



March1-overexpressed dendritic cells downregulate Th1/Th2 ratio in asthma through promoting OX40L

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ABSTRACT

Asthma is an inflammatory disease. Th2 differentiation plays an important role in the pathogenesis of asthma. We explored the role and action mechanism of membrane-associated RING-CH 1 (March1) in the Th2 differentiation regulated by dendritic cells (DCs). Our data showed that the expression of March1 was higher in asthmatic children-derived DCs, asthmatic mice-derived DCs and house dust mites (HDMs)-treated DCs than that in control DCs. Increasing of March1 promoted the production of pro-inflammatory cytokines from HDMs-treated DCs, and enhanced the promotion of HDMs-treated DCs to CD4⁺T cell proliferation and Th2 differentiation, whereas decreasing of March1 resulted in opposite effects. Furthermore, our data indicated that March1 positively regulated the expression of OX40 ligand (OX40L) and facilitated DCs-induced Th2 differentiation through OX40L. In asthmatic mice, March1-overexpressed DCs significantly aggravated the injury in lung tissues and promoted Th2 differentiation. Overall, our data proved that highly expressed March1 in DCs facilitated asthma development through inducing Th2 differentiation by facilitating OX40L expression. Our data might provide a new idea for the treatment of asthma.

1. Introduction

Asthma is one of chronic inflammatory disorder. Asthma seriously affects the physical and mental health, and reduces the quality of life of patients [1]. According to the message from previous study, prevalence of asthma in the children aged 5 to 11 years is 9.6%, and in the children aged 12 to 17 years is 10.5%. Asthmatic children exhibit notably higher eosinophils number in serum, higher serum IgE level and allergen sensitization when compared to adults [2]. Currently, although multiple drugs can be used for the treatment of asthma, such as omalizumab, inhaled corticosteroids, long-acting β -agonists, leukotriene antagonists and tiotropium, these drugs remain can't meet the needs of treatment [3]. It is very meaningful to explore the pathogenesis and find new therapeutic target of asthma.

T helper (Th) cells, especially CD4⁺T cells, are an important class of immune cells. Th cells participate in the pathogenesis of almost all of disorders, including asthma. Activated CD4⁺T cells can differentiate

into Th1 and Th2 effector cells. Previous studies indicated that imbalanced Th1 and Th2 cells ratio is a crucial factor for the development of asthma [4]. Th1 cells secrete interferon- γ (IFN- γ) and other cytokines, and Th2 cells secrete interleukin-4 (IL-4), IL-5 and other cytokines. A large number of activated Th2 cells exist in the airway biopsies and bronchoalveolar lavage fluids of the patients with asthma [5]. It was reported that the activation and differentiation of Th2 cells is associated with the co-stimulation signal which on the surface of dendritic cells (DCs), such as OX40-OX40L co-stimulation signal [6]. In our previous studies, we indicated that exosome-activated DCs could facilitate Th2 cell differentiation via increasing the expression of OX40 ligand (OX40L), thus to contribute the development of asthma. Meanwhile, we also found that blocking the co-stimulatory molecule OX40 and OX40L (a ligand of OX40) through anti-OX40L mAb or miR-138 could notably inhibit Th2 cell differentiation, thus to improve asthma [7–9]. Our previous studies revealed that OX40L plays an important role in Th2 cell differentiation in asthma.

Abbreviations: DCs, dendritic cells; HDMs, house dust mites; IFN- γ , interferon- γ ; IL-4, interleukin-4; March1, membrane-associated RING-CH-1; OX40L, OX40 ligand; OVA, ovalbumin; PBMCs, peripheral blood mononuclear cells; Th, T helper.

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Membrane-associated RING-CH-1 (March1) is a member of E3 ubiquitin ligases family. Wilson et al. reported that silencing of March1 in DCs will decline the level of major histocompatibility complex class I (MCH I), and then affect the antigen presentation function of DCs [10]. In addition, a recent study demonstrated that March1^{-/-} asthmatic mice exhibit lower airway hyper-responsiveness and lower IFN- γ , IL-10, IL-6 and IL-13 levels in lung tissues when compared to the asthmatic mice base on normal mice, suggesting March1 might contribute the development of asthma [11]. However, the role of March1 and its action mechanism in asthma remain not fully understand. In this present study, we detected the expression levels of March1 in asthmatic children-derived DCs, ovalbumin (OVA)-induced asthmatic mice-derived DCs and house dust mites (HDMs)-treated DCs. Then, we explored the regulatory mechanism of March1-overexpressed DCs to Th2 cell differentiation in asthma. Our results might provide a new idea for the treatment of asthma.

