



Ovatodiolide inhibits the maturation of allergen-induced bone marrow-derived dendritic cells and induction of Th2 cell differentiation

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ABSTRACT

Ovatodiolide was a unique macrocyclic diterpenoid isolated from the traditional Chinese medicinal herb *Anisomeles indica*. The present study attempted to examine the ovatodiolide effects on dendritic cell (DC) maturation and immuno-stimulatory activities. The effects of ovatodiolide on DC surface molecule expression, cytokine production, and capacity to induce T-cell differentiation were examined in ovalbumin (OVA)/thymic stromal lymphopoietin (TSLP)-stimulated DCs. Ovatodiolide attenuated the expression of DC surface molecules CD80, CD86, histocompatibility complex (MHC) class II, and Th2 subset of CD4⁺ T cells co-stimulatory molecule- OX40 ligand production. Additionally, ovatodiolide suppressed the CD4⁺ T cells proliferation, and production of inflammatory cytokines interleukin (IL)-4, IL-5, and tumor necrosis factor (TNF)-α. This study may be useful to develop ovatodiolide as a therapeutic adjuvant.

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1. Introduction

The incidence of immunological allergic diseases and asthma has been increased over the last five decades worldwide [1]. Among the immune cells, dendritic cells (DCs) are bone marrow-derived leucocytes and play a critical role in the initial immune response [2]. The over-production and the activation of T-helper type 2 (Th2) lymphocytes play an important role in allergic inflammation [3]. Immature DCs are derived from bone marrow progenitor cells and resides in blood or tissues in contact with the external environment. These cells exhibit high endocytic activity and low T-cell activation potential. However, DCs are activated when they are exposed to antigens and become mature DCs [2,3]. Mature DCs can pass through several maturation stages including the upregulation of cell-surface molecules [histocompatibility complex (MHC), costimulatory (CD40, CD80 and CD86)], and secretion of proinflammatory mediators [tumor necrosis factor-α (TNF-α), interleukin (IL)-4 and IL-5] [4]. Aberrant activation of DCs is implicated in the pathogenesis of acute and chronic inflammation

and autoimmune diseases. Therefore, identification and development of therapeutic agents that can modulate functions of DCs are of clinical relevance [4].

Natural products are rich source of various bioactive drugs including immunomodulators. Traditional Chinese medicine (TCM) has become an accepted and increasingly used modality for immunomodulation, especially in Asia [5]. The natural compound ovatodiolide was a macrocyclic diterpenoid isolated from Chinese medicinal herb *Anisomeles indica* (L.) Kuntze [6]. The decoctions of *A. indica* commonly used in traditional medicines for inflammatory skin disorder and immune system deficiencies [7]. Previous studies indicate that ovatodiolide possesses a wide range of biological activities including anti-cancer [8–10], anti-inflammatory [11], bactericide against *Helicobacter pylori* [6], and anti-HIV [12]. However, ovatodiolide effects on the functions of DC maturation have not been reported so far.

The maturation of DCs is a prerequisite for the induction of adaptive immune responses [4]. Several stimuli initiated DC maturation results in the upregulation of costimulatory and MHC molecules, and production of inflammatory cytokines [4]. Thymic stromal lymphopoietin (TSLP) is a DC activator, which is an interleukin (IL)-7-like cytokine produced by epithelial cells [13]. TSLP promotes the migration of myeloid DCs to the regional lymph nodes and induce naive T cells to develop into the Th2 cells via OX40 ligand and OX40 co-stimulatory interactions [14]. TSLP stimulated DCs also produce allergic inflammatory chemokines [14]. On the other hand, ovalbumin (OVA)-induced allergic airway

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inflammation is a model which exhibits symptoms of acute allergic asthma [4,14]. Recent studies show that OVA combined with TSLP (OVA/TSLP) can effectively induce maturation of DCs [4]. In the present study, we sought to characterize the effects of a non-cytotoxic dose of ovatodioidide on murine bone marrow-derived DC surface molecule expression, cytokine production, and capacity to induce T-cell differentiation.

2. Materials and methods

2.1. Reagents and chemicals

Ovalbumin (OVA) (grade V) obtained from Sigma Chemical Co. (St. Louis, MO, USA). RPMI 1640 medium, Hank's balanced salt solution (HBSS), penicillin, streptomycin, L-glutamine, and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA, USA). Thymic stromal lymphopoietin (TSLP) purchased from R&D Systems, Inc. (Minneapolis, MN, USA).

2.2. Plant material, extraction and isolation of ovatodioidide

Whole plants of *A. indica* were collected in October 2009 from the Yuli, Hualien County in Eastern Taiwan, and a botanically identified voucher specimen (YMT-09-02) was deposited in the Herbarium of the Institute of Biochemical Sciences and Technology, Chaoyang University of Technology, Taiwan. The compound ovatodioidide was obtained followed by the extraction and isolation procedures as we reported previously and its purity (>95%) was confirmed by HPLC [6]. Ovatodioidide used in this study was endotoxin free. The chemical structure of ovatodioidide was shown in Fig. 1A.

2.3. Generation of murine bone marrow-derived dendritic cells (DCs)

DCs were prepared followed by a method described previously [15]. Briefly, bone marrow cells from mouse femurs and tibias were first depleted of red cells with lysis buffer. Approximately 1×10^6 cells/ml were placed in 24-well plates in 1 ml of RPMI-1640 medium supplemented with 5%FBS, recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF) (500 U/ml) (PeproTech Inc., Rocky Hill, NJ, USA), 4 mM L-glutamine, 25 mM Hepes (pH 7.2), 50 μ M 2-mercaptoethanol, 0.25 μ g/ml amphotericin, 100 U/ml penicillin and 100 μ g/ml streptomycin. Every other day, fresh medium containing GM-CSF was replaced and non-adherent cells were transferred to a new plate to decrease the contamination by macrophages. On day 6 of culture, non-adherent cells (DCs) were collected and treated with ovatodioidide for 48 h. Then, DCs were analyzed by

flow cytometry to examine their surface marker expressions and used for other experiments.

