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# Attenuation of allergic responses following treatment with resveratrol in anaphylactic models and IgE-mediated mast cells

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Resveratrol exists widely in plant species and has a variety of anti-oxidant, anti-inflammatory, and immunomodulatory properties. However, there have been few reports regarding its anti-food allergic activity. In this study, we demonstrated that resveratrol (isolated from *Abies georgei*) could decrease the release of  $\beta$ -hexosaminidase and histamine in rat basophilic leukemia-2H3 cells. Resveratrol was not only found to suppress the development of diarrhea, up-regulate the rectal temperature of ovalbumin-allergic mice, and decrease the serum level of specific immunoglobulin E, mouse mast cell protease-1 and histamine, but also found to decrease the population of dendritic cells, B cells and mast cells of ovalbumin-allergic mice in the spleen or mesenteric lymph node. Furthermore, resveratrol inhibited the release of  $\beta$ -hexosaminidase and histamine in bone marrow-derived cells and alleviated mast cell-mediated passive cutaneous anaphylaxis reactions. These findings indicated that resveratrol isolated from *Abies georgei* might have the potential to alleviate food hypersensitivity or allergic disease.

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## Introduction

Food allergy involves an abnormal immune response to specific food proteins, characterized by an adverse reaction mediated by immunoglobulin (Ig)E or non-IgE antibodies.<sup>1,2</sup> In industrialized countries, IgE-mediated hypersensitivity (type I allergy) is the most common allergic disease.<sup>3</sup> Moreover, in several countries, food allergies affect a substantial proportion of the population, with a prevalence as high as 8% in children and 2%–3% in adults.<sup>4</sup> Furthermore, egg allergies are common among young children, with a prevalence rate of 1.2%–2.0%, and the ovalbumin (OVA) allergy model is often used to evaluate the anti-allergic activities of various materials.<sup>2,5,6</sup> Therefore, there is a clear need for effective therapeutic approaches to food allergies.

There is a wide range of immune cells that can trigger or inhibit the allergic reaction following stimulation with food allergens.<sup>7</sup> Dendritic cells (DCs) are a type of antigen-presenting cell, and have been shown to play an important role in both innate and adaptive immunity; thus, aberrant DC activation can induce detrimental immune responses.<sup>8</sup> Moreover, DCs are thought to be central to various strategies of therapeutic manipulation. For example, Liu *et al.* demonstrated that the enzymatic cross-linking product of horseradish peroxidase and tropomyosin has the potential to reduce the allergenicity of tropomyosin *via* altering the protein morphology, changing the method of antigen presentation by CD11c<sup>+</sup> DCs and inducing oral tolerance to the allergen, tropomyosin.<sup>9</sup> In addition to DCs, regulatory T (Treg) cells are also thought to be associated with oral tolerance to allergens.<sup>10</sup> Furthermore, Treg cells are generally believed to alleviate the clinical symptoms of hypersensitivity to dietary food allergens by modulating the priming of allergen-specific T and B cell responses during oral sensitization.<sup>11</sup> Activated CD4<sup>+</sup> T helper (Th)2 cells produce interleukin (IL)-4 and IL-5 and IL-13, which facilitate an allergic response.<sup>12</sup> Furthermore, upon stimulation with cytokines secreted by CD4<sup>+</sup> Th2 cells, B cells produce antigen-specific IgE, which is involved in the activation process of mast cells (MCs) and basophils.<sup>7</sup>

MCs and basophils are inflammatory effector cells which play a unique role in IgE-mediated allergic reactions.<sup>13</sup> These cells produced several allergic mediators, including proinflam-

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matory cytokines, chemokines, cytoplasmic granule-derived mediators, and lipid-derived mediators following activation by the cross-linking of the high affinity receptor for IgE (FcεRI).<sup>13</sup> Moreover, these mediators can trigger both acute and chronic inflammation.<sup>14</sup> Several studies have demonstrated that targeting MCs may be a promising strategy for the treatment of allergic disorders.<sup>13,15</sup> It has also been reported that human milk oligosaccharides can suppress the symptoms of food allergies by indirectly inhibiting the activation of MCs and inducing the production of IL-10<sup>+</sup> Treg cells.<sup>6</sup> *In vitro*, rat basophilic leukemia (RBL-2H3) and a bone marrow-derived mast cell (BMMC) assay have been established to assess the anti-allergic activities of various materials.<sup>16,17</sup> *In vivo*, passive cutaneous anaphylaxis (PCA) and passive systemic anaphylaxis are commonly used to further estimate MC-mediated anaphylaxis.<sup>18,19</sup> Although studies have shown that certain substances can alleviate allergic symptoms by modulating immune cells, few reports have determined which types of immune cells are primarily regulated following such treatment.

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) is a non-flavonoid polyphenolic compound belonging to the stilbene group that exists naturally in many plant species, including grapes, berries, and peanuts.<sup>20</sup> It has been previously shown that resveratrol can mediate a series of biological activities, including anti-oxidant, anti-inflammatory and immunomodulatory effects.<sup>21–23</sup> For example, resveratrol was found to effectively inhibit lipopolysaccharide-induced inflammation of RAW 264.7 macrophages.<sup>23</sup> Resveratrol typically exists naturally in *cis* and *trans* forms. Some studies have shown that the resveratrol *trans* isomer is more biologically active and stable.<sup>24,25</sup> According to a previous report, *trans* but not *cis* resveratrol was found to impair angiotensin-II-mediated vascular inflammation through the inhibition of NF-κB activation.<sup>25</sup> However, the anti-food allergic activities and related mechanisms of resveratrol have rarely been reported.