2. Materials and methods

2.1. Clinical samples

A total 15 asthmatic children were recruited from our hospital, and 15 healthy children were recruited as healthy control (HC). Peripheral blood samples were obtained from participants. The informed consent were written by their family members of all participants. Besides, this study was approved by the ethics committee of the Children's Hospital of Soochow University (approve number: 2017014).

2.2. Establish of asthmatic mouse model

A total of 30 healthy female BALB/c mice (6–8 weeks, 18–20 g) were purchased from Charles River (Beijing, China). The raising conditions of all mice were as follows: temperature at 20–22 °C, 50–60% relative humidity, 12 h of dark/light cycle, and enough food and water. All animal experiments were approved by the Animal Ethics Committee of the Soochow University (approve number: JP-2018-1). All experiments were carried out strictly according to the request of the Recommended Guideline for the Care and Use of Laboratory Animals formulated by the Chinese Council on Animal Research.

To induce experimental model of asthma, at day 1, day 7, and day 14, partly mice were separately sensitized with 0.2 mg OVA (Sangon Biotech, Shanghai, China) and 1 mg aluminum hydroxide (Solarbio, Beijing, China) through intraperitoneal injection. OVA and aluminum hydroxide were resolved in 0.2 ml normal saline. After that, from day 21 to day 25, the mice were administered aerosolized with 5% (v/v) OVA for 30 min daily by inhalation in a whole-body exposure chamber. The mice sensitization with 200 μ L saline, and then challenging with normal saline served as control. At 24 h after the last challenge, the peripheral blood samples were obtained from the control mice and asthmatic mice, and then they were used to separate DCs.

2.3. DCS and CD4⁺T cells isolation and culture

Peripheral blood mononuclear cells (PBMCs) were separated from the peripheral bloods from healthy children, asthmatic children, control mice, and asthmatic mice using lymphocyte separation medium (Solarbio). Then, DCs were sorted and purified from the PBMCs using an anti-CD31 microbead isolation kit (Miltenyi Biotec, Auburn, CA, USA) in accordance with the manufacture's protocol. In addition, bone marrow cells were isolated from healthy mice. Then, bone marrow cells were cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco), 10 ng/ml recombinant mouse GM-CSF (PeproTech, USA) and 2 ng/ml of recombinant mouse IL-4 (PeproTech) at 37 °C for seven days. At day 7, automated magnetic cell sorting kit was used to sort and purify DCs as our previous study [9]. *In vitro*, 100 μ g/mL HDMs were used to treat the bone marrow

cells-derived DCs.

CD4⁺T cells were isolated from the PBMCs of healthy mice using an anti-CD4 microbead isolation kit (Miltenyi Biotec) according to the manufacturer's protocol. CD4⁺T cells were cultured in RPMI-1640 medium supplemented with 10% FBS at 37 °C in an incubator with 5% CO₂.

2.4. Lentivirus infection

March1 gene coding sequence was amplified by PCR, and was sub-cloned into a lentivirus expression plasmid with BamH I and Mlu I restriction sites to establish the lentivirus expressing March1 (LV-March1). Meantime, two different shRNAs were separately cloned into pTY vector (a type of lentiviral vectors) to establish the lentivirus expressing March1 shRNAs. Lentivirus vector, LV-March1, lentivirus expressing March1 shRNA-1 (LV-shMarch1-1), lentivirus expressing March1 shRNA-2 (LV-shMarch1-2), and the lentivirus expressing negative control shRNA (LV-shRNA) were obtained from GeneChem (Shanghai, China). The original lentivirus (LV-mock) served as control of LV-March1. DCs at a density of 5×10^3 cells/well were planted into a six-well plates, and then were infected with lentivirus. At 24 h after lentivirus infection, qRT-PCR, western blot and other experiments were carried out.

2.5. qRT-PCR

Total RNA was isolated from DCs using TRIzol reagent (Invitrogen, USA) and subsequently served as the template for reverse transcription. After complementary DNA synthesis, qPCR was carried out on a Step OnePlus™ Real-Time PCR System (Applied Biosystems) using SYBR Green real-time PCR Master Mix (Invitrogen). *GAPDH* acted as the internal reference of March1, IFN- γ , IL-4, and OX40L gene. The relative expression levels of gene were analyzed as the method of $2^{-\Delta\Delta Ct}$.