2.4. Flow cytometry analysis

DCs (1×10^6 cells/ml) were stained with fluorescein isothiocyanate [FITC]-conjugated anti-I-A/I-E (MHC class II) or phycoerythrin [PE]-conjugated anti-CD80 and anti-CD86 or PE-cyanine (Cy)7-conjugated anti-CD11c (eBioscience Inc., San Diego, CA, USA) at 4 °C for 30 min. The cells were washed and suspended in 0.5 ml of PBS with 0.1% sodium azide and analyzed by flow cytometry (FACScan, Becton Dickinson, Mountain View, CA). A total of 2×10^5 cells/ml were counted and the frequency of each cell surface marker was determined using commercial software. Control cells were suspended in medium only. The flow cytometer was regularly calibrated with CaliBRITE beads (Becton Dickinson, Mountain View, CA, USA).

2.5. Cytotoxicity assay

DCs were pre-treated with different concentrations of ovatodioidide for 10 min and cultured with or without OVA (100 μ g/ml) combined with TSLP (20 ng/ml) for 48 h. At this time point, the number of viable cells was determined using trypan blue staining [16].

2.6. Coculture of DCs with naïve CD4⁺ T cells derived from DO11.10 mice

On day 6 DCs were treated with different concentrations of ovatodioidide for 10 min and cultured with or without OVA (100 μ g/ml) combined with TSLP (20 ng/ml) for 48 h. On day 8, DCs treated with mitomycin C (50 μ g/ml) for 30 min and washed three times with HBSS, and then DCs were collected for the following proliferation assay. Freshly isolated DO11.10 spleen cells and purified by positive selection using anti-CD4⁺ microbeads according to the manufacturer's instructions (STEMCELL Technologies Inc. Canada). The purity of CD4⁺ T cells was analyzed by flow cytometry (CD4⁺ T cells: >95%). Freshly isolated CD4⁺ T cells (2×10^5 cells/ml) cocultured with DCs at the 1/10 DC/T cell ratio in the round bottom 96-well microtiter plates. After 4 days, mixed cell cultures were pulsed with 5-Bromo-2-deoxyuridine (BrdU) for measurement cell proliferation. Briefly, 2 nmol of BrdU was added to each well, and the plate was incubated at 37 °C for 8 h. The amount of BrdU incorporated into the T cells was measured using the anti-BrdU monoclonal antibody in the ELISA kit according to the manufacturer's instructions (Roche Diagnostics GmbH, Roche Applied Science, Germany).

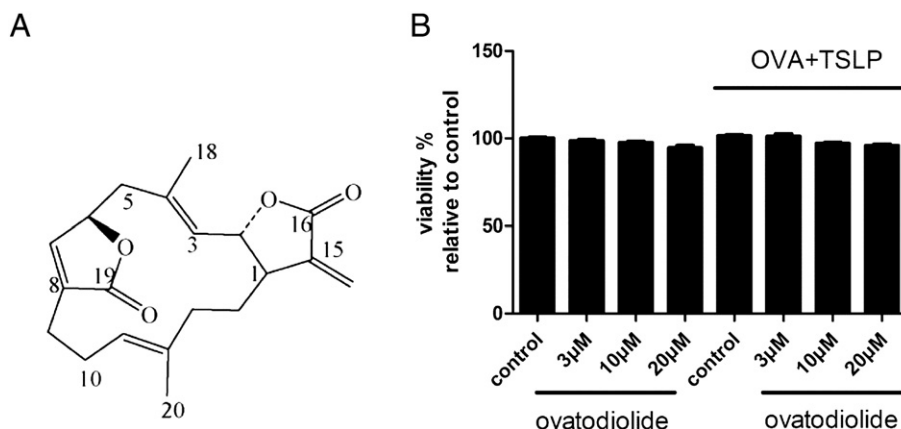


Fig. 1. Ovatodioidide did not affect the DC viability. (A) Chemical structure of ovatodioidide. (B) DCs treated with various doses of ovatodioidide or then added with OVA (100 μ g/ml) + TSLP (20 ng/ml) for 48 h. Cell viability was assessed by trypan blue exclusion assay. Data were expressed as mean $\pm$ SEM of six independent experiments. Control cells contained 0.1% DMSO in medium.

2.7. Cytokine assays

Cell culture supernatants were collected after different doses of ovatodioid treatments and stored at -20°C prior to analysis by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Standards were prepared from recombinant mouse IFN- γ , IL-4, IL-5, and TNF- α (R&D systems, Minneapolis, MN, USA).

2.8. Statistical analysis

Results are given as means $\pm$ S.E.M. ($N \geq 3$). Group comparisons were by one-way ANOVA followed by Newman–Keuls post-hoc test. $p < 0.05$ was considered significant.

3. Results

3.1. Effect of ovatodioid on viability of DCs

In the initial series of experiments, the relative cytotoxic effect of ovatodioid (its chemical structure was shown in Fig. 1A) against murine bone-marrow-derived dendritic cells (DCs) was assessed by trypan blue staining assay. DCs were successfully induced from bone marrow precursor of mice after 6 day culture. The DCs were preincubated in the absence or presence of the ovatodioid (0, 3, 10 and 20 μM) and unstimulated or stimulated with OVA (100 $\mu\text{g}/\text{ml}$) combined with TSLP (20 ng/ml) (OVA/TSLP) for 24 or 48 h. The

results showed that ovatodioid did not affect the viability of either unstimulated or stimulated DCs at concentration up to 20 μM for 24 h (Fig. 1B). Additionally, at 20 μM ovatodioid the relative cell viability still remained $\sim 95\%$ after 48 h of incubation (Fig. 1B). Based on the above results, we choose the concentration of ovatodioid $\leq 20 \mu\text{M}$ as the experimental dose in the following experiments.