In the present study, resveratrol was isolated from *Abies georgei* by Yang *et al.*<sup>26</sup> and the toxicity and anti-allergic properties of resveratrol were measured in RBL-2H3 cells. Furthermore, the anti-food allergic activities of resveratrol were assessed using an OVA-induced mouse model, and its regulatory effects on associated immune cells were then explored. Moreover, a BMMC assay and PCA model were employed to verify the potential mechanism of the anti-food allergic activity of resveratrol.

## Materials and methods

### Materials

Imject alum was purchased from Thermo Fisher Scientific Inc (Waltham, MA, USA). OVA, Evans blue, 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide, loratadine, and anti-dinitrophenyl (DNP)-IgE were purchased from Sigma (St Louis, MO, USA), and DNP-BSA was purchased from Biosearch (Petaluma, CA, USA). Fetal bovine serum (FBS), penicillin–streptomycin solution, trypsin 0.25% (1X) solution, RPMI 1640 and Eagle's minimum

essential medium were purchased from Hyclone (Logan, UT, USA). Recombinant mouse IL-3 protein and mouse stem cell factor were obtained from PeproTech (Rocky Hill, NJ, USA). ELISA kits for histamine, mouse mast cell proteinase (mMCP)-1, and anti-OVA-IgE were obtained from IBL (Hamburg, Germany), R&D Systems (Minneapolis, MN, USA), and Cayman (Ann Arbor, MI, USA), respectively. Goat anti-mouse IgG1 and IgG2a antibodies were obtained from Abcam (Cambridge, UK). All antibodies for flow cytometry analysis were purchased from BioLegend (San Diego, CA, USA). Resveratrol was isolated from the aerial parts of *Abies georgei*, as described by Yang *et al.*<sup>26</sup> The purity of resveratrol was 98% as assessed by HPLC.

Female BALB/c mice (6–8 weeks) were obtained from the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Shanghai, China), and housed in an SPF environment with a relatively stable temperature (22 ± 1 °C) and humidity (55 ± 10%) for one week before experimentation. This study was performed in strict accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985) and was approved by the Institutional Animal Care and Use Committee of Animal Laboratory Center of Jimei University (Xiamen, China, No. SCXK 2012-0005). In the mouse model, five mice were included in each group.

### RBL-2H3 assay

RBL-2H3 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in an incubator with 5% CO<sub>2</sub> at 37 °C. The cytotoxic effects of resveratrol on RBL-2H3 cells were first determined by the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) assay. β-Hexosaminidase and histamine in the cell supernatant, released by RBL-2H3 cells pretreated with or without resveratrol, were quantified as previously described.<sup>17</sup>

### OVA-induced model of food allergy

After one-week of acclimation, age-matched mice (7–9 weeks old) were sensitized with 100 μg OVA and 2 mg alum in 200 μL phosphate-buffered saline (PBS) by intraperitoneal (i.p.) injection on Days 0 and 14. From Days 28 to 40 of the protocol, mice were challenged with 50 mg OVA (in 200 μL of PBS) by gavage every three days, for a total of five challenge events. OVA-sensitized and OVA-challenged mice were treated without (OVA group) or with resveratrol (resveratrol groups) every day starting from Day 27. As a positive control, OVA-sensitized and OVA-challenged mice were treated with 20 mg kg<sup>−1</sup> loratadine (loratadine group) by gavage. Mice sensitized with 2 mg alum in 200 μL PBS and challenged with PBS served as the controls (PBS group). Diarrhea, anaphylaxis, and rectal temperature were measured as indices of food allergy in accordance with a previous study.<sup>27</sup> Thirty minutes after the last challenge, serum was collected *via* the tail vein. One day after the last challenge, mice were sacrificed, and the serum, spleen (SPL) and mesenteric lymph node (MLN) were collected. Sera were stored at −80 °C after centrifugation at 4000g for 10 min. Serum anti-OVA-IgE was determined using an anti-OVA-IgE

ELISA kit in accordance with the manufacturer's instructions. Specific IgG1 and IgG2a were measured using an indirect ELISA as previously reported.<sup>28</sup> Serum histamine and mMCP-1 were quantified using commercial ELISA kits as described by the manufacturer.

### BMMC assay

BMMCs from a normal mouse were obtained and cultured as described previously.<sup>17</sup> After four weeks, more than 90% of the cells expressed MC-specific surface receptors (c-Kit and FcεRI α-chain) as identified by flow cytometric analysis. BMMCs were sensitized with 0.1 μg mL<sup>-1</sup> anti-DNP-IgE overnight and treated with or without resveratrol for 1 h. The cells were then stimulated with DNP-BSA (0.5 μg mL<sup>-1</sup>) for 15 min to detect the levels of histamine. Cells were stimulated with DNP-BSA for 1 h to determine the level of β-hexosaminidase.

### Flow cytometry

Single-cell suspensions were isolated from the SPL and MLN in the OVA-induced food allergic mice 24 h after the last challenge. These cells were labeled with anti-CD3, anti-CD4, anti-CD19, anti-Foxp3, anti-MHC-II, anti-CD11c, anti-c-Kit, and anti-FcεRIα antibodies, and analyzed on a Guava easyCyte 6-2L system using ACS Paragon Plus Environment Guava Soft 3.1.1 software (Millipore, MA, USA).

### PCA model

An IgE-mediated PCA model was used based on the methods of a previous study,<sup>29</sup> with the following modifications. Anti-

DNP-IgE (0.1 μg) was subcutaneously injected into the ears of BALB/c mice. After 24 h, the mice were treated with resveratrol (20 mg kg<sup>-1</sup>) by gavage. One hour later, the mice were injected DNP-BSA (0.2 mg) in PBS containing Evans blue (5 mg mL<sup>-1</sup>), *via* the tail vein. Thirty minutes later, the mice were sacrificed, the ears were removed and soaked in 3 M KOH at 37 °C overnight, and a mixture of acetone and phosphoric acid (13:5) was added. The samples were centrifuged at 3000g for 15 min, and supernatants were measured with a microplate reader at 620 nm.