2.6. Western blot

Western blot was accomplished to detect the levels of March1 and OX40L in DCs. Total protein was extracted from DCs using RIPA lysis (Solarbio). Next, concentration of protein sample was ensued by a NanoDrop 2000 (Thermo Fisher Scientific). Equal quantify of proteins (25 μ g) from each sample were separated on a 12% SDS-PAGE gel, and then were transferred onto PVDF membranes. The membranes were then maintained with 5% non-fat milk for 1 h at room temperature, and were incubated with primary antibodies (1:1000, Abcam), including anti-March1 and anti-OX40L, overnight at 4 °C. After that, the membranes were maintained with secondary antibody (1:3000, Proteintech, Wuhan, China) for 1 h at room temperature. At last, an Enhanced chemiluminescence reagent (Thermo Scientific, Shanghai, China) was utilized to analyze the blots.

2.7. ELISA assay

The concentrations of IL-12, IL-1 β , IL-6, and TNF- α in cell culture supernatant were measured by ELISA assay using mouse IL-12 ELISA kit, mouse IL-1 β ELISA kit, mouse IL-6 ELISA kit, and mouse TNF- α ELISA kit, respectively. Meantime, the concentrations of IL-4 and IFN- γ in serum were determined by ELISA assay using mouse IL-4 ELISA kit and mouse IFN- γ ELISA kit, respectively. All ELISA kits were obtained from R&D Systems. All experiments were carried out according to the specific manufacture's introductions.

2.8. Mixed lymphocyte reaction assay

Mixed lymphocyte reaction assay was performed to detect the effect of DCs infected with different lentiviruses on the proliferative capacity of CD4⁺T cells. At 24 h after lentivirus infection, DCs were collected and subsequently resuspended in RPMI-1640 medium at a density of $2 \times$

10^5 /mL. Then, the DCs cell suspension were maintained with mitomycin C with a final concentration of 25 μ g/mL for 30 min at 37 °C. Next, the DCs were resuspended again in RPMI-1640 medium at a density of 5×10^5 /mL. After that, 20 μ L of DCs suspension and 1×10^5 CD4⁺T cells were planted into 96-well plate, and then were incubated in an incubator for 48 h. Subsequently, the cells were incubated with 10 μ L of CCK-8 solution (Solarbio) for another 4 h. The absorbance value was measured at 450 nm. Stimulation index (%) = [(experimental absorbance value-RPMI-1640 absorbance value) - (control absorbance value-RPMI-1640 absorbance value)] $\times$ 100%.

2.9. Flow cytometry

Flow cytometry was used to analyze the ratio of Th1/Th2 cells. At 48 h after incubation with DCs or 10 μ g/mL anti-mouse OX40L mAb treatment, CD4⁺T cells were washed, and then were incubated with FITC-labeled anti-IFN- γ (BioLegend) and PE-labeled anti-IL-4 (BioLegend) to determine Th1 cells and Th2 cells. Next, the Th1/Th2 cell ratio was analyzed.

2.10. Experimental of animal experiments

Asthmatic mice were randomly divided into four groups (n = 7): OVA, OVA + DCs, OVA + DCs^{mock}, and OVA + DCs^{March1}. In OVA + DCs, OVA + DCs^{mock}, and OVA + DCs^{March1} groups, the asthmatic mice were injected with 2×10^5 DCs, adenovirus-infected DCs, and adenovirus-March1-infected DCs, respectively, before OVA challenge. At 24 h after the last OVA challenge, airway hyper-responsiveness of mice were measured. Besides, the lung tissues of mice were obtained, and were embedded into paraffin. Next, the tissues were cut into 4- μ m of slices. HE staining and PAS staining were carried out using H&E staining kit (Solarbio) and PAS staining kit (Solarbio) in accordance with the manufacture's introduction, respectively. HE staining and PAS staining was used to analyze the pathological changes in lung tissues.

2.11. Statistical analysis

GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA) was used for data analysis, which was shown as mean $\pm$ standard deviation (SD). Student's *t*-test and one-way ANOVA followed by Tukey's post hoc test were utilized to analyze the significant difference between two independent groups and multiple groups, respectively. All experiments were repeated at least three times. A value of *P* lower than 0.05 was recognized significant.