3.2. Effect of ovatodioid on surface molecule expression in OVA/TSLP-treated DCs

Mature or activated DCs are characterized by an increase of co-stimulatory molecules (e.g., CD80, CD86) and MHC II on their surface [17]. We next analyzed the maturation status of DCs based on expression of CD80, CD86, MHC II, and OX40 ligand. The DCs were cultured in the presence or absence of ovatodioid and then exposed to OVA/TSLP for 48 h. The expression of co-stimulatory molecules OVA/TSLP-stimulated DCs was assessed by flow cytometry. The results revealed that OVA/TSLP-stimulated DCs resulted an increased expression of CD80 [gated % and mean fluorescence intensity (MFI), (Fig. 2)], CD86 (gated % and MFI, Fig. 3), and MHC II (gated and MFI, Fig. 4) as compared with DCs cultured in medium alone. However, the presence of ovatodioid dose-dependently (0, 3, 10 and 20 μM) inhibited the activation-induced upregulation of CD80 (gated % and MFI), CD86 (gated % and MFI), and MHC II (gated % and MFI) as compared with OVA/TSLP-treated DC (Figs. 2–4). On the other hand, OVA/TSLP-activated DC can induce Th2 cell differentiation and initiate Th2 cell responses through up-regulation of OX40 ligand (OX40L) expression [18].

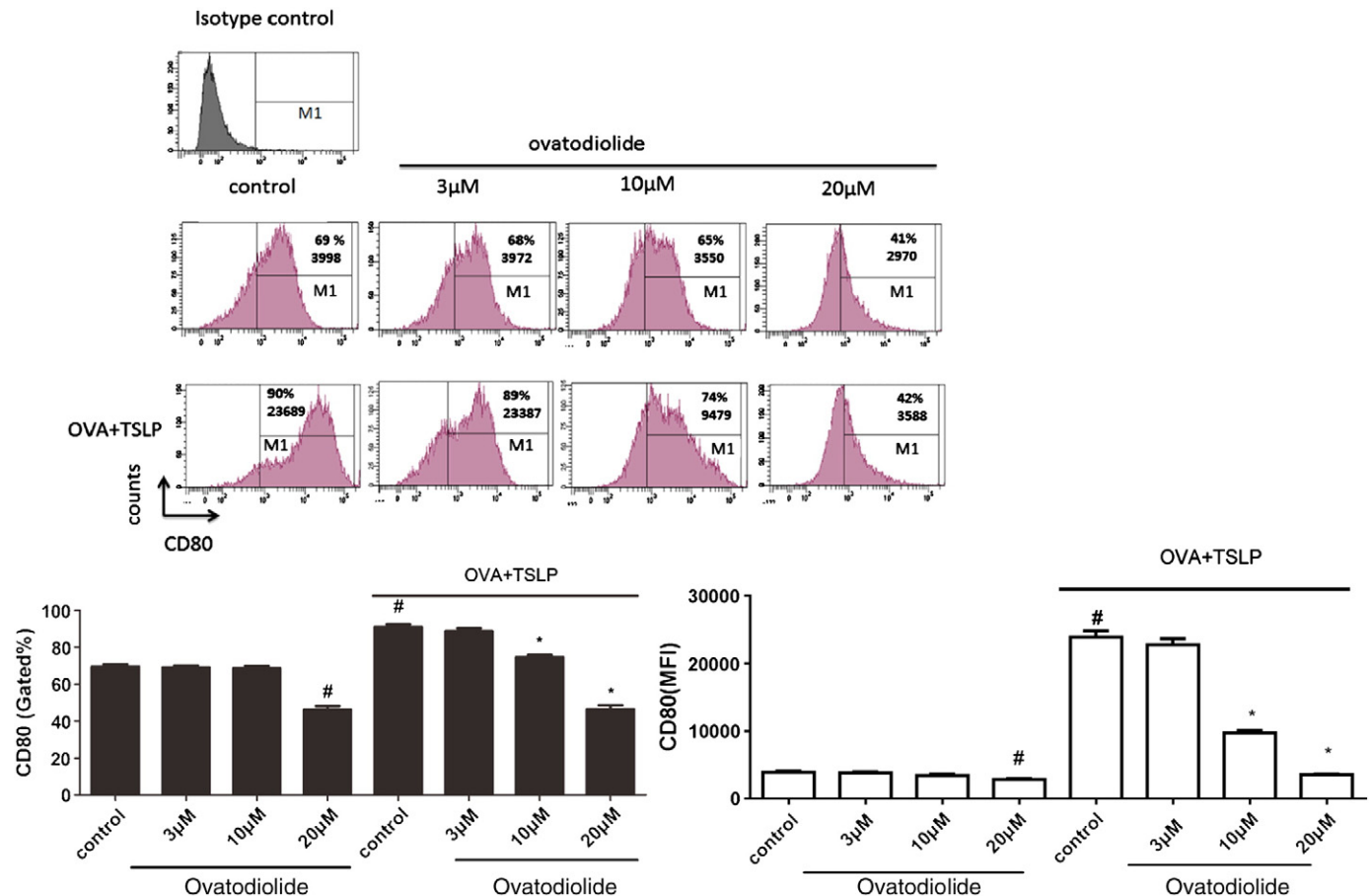


Fig. 2. Ovatodioid inhibited the CD80 expressions in stimulated DCs. DCs treated with various doses of ovatodioid or then added with OVA (100 $\mu\text{g}/\text{ml}$) + TSLP (20 ng/ml) for 48 h. Detection gates % and mean fluorescence index (MFI) of CD80 expressions on DCs were determined by flow cytometry. Data were expressed as mean $\pm$ SEM of six independent experiments. Control cells contained 0.1% DMSO in medium. Significant difference was set at $p < 0.05$ and $^{\#}p < 0.05$ when compared with control group treatment without OVA/TSLP and with OVA/TSLP, respectively.