### Statistical analysis

Experimental results are expressed as the mean ± standard deviation. Differences between the groups were analyzed with an ANOVA. Significant differences between the drug-treated groups (resveratrol or loratadine group) and the OVA group were determined using a Duncan highest significance difference test. The *P* value for significance was set at 0.05. Each experiment was repeated at least three times.

## Results

### Resveratrol decreased the release of β-hexosaminidase and histamine in RBL-2H3 cells

In this study, *trans*-resveratrol (100.9 mg) was isolated by Yang *et al.* from *Abies georgei* plant material (22 kg). The chemical structure of resveratrol was then identified, as shown in Fig. 1A. The effect of resveratrol on RBL-2H3 cell cytotoxicity

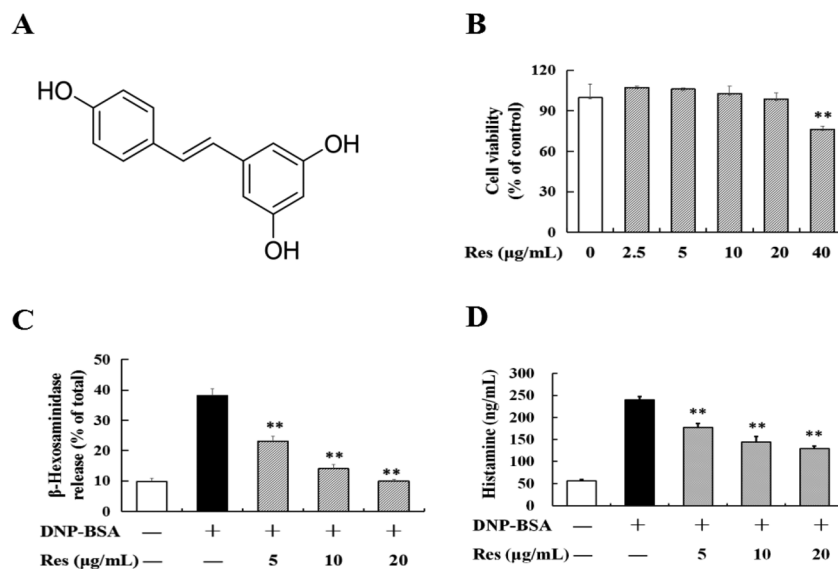


**Fig. 1** Chemical structure of resveratrol and its effects on RBL-2H3 cells. A. The chemical structure of resveratrol isolated from *Abies georgei*. B. The cytotoxicity of resveratrol on RBL-2H3 cells was evaluated using the MTT assay. RBL-2H3 cells were incubated with resveratrol at a dose of 2.5–40 μg mL<sup>-1</sup>. C. The effect of resveratrol on the level of β-hexosaminidase released from IgE-sensitized RBL-2H3 cells. RBL-2H3 cells (1 × 10<sup>6</sup> cells per mL) sensitized overnight by 0.1 μg mL<sup>-1</sup> anti-DNP-IgE in 48-well plates were treated with concentrates of resveratrol (5, 10, and 20 μg mL<sup>-1</sup>) for 1 h and stimulated with 0.1 μg mL<sup>-1</sup> DNP-BSA for 1 h. Then, the quantities of β-hexosaminidase in the cell supernatant and lysate were measured. D. The effect of resveratrol on the level of histamine released from IgE-sensitized RBL-2H3 cells. Cells were sensitized and treated the same as C, except for stimulation with DNP-BSA for 15 min and using ELISA to measure the level of histamine in the cell supernatant. \**P* < 0.05, \*\**P* < 0.01. The data represent the mean ± SD of triplicate determinations. Res, resveratrol.

was assessed using the MTT assay. Resveratrol ( $<20 \mu\text{g mL}^{-1}$ ) had no significant effect on RBL-2H3 cell viability; however, resveratrol at a concentration of  $40 \mu\text{g mL}^{-1}$  resulted in 23% cytotoxicity (Fig. 1B). Therefore, we assessed the anti-allergic effects of resveratrol at doses of 5, 10, and  $20 \mu\text{g mL}^{-1}$ . As shown previously,  $\beta$ -hexosaminidase and histamine are the iconic products of activated MCs and basophils, and have long been demonstrated to be critically associated with allergic responses.<sup>13,14</sup> As presented in Fig. 1C and D, resveratrol significantly inhibited the release of  $\beta$ -hexosaminidase and histamine from RBL-2H3 cells in a dose-dependent manner.

## Resveratrol alleviated allergic reactions in an OVA-induced mouse model

Then, resveratrol was used to evaluate its anti-allergic biological activities. The protocol for the OVA-induced allergy model is shown in Fig. 2A. As shown previously, successive oral challenges with an allergen in sensitized mice led to diarrhea, hypothermia, and a high anaphylactic score.<sup>30</sup> We determined the anti-allergic activities of resveratrol in comparison to loratadine, which demonstrated that it could inhibit the development of diarrhea and decrease the severity of symptom scores at a dose of  $20 \text{ mg kg}^{-1}$ .<sup>31</sup> After daily treatment with