3. Results

3.1. March1 was highly expressed in asthmatic DCs

Our results showed that the levels of March1 mRNA (Fig. 1A) and March1 protein (Fig. 1B) were higher in the DCs from asthmatic patients when compared to the DCs from healthy individuals. Also, the expression of March1 mRNA (Fig. 1C) and March1 protein (Fig. 1D) was notably facilitated in the DCs from asthmatic mice compared with the DCs from healthy mice. Besides, we isolated bone marrow cells from normal mice, and then DCs were separated from the bone marrow cells. HDM-treated DCs were used for the study *in vitro*. Compared with control group, the levels of March1 mRNA (Fig. 1E) and March1 protein (Fig. 1F) were markedly increased in HDM-stimulated DCs.

3.2. March1 promoted DCs-induced declining in Th1/Th2 cells ratio in asthma

We then explored whether abnormally expressed March1 in asthmatic DCs affects the interaction between DCs and Th1/Th2 cells. The levels of March1 mRNA (Fig. 2A) and March1 protein (Fig. 2B) in DCs

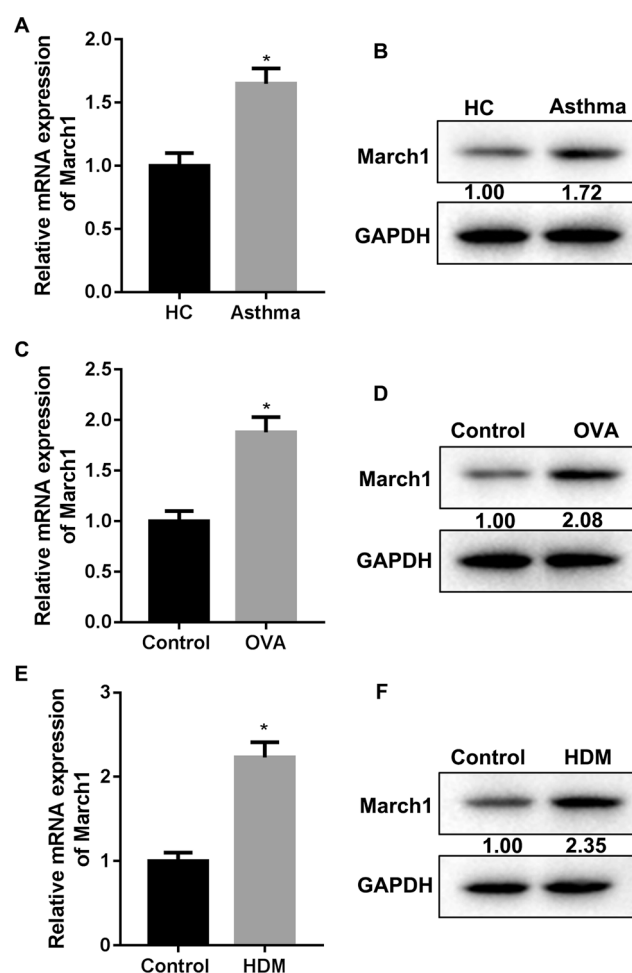


Fig. 1. Detection of March1 mRNA and protein levels in DCs. DCs were isolated from the peripheral bloods of healthy volunteers and asthmatic patients. (A) The level of March1 mRNA in DCs was measured by qRT-PCR. (B) The level of March1 protein in DCs was ensured by western blot. DCs were isolated from the peripheral bloods of healthy mice and asthmatic mice. (C) qRT-PCR was used to detect the level of March1 mRNA in DCs. (D) Western blot was carried out to examine the level of March1 protein in DCs. We next isolated bone marrow cells of normal mice, and then DCs were separated from the bone marrow cells. HDM-treated DCs were used for the study *in vitro*. (E) The levels of March1 mRNA in both normal saline- and HDM-treated DCs were ensured utilizing qRT-PCR. (F) Western blot was carried out to measure the level of March1 protein. **P* < 0.05 compared with HC or Control groups.