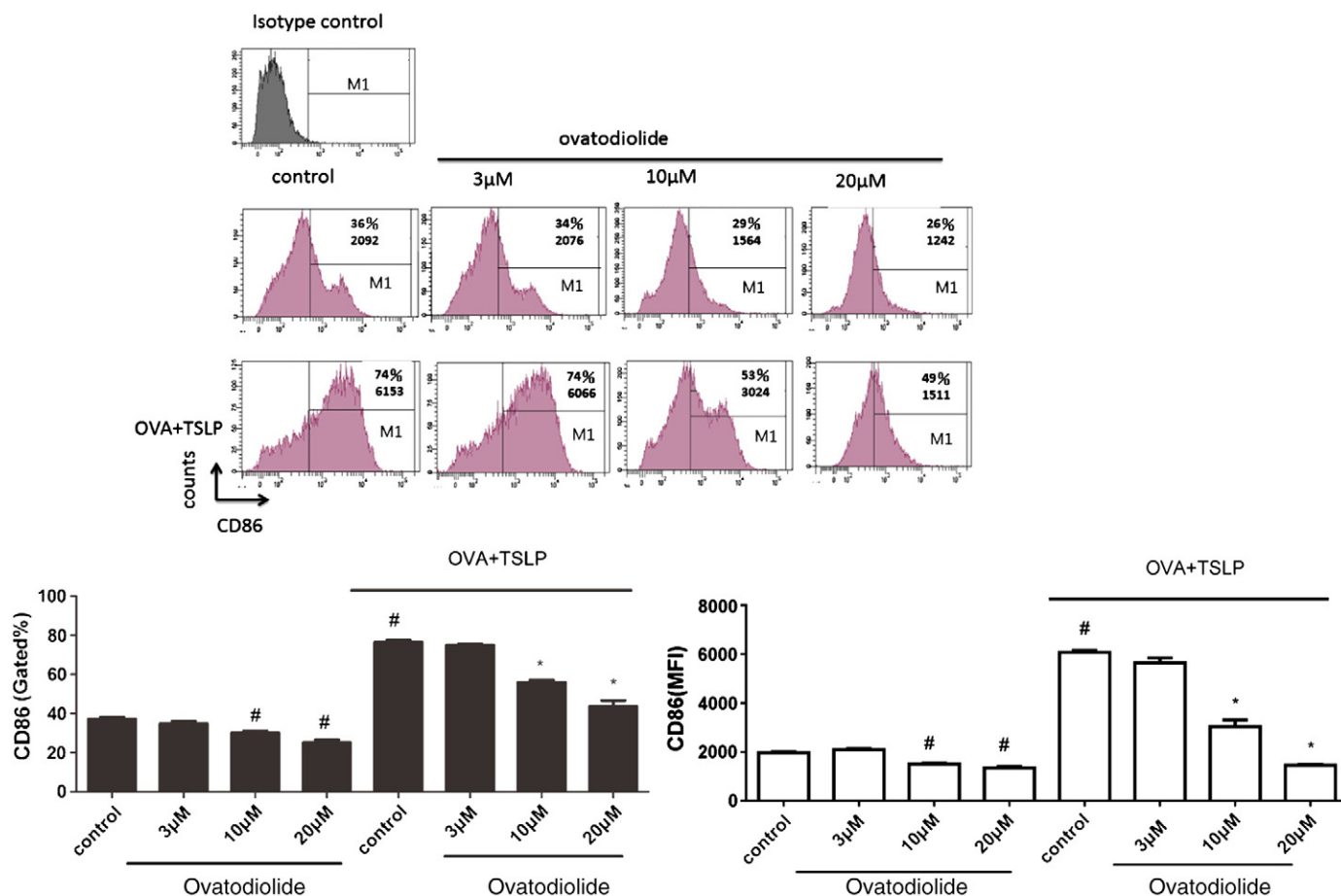


Fig. 3. Ovatodioidide inhibited the CD86 expressions in stimulated DCs. DCs treated with various doses of ovatodioidide or then added with OVA (100 μg/ml) + TSLP (20 ng/ml) for 48 h. Detection gates % and mean fluorescence index (MFI) of CD86 expressions on DCs were determined by flow cytometry. Data were expressed as mean ± SEM of six independent experiments. Control cells contained 0.1% DMSO in medium. Significant difference was set at #*p* < 0.05 and **p* < 0.05 when compared with control group treatment without OVA/TSLP and with OVA/TSLP, respectively.

Therefore, we next examined the effect of ovatodioidide on OX40L expression in stimulated DCs. As shown in Fig. 5, ovatodioidide (up to 20 μM) did not affect the OX40L expression in un-stimulated DCs. Next, it was observed that OVA/TSLP-stimulated DCs showed an increased expression of OX40L (Fig. 5). However, ovatodioidide dose dependently (3, 10, and 20 μM) attenuated the OVA/TSLP-induced upregulation of OX40L expression (Fig. 5). These data indicate that ovatodioidide attenuated the maturation of activated DCs.