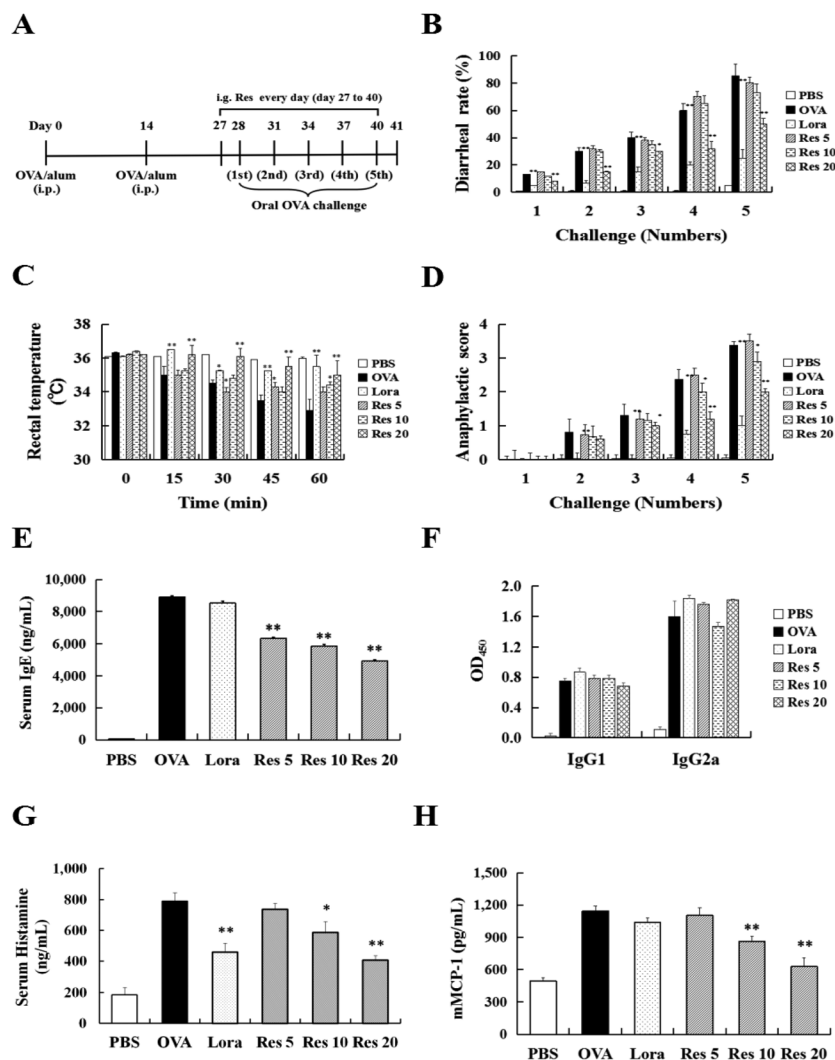


**Fig. 2** Protocol for the food allergy model and the effects of resveratrol on the allergic reactions. A. Protocol for the food allergy model. Mice were sensitized with  $100 \mu\text{g}$  OVA and  $2 \text{ mg}$  alum in  $200 \mu\text{L}$  of PBS, orally challenged with  $50 \text{ mg}$  OVA in  $200 \mu\text{L}$  of PBS, and concentrates of resveratrol ( $5, 10$ , and  $20 \text{ mg kg}^{-1}$ ) or loratadine ( $20 \text{ mg kg}^{-1}$ ) were administered by gavage ( $n = 5$ ). B. The effect of daily treatment with resveratrol on diarrhea severity. The rate of diarrhea was measured 1 h after every OVA challenge. C. The effect of daily treatment with resveratrol on the rectal temperature of allergic mice. The rectal temperature of mice was measured after every OVA challenge, within one hour. D. The effect of daily treatment with resveratrol on the anaphylactic score measured for 1 h after every OVA challenge. E. The effect of daily treatment with resveratrol on serum OVA-specific IgE. The serum from the eyeball was collected on day 41 of the food allergy protocol. F. The effect of daily treatment with resveratrol on serum OVA-specific IgG1 and IgG2a. The serum from the eyeball was collected on day 41 of the food allergy protocol. G. The effect of daily treatment with resveratrol on serum histamine. The serum from the tail veins of mice was collected 1 h after the last challenge. H. The effect of daily treatment with resveratrol on serum mMCP-1. The serum from the tail veins of mice was collected 1 h after the last challenge. \* $P < 0.05$ , \*\* $P < 0.01$ . The data represent the mean  $\pm$  SD of triplicate determinations. Res, resveratrol; Lora, loratadine.

resveratrol, the levels of allergic diarrhea, hypothermia, and anaphylactic score in mice were attenuated. Moreover, we demonstrated that 20 mg kg<sup>-1</sup> resveratrol displayed approximately equal activity for suppressing the development of diarrhea, up-regulating the rectal temperature and decreasing the severity of symptom scores compared with loratadine (Fig. 2B–D).

Increased allergic mediators are thought to be associated with more severe allergic reactions.<sup>28</sup> In this study, the level of OVA-specific IgE, IgG1, and IgG2a increased robustly in the OVA group (Fig. 2E and F). Following treatment with 5, 10, or 20 mg kg<sup>-1</sup> resveratrol, the level of OVA-specific IgE was decreased significantly to 6341.8 ng mL<sup>-1</sup>, 5877.8 ng mL<sup>-1</sup>, and 4926.5 ng mL<sup>-1</sup>, respectively, while the level of OVA-specific IgE in the PBS group was only 5.78 ng mL<sup>-1</sup>. Compared with the loratadine group, the inhibitory effect of resveratrol on OVA-specific IgE was more significant at the lowest concentration (5 mg kg<sup>-1</sup>) (Fig. 2E). However, resveratrol and loratadine exhibited no significant effect on the release of OVA-specific IgG1 and IgG2a (Fig. 2F). Resveratrol decreased the release of histamine induced by OVA in the serum in a dose-dependent manner, and resveratrol (20 mg kg<sup>-1</sup>) decreased the level of serum histamine to 407.9 ng mL<sup>-1</sup>, compared with loratadine (459.9 ng mL<sup>-1</sup>). In a word, the inhibitory effect on the histamine release of resveratrol (20 mg kg<sup>-1</sup>) was nearly equal to that observed with loratadine (Fig. 2G). At a dose of 10 or 20 mg kg<sup>-1</sup>, resveratrol significantly down-regulated the level of mMCP-1; however, this effect was not observed with loratadine (Fig. 2H).

