) against CD86 has also been confirmed to restrain the severities of eosinophilia and AHR induced by OVA. Meanwhile, declines of IgE in the sera, and decreases of IL-5, IL-13 and CCL17 in BALF were also observed (Asai-Tajiri et al., 2014). In our study, OPCs administration markedly alleviated airway eosinophil inflammation, accompanied by a notable decrease of CD86 on pulmonary DCs. Since CD86 has been well established as a crucial marker for DCs maturation, the evidence presented here indicated that OPCs markedly suppressed DCs maturation, which may contribute to the amelioration of airway inflammation.

BMDCs, derived from bone marrow progenitors, have been used for the study of DCs migration and T cell priming for their similar capabilities with antigen-presenting-cell populations (Lambrecht et al., 2000a). In the present work, by using BMDCs primary cultures, we investigated possible pathways that regulated DCs maturation. CREB, a

dimeric transcription factor, is verified to be activated through a phosphorylation at Ser133 and/or nuclear translocation of transducer of regulated CREB activity coactivators. This activation of CREB promotes the transcription of more than 5000 target genes. It has been well documented that CREB was quite crucial for butyrate-dependent CD86 transcription, and there was a binding site for CREB in CD86 promoter sequence (Suzuki et al., 2003). Another cAMP responsive element binding protein CREM is also implicated in CD86 transcription and has been proved to suppress CD86 expression (Ahlmann et al., 2009). CREM has been shown as a negative regulator of Th2 response, determining the development of allergic asthma (Verjans et al., 2015). In our study, we examined whether CREB and/or CREM were involved in the OPCs-elicited downregulation of CD86. Our data elucidated that pretreatment with OPCs evidently mitigated CREB phosphorylation with little effect on CREM level. Since CREB and CREM are so crucial in an array of physiological events, our findings provided assertive evidence that OPCs notably suppress DCs maturation via CREB signaling.

In our adoptive transfer model, transfer of OVA-challenged DCs pretreated with OPCs markedly inhibited the infiltration of inflammatory cells in BALF and around airway. Compared to the mice challenged by OVA alone, those pretreated with OPCs exhibited a reduced proliferation of CD4⁺ T cells in lungs. These findings further confirmed the direct effects of OPCs on DCs functions, hinting a diminished capacity of DCs to prime T-cell response.

Taken together, this is the first report to demonstrate that OPCs effectively inhibited airway inflammation and AHR in a murine model of asthma. The effects of OPCs could be mediated by the regulation of DCs function. *In vitro*, OPCs potently suppressed BMDCs maturation via CREB pathway. Adoptive transfer with DCs pretreated with OPCs significantly alleviated Th2 inflammation. These results pave the way for our further understanding of immunoregulatory function of OPCs. Combined with our previous findings as to OPCs potency in asthma management, our results suggest that OPCs might be potential options to modulate DCs function as a promising strategy for asthma.

Conflicts of interest statement

The authors declare no conflict of interest.

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