3.3. Effect of ovatodioidide on cytokine production in OVA/TSLP-stimulated DCs

Next, we examined whether OVA/TSLP-treated DCs can enhance the proliferation of CD4⁺ regulatory T (CD4⁺ T) cells. The CD4⁺ T cells were isolated from DO.11.10 mice which T cell receptors recognize OVA peptide and co-cultured with OVA/TSLP treated DCs which show the capability of priming CD4⁺ T cells into Th2 cells. As shown in Fig. 6, the proliferation of CD4⁺ T cells was noticeably increased when OVA/TSLP-treated DCs were used as an accessory cell. However, ovatodioidide dose dependently (3, 10 and 20 μM) attenuated the proliferation of CD4⁺ T cells (Fig. 6). Next, it is known that CD4⁺ T cells secrete various cytokines when they were stimulated in the presence of OVA/TSLP-treated DCs [19]. Therefore, we tested the cell-culture supernatants of CD4⁺ T cells stimulated with OVA/TSLP-treated DCs with or without ovatodioidide for IL-4, IL-5, TNF-α, and IFN-γ. Unstimulated immature CD4⁺ T cells did not produce detectable levels of IL-4, IL-5 or TNF-α (Fig. 7). However, CD4⁺ T cells stimulated

with OVA/TSLP-treated DCs secreted detectable amounts of the proinflammatory cytokines IL-4 (146 pg/ml), IL-5 (76 pg/ml), and TNF-α (54 pg/ml) (Fig. 7). Ovatodioidide dose dependently (3, 10 and 20 μM) inhibited the IL-4, IL-5, and TNF-α secretion (Fig. 7). In particular, at 20 μM of ovatodioidide the concentrations of IL-4 (146 pg/ml) and IL-5 (76 pg/ml) of the positive control group was reduced to IL-4 (8 pg/ml) and IL-5 (11 pg/ml), respectively (Fig. 7). Similarly, the TNF-α concentration was reduced to 13 pg/ml by 20 μM of ovatodioidide treatment as compared to the positive control group (54 pg/ml) (Fig. 7). We did not observe a significant (statistically significant decrease, *P* < 0.05) suppression of a key Th1 cytokine IFN-γ secretion by ovatodioidide (Fig. 7). These data demonstrate that ovatodioidide suppressed DC-mediated CD4⁺ T-cells proliferation and cytokine secretion.

4. Discussion

In this study, we examined the effects of ovatodioidide on the functional maturation of murine bone marrow-derived DCs. In general, natural product-based compounds are better than synthetic compounds because of their devoid of severe side effects [20]. Ovatodioidide is a natural diterpenoid compound from *A. indica* [11]. The herbal species *A. indica* contains several constituents such as flavonoids, phenyl propanoids, benzenoids, and sterols [11]. Among them, ovatodioidide is previously reported as potential inhibitor of *H. pylori*-induced inflammation [6]. However, the effects of ovatodioidide on maturation and immunological response of DCs remain largely unknown. Therefore, we

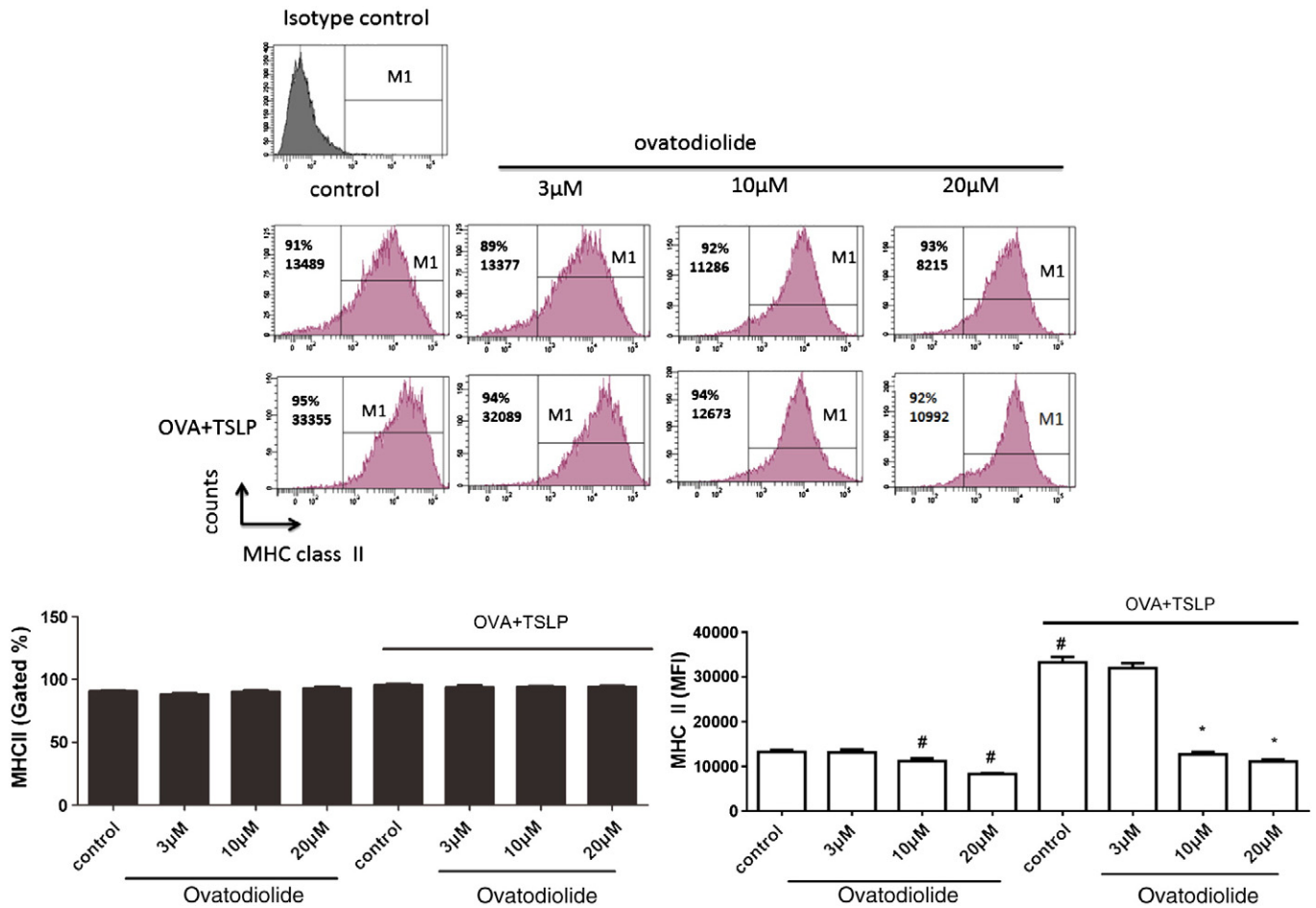


Fig. 4. Ovatodioidide inhibited the MHC II expressions in stimulated DCs. DCs treated with various doses of ovatodioidide or then added with OVA (100 μg/ml) + TSLP (20 ng/ml) for 48 h. Detection gates % and mean fluorescence index (MFI) of MHC II expressions on DCs were determined by flow cytometry. Data were expressed as mean ± SEM of six independent experiments. Control cells contained 0.1% DMSO in medium. Significant difference was set at # $p < 0.05$ and * $p < 0.05$ when compared with control group treatment without OVA/TSLP and with OVA/TSLP, respectively.