As shown in Fig. 2, the effects of resveratrol on the allergic symptoms and common allergic mediators of OVA-allergic mice were initially identified by comparing it with loratadine, known as an H1-antihistamine.<sup>31,32</sup> We didn't set a positive group in the experiment to explore the regulation effects of resveratrol on immune cells. Because these sections were used to further investigate the primary target cells of resveratrol, few substances have the same mechanism of action. In addition, since we

observed that 20 mg kg<sup>-1</sup> resveratrol exhibited the greatest anti-allergic effect on OVA-allergic mice, we will only present the data for 20 mg kg<sup>-1</sup> resveratrol for the remaining experiments.

### Effects of resveratrol on DCs in the SPL and MLN

Recently, the primary method of treating food allergies has been to interfere with the allergic responses induced by immune cells.<sup>9,10,13</sup> Based on the unique ability of DCs to initiate primary immune responses, the effect of resveratrol on DCs was investigated in this study. In the OVA group, the percentage of DCs in the SPL increased by as much as 12.74%. Following treatment with resveratrol, the number of DCs decreased sharply to 6.85%. However, there were no significant regulatory effects on DCs in the MLN following treatment with resveratrol (Fig. 3).

### Effects of resveratrol on Th cells and Treg cells in the SPL and MLN

Th cells are important for activating B cells and promoting the production of allergic mediators that lead to allergic reactions.<sup>12</sup> In contrast, Treg cells are thought to inhibit CD4<sup>+</sup> cells and are involved in the regulatory response to allergic inflammation.<sup>33</sup> Thus, we analyzed the effect of resveratrol on Th and Treg cells in the SPL and MLN. Compared with the PBS group, the CD3<sup>+</sup> CD4<sup>+</sup> Th cell population in the SPL and MLN of the OVA group increased by about 5.53% (from 65.50% to 71.03%) and 0.44% (from 80.07% to 80.51%), respectively, which indicated that Th cells increased in response to an OVA-induced allergic reaction. We found that resveratrol had a weak inhibitory effect on the percentage of Th cells only in the SPL (Fig. 4A). An increased Treg cell population is believed to prevent the development of an allergic response.<sup>6</sup> However, there was no effect of resveratrol on Treg cells in the SPL or MLN (Fig. 4B). Therefore, resveratrol appears to have little effect on Th and Treg cells. These results were consistent with the levels of OVA-specific IgG1 and IgG2a (Fig. 2F). Taken together, resveratrol appears to alleviate the

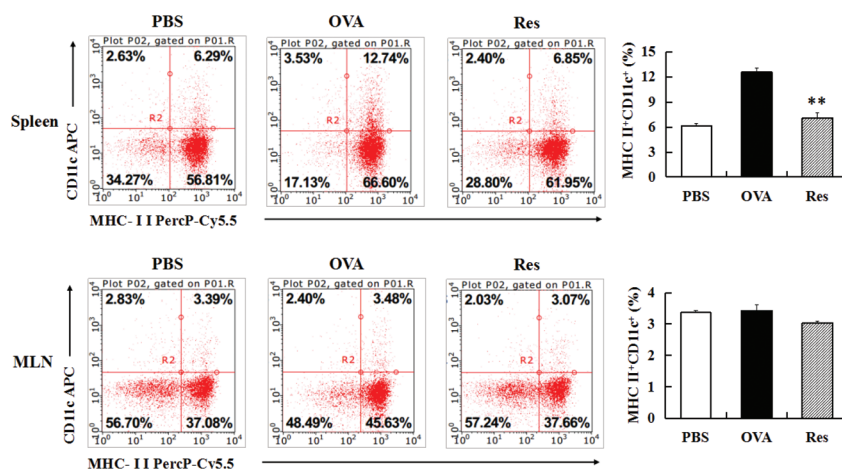


**Fig. 3** Effects of resveratrol on the population of DCs in the SPL and MLN. The scatter diagrams of DC FACS analysis. Spleens and MLNs were isolated from each group of mice 24 h after the last challenge and labeled with anti-CD11c and anti-MHC II antibodies. \**P* < 0.05, \*\**P* < 0.01. The data represent the mean ± SD of triplicate determinations. Res, resveratrol.