were upregulated by the lentivirus expressing March1. Two types of March1 shRNA (shMarch1-1 and shMarch1-2) were designed. The levels of March1 mRNA (Fig. 2C) and March1 protein (Fig. 2D) in DCs were declined by the lentivirus expressing March1 shRNA. According to the results showed in Fig. 2C and D, shMarch1-2 was used in subsequent experiments. DCs were administered with HDM following lentivirus infection. Our results proved that the production of IL-12 was down-regulated, whereas IL-1 β , IL-6 and TNF- α were upregulated in the cell culture supernatants of HDM-treated DCs when compared to the cell culture supernatants of control DCs. Increasing of March1 further suppressed the production of IL-12 and facilitated the production of IL-1 β , IL-6 and TNF- α in HDM-treated DCs. Oppositely, decreasing of March1 promoted the production of IL-12, while suppressed the production of IL-1 β , IL-6 and TNF- α in HDM-treated DCs (Fig. 2E-H). Next, mixed lymphocyte reaction assay was used to detect the effect of DCs on CD4⁺T cells proliferation and Th1/Th2 cells ratio. Our data showed that HDM-treated DCs significantly promoted the proliferation of CD4⁺T cells. The promotion of HDM-treated DCs to CD4⁺T cells proliferation was

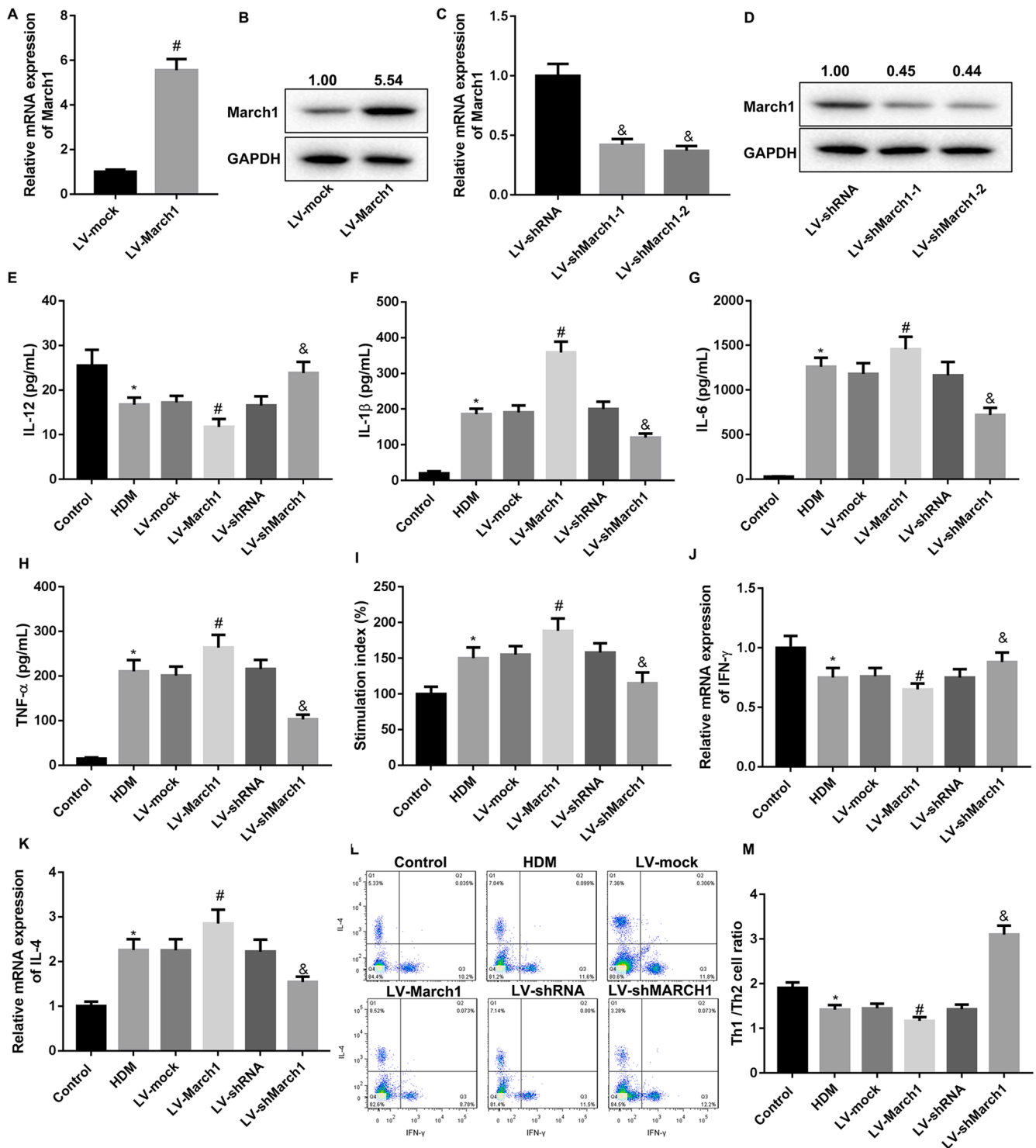


Fig. 2. Effect of March1 on the regulation of HDM-treated DCs to Th1 and Th2 differentiation. (A-B) qRT-PCR and western blot were carried out to detect the levels of March1 mRNA and March1 protein in DCs, thereby to detect the overexpression efficiency. (C-D) qRT-PCR and western blot were performed to determine the levels of March1 mRNA and March1 protein in DCs, thus to verify the knockdown efficiency. (E-H) DCs were infected with the lentiviruses expressing March1, vector (mock), March1 shRNA (shMarch1) or shRNA negative control (shRNA) followed by HDM treatment. Then, ELISA assay was performed to measure the concentrations of inflammatory cytokines, including IL-12, IL-1 β , IL-6 and TNF- α . CD4⁺T cells were co-cultured with the DCs