investigated ovatodioidide-exposed DCs with regard to their capacity to sensitize recipients for cell-mediated immune responses. To be sure the observed effects of ovatodioidide could be attributed to DCs and not to contaminating cells in the murine bone marrow-derived cell cultures as the DCs were purified at greater than 95%. Our results indicated that ovatodioidide was a potent inhibitor of OVA/TSLP-induced DC maturation and impairs the Th2 immune response, and provides new insights into the immunopharmacological potential of ovatodioidide.

At the beginning, we found that ovatodioidide at concentrations from 3 to 20 μM had no effect on the viability of murine bone marrow-derived DCs compared to the control DCs (Fig. 1B). In contrast, the concentrations of ovatodioidide higher than 20 μM (75 and 150 μM) were cytotoxic for DCs as estimated by trypan blue staining assay (data not shown). It is previously reported that ovatodioidide is potent cytotoxic toward renal cell carcinoma cell lines 786-O, Caki-1, ACHN, and A498 (IC_{50} ranging from 16.1 to 38.6 μM), as compared with the non-tumorigenic human kidney epithelial cell line HK-2 ($IC_{50} = 88.2$ μM) [10]. It is also reported that ovatodioidide is cytotoxic to human squamous cell carcinoma Ca9-22 cells with an IC_{50} value of 4.4 and 2.5 μM at 24 and 48 h after treatment, respectively [8]. In contrast, it is interesting to note that ovatodioidide at a concentration between 0 and 40 μM exhibited no cytotoxicity against highly metastatic human breast cancer MDA-MB-231 cells [9]. In our previous report we also found that the concentrations of ovatodioidide up to 30 μM were non-cytotoxic for estrogen receptor α -positive human breast adenocarcinoma cell line MCF-7 [21]. These discrepancies might be

attributed from different disease models, tested cell lines, and culture conditions. Finally, the present study demonstrated that ovatodioidide (≤ 20 μM) did not show any apparent sign of toxicity toward DCs as proved by trypan blue staining assay.

DCs are crucial regulators of immune response to foreign and self antigens and are ideal therapeutic targets for pharmacological modulation [4]. The expression levels of co-stimulatory molecules such as CD80, CD86 and MHC II, and specific cytokines are lower in immature DCs. However, they become mature DCs when inflammatory stimuli are engaged and secrete specific cytokines to activate innate effector cell, and direct the development and differentiation of specific T helper cell subsets [4]. It is known that CD80 and CD86 belong to the B7 group of costimulatory molecules expressed on the DC surface that are essential for T-cell activation [22]. Additionally, induction of CD80 and CD86 on DC surface is an important step in the initiation of allergen-specific $CD4^+$ T cells activation [22]. The function of MHC II is the presentation of short, pathogen-derived peptides to T cells [22]. It is known that structurally diverse immunosuppressive agents such as rapamycin, quercetin, cyclosporine, lycopene, dexamethasone, aspirin, and mycophenolate mofetil reported to inhibit the DC maturation and functions [23]. Our results demonstrated that ovatodioidide effectively reduced the expression of MHC II, CD80 and CD86 molecules in stimulated DCs (Figs. 2–4). On the other hand, OX40L-OX40 signaling is critical for Th2 differentiation and Th2-mediated airway inflammatory hyper responsiveness [18]. The OX40 receptor is preferentially expressed on the surface of activated