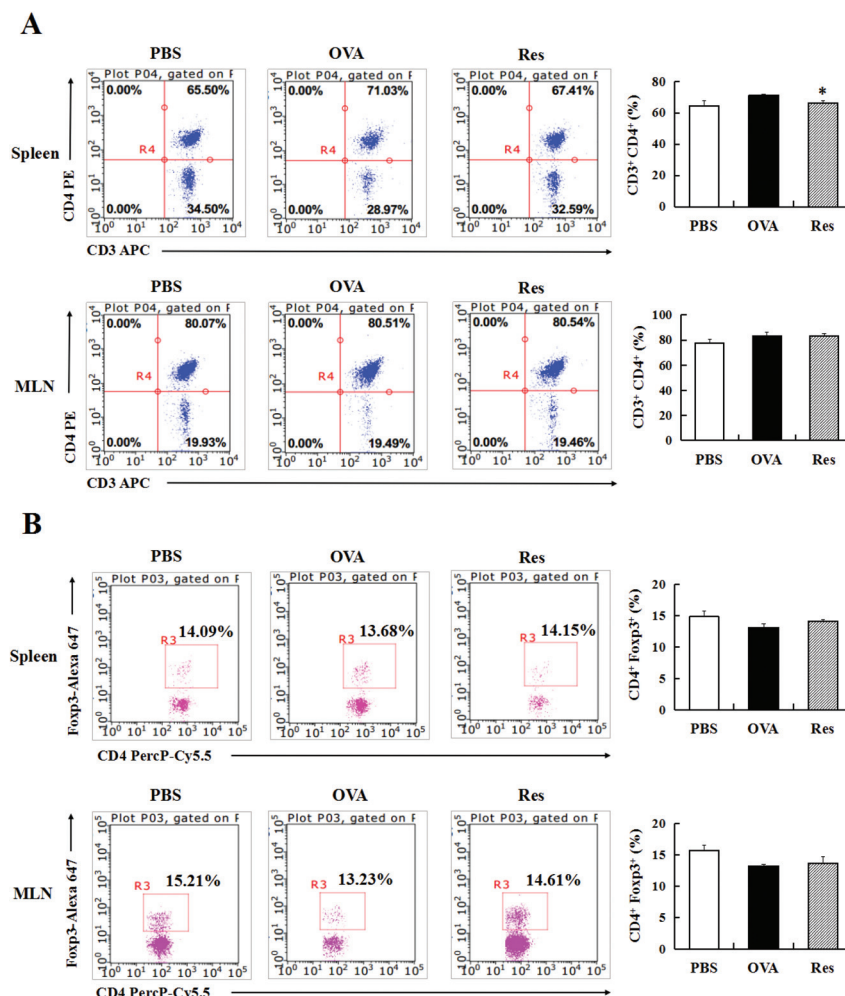


**Fig. 4** Effects of resveratrol on the population of Th cells and Treg cells in the SPL and MLN. A. The scatter diagrams of Th cells FACS analysis. Spleens and MLNs were isolated from each group of mice 24 h after the last challenge and labeled with anti-CD3 and anti-CD4 antibodies. B. The scatter diagrams of Treg cell FACS analysis. Spleens and MLNs were isolated from each group of mice 24 h after the last challenge and labeled with anti-CD4 and anti-Foxp3 antibodies. \* $P < 0.05$ , \*\* $P < 0.01$ . The data represent the mean  $\pm$  SD of triplicate determinations. Res, resveratrol.

allergic response primarily *via* the allergen presentation capacity of DCs, rather than targeting the functionality of CD4<sup>+</sup> Th and CD4<sup>+</sup>Foxp3<sup>+</sup> Treg cells in an OVA-induced mouse model.

### Resveratrol decreased the population of B cells and MCs in the SPL and MLN

Considering the suppressive effects of resveratrol on serum OVA-specific-IgE, lymphocytes from the SPL and MLN from each treatment group were labeled with anti-CD19 and analyzed by flow cytometry. Compared with the PBS group, the population of CD19<sup>+</sup> B cells in the SPL and MLN of the OVA group increased by approximately 14% and 10%, respectively. Moreover, there was a significant decrease in the percentage of CD19<sup>+</sup> cells in the SPL and MLN of the resveratrol group compared with the OVA group (56.32% *vs.* 70.14% and 29.12% *vs.* 39.62%, respectively) (Fig. 5A).

MCs release allergic mediators more robustly and rapidly compared to CD4<sup>+</sup> Th cells, thus leading to more serious allergic reactions.<sup>12</sup> Moreover, MCs play an important role in allergic dis-

eases due to their wide distribution in various tissues.<sup>34</sup> In this study, mice challenged with OVA showed a significant increase in the percentage of MCs in the SPL and MLN compared with the sham-sensitized mice (2.04% *vs.* 0.13% and 2.62% *vs.* 1.82%, respectively). Moreover, resveratrol treatment led to a significant decrease in the percentage of MCs in the SPL and MLN (1.64% and 1.08%, respectively) (Fig. 5B). Taken together, resveratrol treatment can decrease the population of B cells and MCs in the SPL and MLN of OVA-allergic mice. Therefore, resveratrol appears to alleviate the allergic response through decreasing the number of B cells and MCs in the SPL and MLN.

### Resveratrol suppressed the allergic response in a BMMC assay and PCA model

To further verify the effects of resveratrol on MCs, we performed a BMMC assay and used a PCA model. As expected, resveratrol inhibited the release of  $\beta$ -hexosaminidase from BMBCs in a dose-dependent manner (Fig. 6A). Furthermore, the changes in histamine levels in each of the different