infected with lentiviruses and treated with HDM. (I) Mixed lymphocyte reaction assay was used to detect the influence of DCs to CD4⁺T cells proliferation. (J and K) qRT-PCR was carried out to measure the levels of IFN- γ and IL-4 mRNAs in CD4⁺T cells. (L and M) Flow cytometry was utilized to analyze the ratio of Th1/Th2 cells. * P < 0.05 compared with Control, # P < 0.05 contrasted with LV-mock, and & P < 0.05 contrasted with LV-shRNA.

enhanced by March1 increasing, but suppressed by March1 decreasing (Fig. 2I). HDM-treated DCs markedly inhibited the expression of Th1-related cytokine (IFN- γ mRNA) and promoted the expression of Th2-related cytokine (IL-4 mRNA) in CD4⁺T cells. Importantly, the effect of HDM-treated DCs on IFN- γ and IL-4 mRNAs expression was exacerbated by March1 increasing and attenuated by March1 decreasing (Fig. 2J and K). Moreover, HDM-treated DCs effectively declined the ratio of Th1/Th2 cells in CD4⁺T cells. HDM-treated March1-overexpressed DCs further downregulated, whereas HDM-treated March1-silenced DCs upregulated the ratio of Th1/Th2 cells (Fig. 2L and M). In summary, increasing of March1 promoted HDM-stimulated DCs-induced Th1/Th2 cell ratio declining, and decreasing of March1 reversed HDM-stimulated DCs-induced Th1/Th2 cell ratio declining.

3.3. March1 declined Th1/Th2 ratio via increasing OX40L expression in DCs

Our data indicated that the levels of OX40L mRNA and OX40L protein were increased in March1-overexpressed DCs, and were decreased in March1-silenced DCs (Fig. 3A and B). To explore whether March1 regulates the influence of DCs to Th1 and Th2 differentiation through promoting OX40L expression, we pre-treated the March1-overexpressed DCs with anti-OX40L mAb. IgG as the negative control of anti-OX40L mAb. Mixed lymphocyte reaction was then used to detect the influence of treated DCs to Th1 and Th2 differentiation. The results demonstrated that increasing of March1 in DCs significantly decreased Th1/Th2 cell ratio, which was partly rescued by anti-OX40L mAb