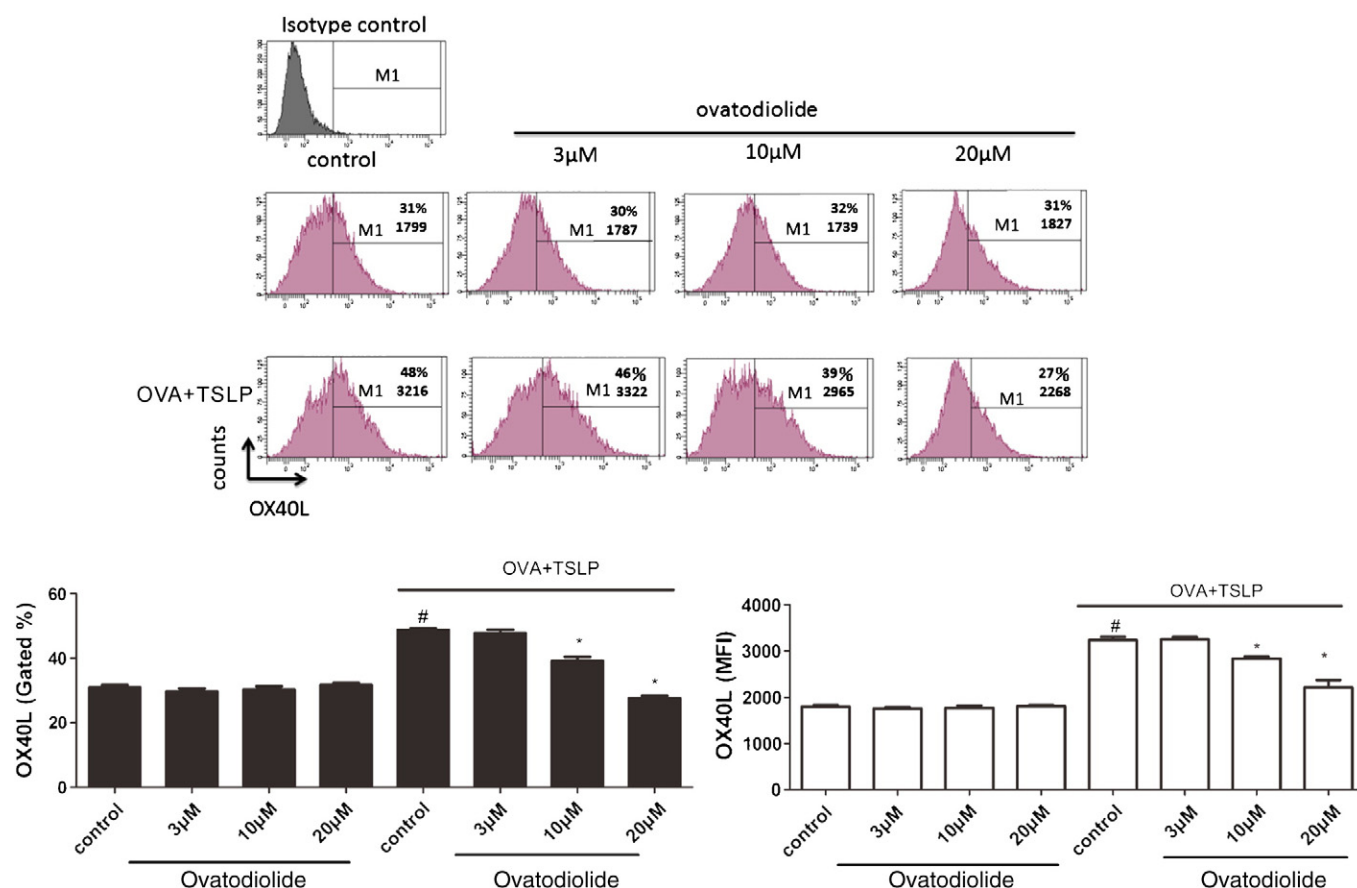


Fig. 5. Ovatodioid inhibited the OX-40L expressions in stimulated DCs. DCs treated with various doses of ovatodioid or then added with OVA (100 μg/ml) + TSLP (20 ng/ml) for 48 h. Detection gates % and mean fluorescence index (MFI) of OX-40L expressions on DCs were determined by flow cytometry. Data were expressed as mean ± SEM of six independent experiments. Control cells contained 0.1% DMSO in medium. Significant difference was set at # $p < 0.05$ and * $p < 0.05$ when compared with control group treatment without OVA/TSLP and with OVA/TSLP, respectively.

regulatory CD4⁺ T cells [18]. Inhibition of OX40L–OX40 signaling by OX40 knockout mice and anti-OX40L mAb reduces Th2 cytokine release, airway inflammation, and airway hyper responsiveness in a murine model of asthma [24]. Previous studies also found that blocking the OX40–OX40L interactions using OX40L-neutralizing antibody inhibits the production of Th2 cytokines in CD4⁺ T cells co-cultured

with TSLP-primed DCs [25]. As OX40–OX40L signaling pathway plays crucial role in Th2 differentiation, therefore, identification of OX40L inhibitors may have potential to improve the efficacy of immunotherapy to promote tolerance. Biologic therapies directed toward OX40/OX40L are in early phases of development, and results from these studies are eagerly awaited [18]. In our study, we found that ovatodioid inhibited OX40L expression in OVA/TSLP-stimulated DCs (Fig. 5). Interestingly, we also found that ovatodioid attenuated the proliferation of CD4⁺ T cells when OVA/TSLP-treated DCs were used as an accessory cell (Fig. 6). These findings suggested that the inhibition of CD4⁺ T cell proliferation by ovatodioid treatment was mediated by the inhibition of DC maturation.

The helper T-cell-mediated immune response toward Th1 or Th2 polarization depends on the specific cytokine secretion by activated/stimulated DCs [4]. Th2 cells are important in recruiting eosinophils and clearing parasitic inflammatory infections. The major cytokines secreted by Th2 cells include IL-4 and IL-5 required for the production of antigen-specific IgE by B cells as well as eosinophilic responses [24,25]. IL-5, on the other hand, is required for the proliferation, differentiation and activation of eosinophils [19]. It is known that TNF-α plays a major role in modulation of the inflammatory response by stimulating secretion of IL-1, IL-6, platelet-derived growth factor and transforming growth factor [19]. As an increased production of cytokines such as IL-4, IL-5, TNF-α, and IFN-γ are closely associated with the pathogenesis of asthma, we next examined their expression levels in OVA/TSLP stimulated DCs. Our experimental results showed that ovatodioid inhibited the secreted inflammatory cytokines IL-4, IL-5 and TNF-α in stimulated DCs (Fig. 7). IFN-γ production by DCs

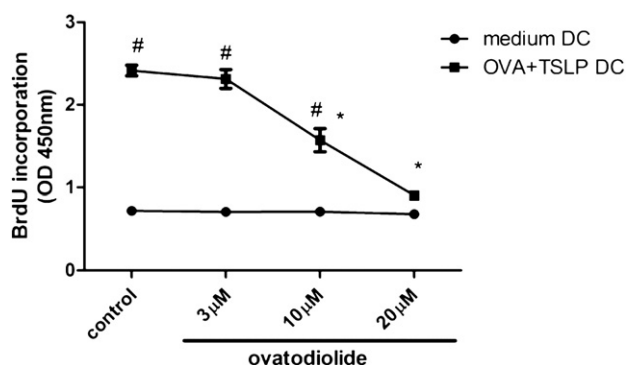


Fig. 6. Ovatodioid suppressed the OVA/TSLP-induced Th2 cell proliferation. DCs treated with various doses of ovatodioid or then added with OVA (100 μg/ml) + TSLP (20 ng/ml) for 48 h. Spleen CD4⁺ T-cells freshly isolated from DO11.10 mice were cultured with various treatments of DCs. (A) CD4⁺ T-cell proliferation was measured by uptake of BrdU with an ELISA kit. Data were expressed as mean ± SEM of five independent experiments. Control cells contained 0.1% DMSO in medium. Significant difference was set at # $p < 0.05$ and * $p < 0.05$ when compared with control group treatment without OVA + TSLP and with OVA + TSLP, respectively.