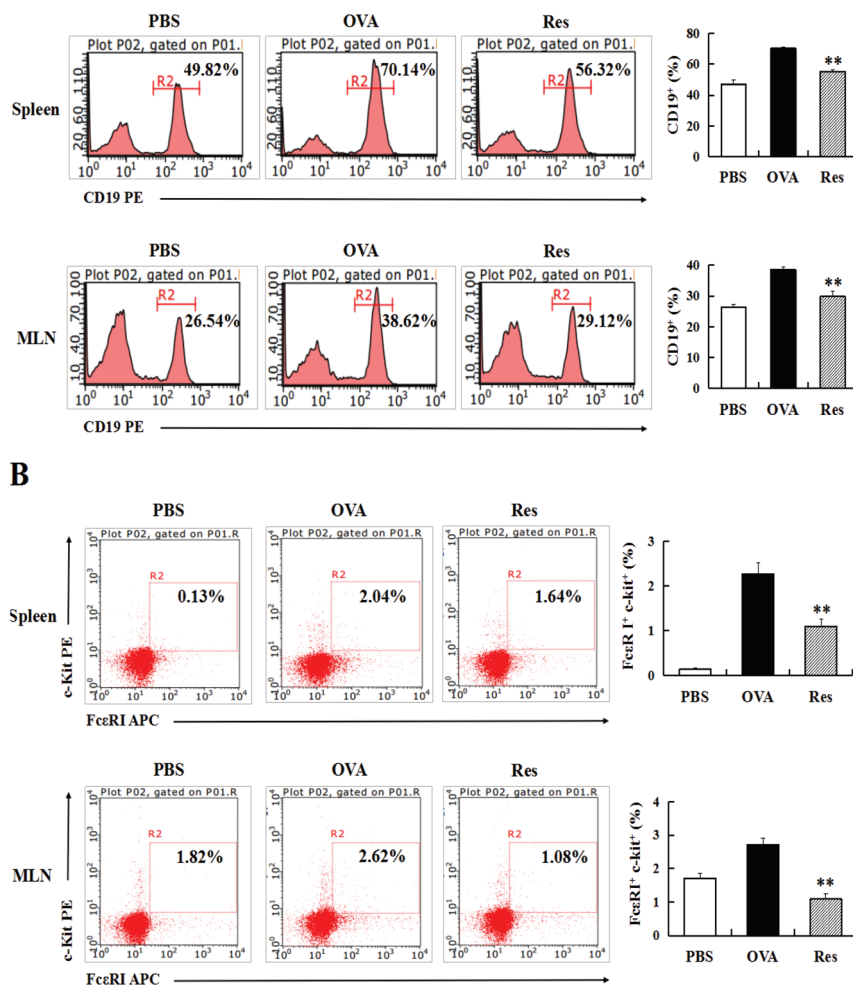


**Fig. 5** Effects of resveratrol on the population of B cells and MCs in the SPL and MLN. A. The histogram of B cell FACS analysis. Spleens and MLNs were isolated from each group of mice 24 h after the last challenge and labeled with anti-CD19 antibodies. B. The scatter diagrams of MC FACS analysis. Spleens and MLNs were isolated from each group of mice 24 h after the last challenge and labeled with anti-c-Kit and anti-FcεRIα antibodies. \* $P < 0.05$ , \*\* $P < 0.01$ . The data represent the mean  $\pm$  SD of triplicate determinations. Res, resveratrol.

groups were also measured. Compared with the PBS group ( $51.2 \text{ ng mL}^{-1}$ ), the level of histamine in the DNP-BSA group was significantly increased ( $224.8 \text{ ng mL}^{-1}$ ). However, the level of histamine in the resveratrol group (5, 10, and  $20 \mu\text{g mL}^{-1}$ ) declined significantly (183.4, 167.1, and  $158.2 \text{ ng mL}^{-1}$ , respectively) (Fig. 6B). In the MC-dependent PCA model, loratadine was also used as a positive control. Resveratrol ( $20 \text{ mg kg}^{-1}$ ) significantly decreased the absorbance of Evans blue dye at 620 nm, with an equivalent inhibitory effect compared with loratadine (Fig. 6C and D). Taken together, these findings indicated that resveratrol could effectively suppress the allergic reactions of IgE-stimulated BMNCs and PCA in mice.

## Discussion

Clinically, although drug treatment can effectively relieve the allergic symptoms of patients, there are some side effects during the process of drug action. Therefore, new therapies

and medications (especially natural active substances) are required to improve the treatment of food allergies.<sup>35</sup> *Abies georgei* has been reported to contain over 100 potential bioactive compounds.<sup>2</sup> Previously, *trans*-resveratrol has been reported to induce a stronger anti-inflammatory effect, compared with *cis*-resveratrol.<sup>25</sup> In this study, *trans*-resveratrol ( $100.9 \text{ mg}$ ) was isolated by Yang *et al.* from *Abies georgei* powdered plant material ( $22 \text{ kg}$ ). At doses of 5, 10, and  $20 \mu\text{g mL}^{-1}$ , resveratrol displayed no cytotoxic effects and exhibited strong inhibitory effects on the levels of  $\beta$ -hexosaminidase and histamine production in an RBL-2H3 assay. Therefore, its strong inhibitory effects on the levels of  $\beta$ -hexosaminidase and histamine may be associated with its *trans* structure.

Recently, one study demonstrated that resveratrol showed effective anti-inflammatory activities through the inhibition of PGE2- or NO-mediated inflammation and NF- $\kappa$ B-mediated inflammatory gene expression.<sup>23</sup> However, its anti-allergic activities have rarely been explored. We determined the anti-allergic activities of resveratrol in comparison to loratadine,

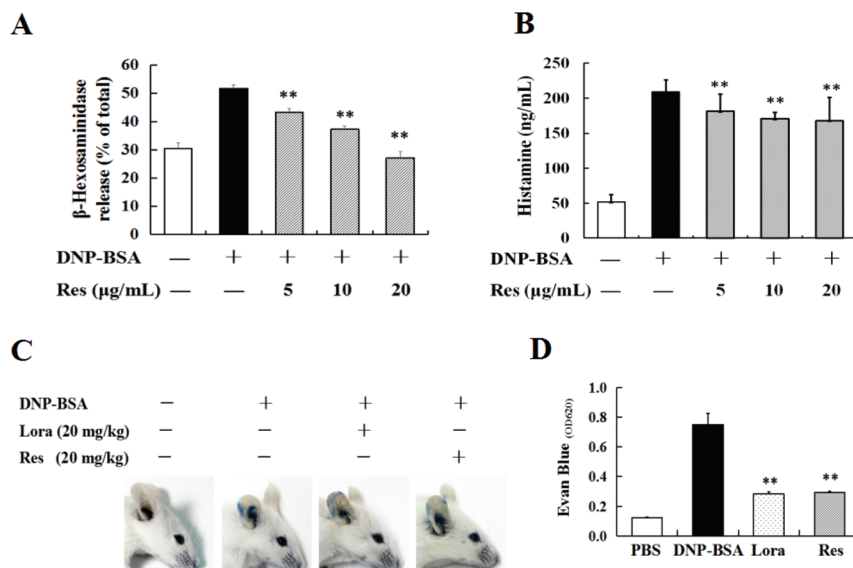


**Fig. 6** Effects of resveratrol on the allergic responses in IgE-mediated BMMCs and PCA model. **A.** The effect of resveratrol on the level of  $\beta$ -hexosaminidase released from IgE-sensitized BMMCs. Mature BMMCs sensitized overnight with anti-DNP-IgE were treated with concentrates of resveratrol (5, 10, 20  $\mu\text{g mL}^{-1}$ ) for 1 h and stimulated with DNP-BSA for 1 h. Then, the quantities of  $\beta$ -hexosaminidase in the cell supernatant and lysate were measured. **B.** The effect of resveratrol on the level of histamine released from IgE-sensitized BMMCs. The same as **A** except for stimulation with DNP-BSA for 15 min and using ELISA to measure the quantities of histamine in the cell supernatant. **C.** The effect of resveratrol on PCA responses. Mice sensitized with anti-DNP-IgE for 24 h were orally administered resveratrol for 1 h, and then were challenged with DNP-BSA. The ears of mice with absorbed Evans blue were photographed ( $n = 5$ ). **D.** The absorbed dye was extracted with 3 M KOH and acetone and phosphoric acid (13 : 5). \* $P < 0.05$ , \*\* $P < 0.01$ . The data represent the mean  $\pm$  SD of triplicate determinations. Res, resveratrol; Lora, loratadine.

which demonstrated that it could inhibit the development of diarrhea and decrease the severity of symptom scores at a dose of 20  $\text{mg kg}^{-1}$ .<sup>31</sup> In this study, we demonstrated that 20  $\text{mg kg}^{-1}$  resveratrol displayed approximately equal activity for suppressing the development of diarrhea, up-regulating the rectal temperature, decreasing the severity of symptom scores, and inhibiting the release of histamine compared with loratadine. Interestingly, resveratrol had a higher inhibitory effect on the level of OVA specific-IgE and mMCP-1 compared to loratadine. It was demonstrated previously that the decreased levels of mMCP-1, histamine, and specific IgE in mice serum were associated with alleviated anaphylaxis in mice.<sup>28</sup> Therefore, resveratrol may have the potential to be a drug for allergic diseases.

CD11c<sup>+</sup> DCs are thought to play an important role in antigen presentation and inducing Treg cells based on the suppression of allergic responses in mouse models of food allergy.<sup>6,36</sup> It has been shown that the intensity of food allergic reactions can be attenuated by DCs and the resulting decreased antigen presentation to T cells may be associated with the decreased number of DCs in the MLN and SPL.<sup>8</sup> In the current study, resveratrol exhibited a significant inhibitory effect on the population of CD11c<sup>+</sup> DCs in OVA-allergic mice in SPL while there are no significant differences between the resveratrol group and the OVA group in MLN. As is previously demonstrated, DCs transport antigens from the MLN to the SPL.<sup>37</sup> Thus, a decrease in the number of DCs in the SPL indicates that the process by which DCs carry antigens from the

MLN to SPL may be disrupted. The results above all indicated that resveratrol alleviated the allergic symptoms which may be associated with the reduced population of DCs, thus affecting the antigen presentation ability of DC to T cells.

In this study, we concluded that the attenuated allergic symptoms observed following the treatment with resveratrol were related to lower serum levels of OVA-specific IgE, histamine and mMCP-1, suggestive of decreased B cell and MC activation, respectively. These results are in accordance with the reduced number of both B cells and MCs (isolated from the MLN and SPL) of OVA-induced allergic mice. Based on these results, we hypothesized that resveratrol contributed to the alleviated allergic responses primarily through suppressing the function of MCs. To test this hypothesis, we performed a BMMC assay and used a PCA mouse model. As expected, resveratrol both suppressed the release of  $\beta$ -hexosaminidase and histamine from BMMCs *in vitro* and attenuated the PCA reaction *in vivo*. As is demonstrated previously, a significantly decreased number of B cells and MCs was associated with the alleviated allergic reactions.<sup>30</sup> These findings are in line with those reported by a previous study, which concluded that egg-specific IgE and basophil activation correlated with the phenotypes of clinical egg allergies.<sup>38</sup> It is thus conceivable that resveratrol suppresses OVA-induced allergic responses by reducing the number of activated MCs and affecting the MC function. While the effect of resveratrol on B cell functionality was not investigated in the present study, it should be explored in the future.

## Conclusion

In conclusion, resveratrol isolated from *Abies georgei* exhibits a potential inhibitory effect on OVA-induced allergic reactions. Our findings suggested that resveratrol alleviated these responses through both reducing the level of serum OVA-specific IgE, histamine, and mMCP-1 and decreasing the number of DCs, B cells, and MCs in the SPL or MLN. Furthermore, resveratrol was capable of suppressing the release of  $\beta$ -hexosaminidase and histamine by BMMCs *in vitro* and attenuating the PCA reaction *in vivo*. Taken together, resveratrol may provide a novel means of treating OVA-induced allergic responses and may have therapeutic potential for allergic disease induced by food allergens.

## Conflicts of interest

The authors declare no competing financial interest.

## Abbreviations

|                  |                                           |
|------------------|-------------------------------------------|
| BMMCs            | Bone marrow derived cells                 |
| DC               | Dendritic cell                            |
| DNP              | Dinitrophenyl                             |
| Fc $\epsilon$ RI | High affinity receptor for IgE            |
| MHC II           | Major histocompatibility complex class II |
| MLN              | Mesenteric lymph node                     |
| OVA              | Ovalbumin                                 |
| PCA              | Passive cutaneous anaphylaxis             |
| RBL              | Rat basophilic leukemia                   |
| SPL              | Spleen                                    |
| Th               | T helper                                  |
| Treg             | Regulatory T                              |

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