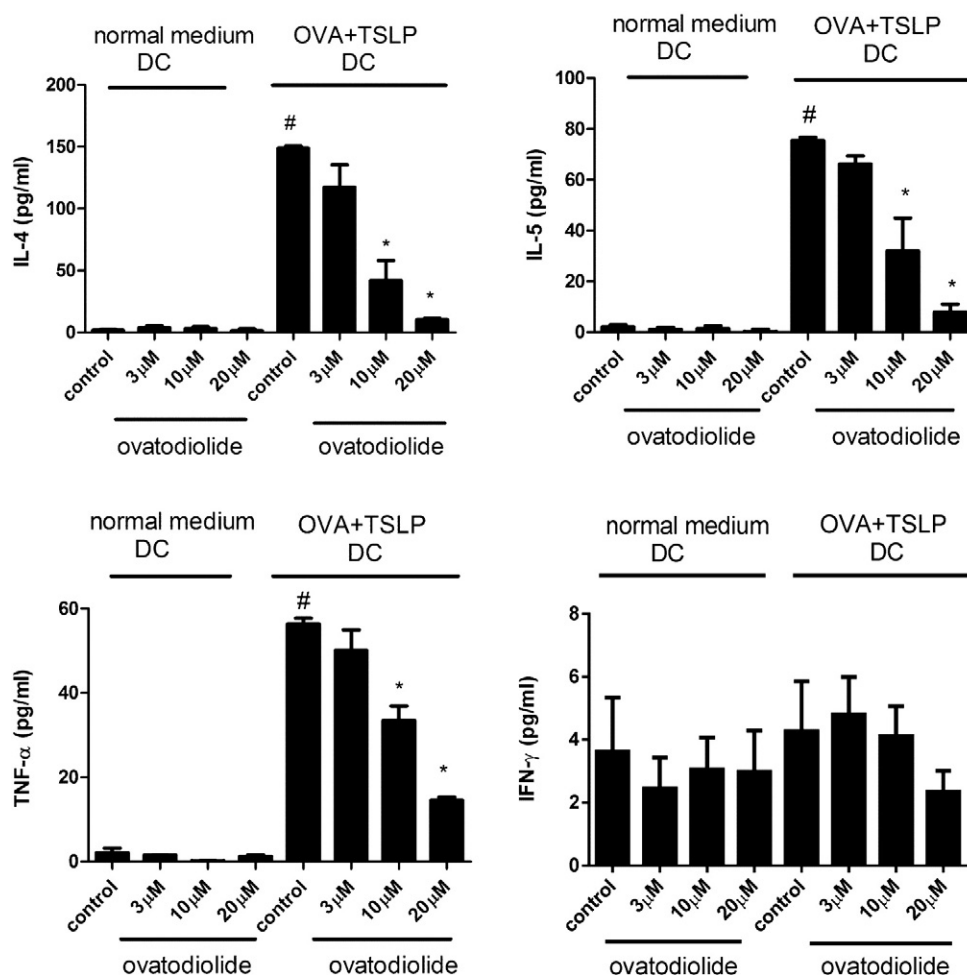


Fig. 7. Ovatodioidide suppressed the OVA + TSLP-induced Th2 cell cytokines production. DCs treated with various doses of ovatodioidide or then added with OVA (100 μg/ml) + TSLP (20 ng/ml) for 48 h. Spleen CD4⁺ T-cells freshly isolated from DO11.10 mice were cultured with various treatments of DCs. Cells medium were analyzed for cytokine production. Data were expressed as mean ± SEM of five independent experiments. Control cells contained 0.1% DMSO in medium. Significant difference was set at #p < 0.05 and *p < 0.05 when compared with control group treatment without OVA + TSLP and with OVA + TSLP, respectively.

could be detected under certain conditions. For example, IL-12 could induce IFN-γ production in DCs, especially when combined with IL-18 [26]. In response to allergenic CD4⁺ T cells, DC production of IFN-γ can also be up-regulated by IL-12 [26]. Conversely, the presence of IL-4 or IL-10 during DC maturation could suppress the expression of IFN-γ in mature DCs [27]. In our study, we did not observe the attenuated IFN-γ production after naïve CD4⁺ T cells co-culture with ovatodioidide treated DCs; the possible reasons may have been the presence of IL-4. Therefore, ovatodioidide inhibited Th2 differentiation in DCs might not associated with induction of Th1 cell differentiation. Recently, the immunomodulatory effects of ovatodioidide have been demonstrated in vitro in macrophages [11]. Our data further provide supporting evidence of ovatodioidide on its hitherto unknown inhibitory effects on functional maturation of DCs. To the best of our knowledge, this is the first report of the immunosuppressive activity of ovatodioidide on DC maturation.

In conclusion, the present study indicates that ovatodioidide was a potent inhibitor of dendritic cell maturation. Thus, ovatodioidide or its derivatives may have great potential as an immunoregulatory agent against various autoimmune diseases and allergy. These results sustain the claims of practitioners of traditional Chinese medicine about the use of herbs containing ovatodioidide in the treatment of inflammatory diseases. Further studies are needed to elucidate the molecular mechanisms and to validate whether ovatodioidide can exert anti-allergic inflammation activity in a murine model of allergic diseases to extend our current findings into clinical implication.

Acknowledgments

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