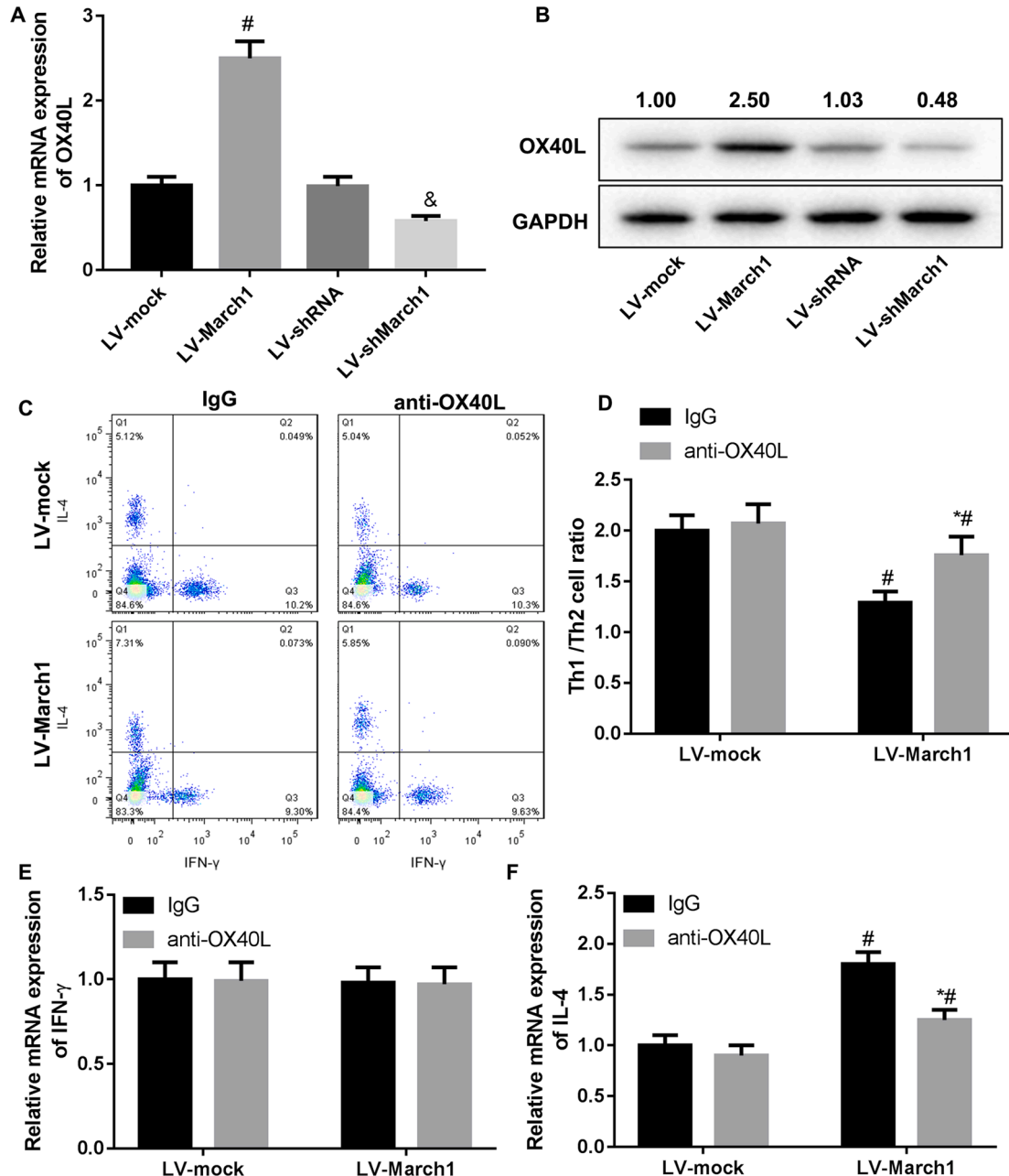


Fig. 3. March1 regulated Th2 differentiation via promoting OX40L expression in DCs. DCs were infected with the lentiviruses expressing March1 or shMarch1, and then (A) qRT-PCR was used to detect the expression of OX40L mRNA in DCs. (B) Western blot was used to measure the level of OX40L in DCs. Anti-OX40L mAb (10 μ g/ml) pre-treated the DCs infected with lentivirus expressing March1, and then above DCs were co-cultured with CD4⁺T cells. (C and D) Flow cytometry was carried out to detect the ratio of Th1/Th2 cells. (E and F) qRT-PCR was used to measure the levels of IFN- γ and IL-4 mRNAs in CD4⁺T cells. * P < 0.05 compared with LV-mock + anti-OX40L group, and # P < 0.05 compared with LV-mock + IgG or LV-mock + anti-OX40L groups.

(Fig. 3C and D). Overexpression of March1 in DCs has no effect on the expression of IFN- γ mRNA in CD4⁺T cells. The level of IFN- γ mRNA still has no changes even using anti-OX40L mAb to rescue the effect of March1 (Fig. 3E). Furthermore, overexpression of March1 in DCs notably promoted the expression of IL-4 mRNA in CD4⁺T cells, which was partly reversed by anti-OX40L mAb (Fig. 3F). In summary, March1 declined Th1/Th2 cell ratio through facilitating the expression of OX40L in DCs.

3.4. March1-overexpressed DCs declined Th1/Th2 cell ratio in asthmatic mice

Then, we further explored the effect of March1 on the regulation of DCs to Th1 and Th2 differentiation in asthmatic mice. DCs were infected with adenovirus vector or the adenovirus expressing March1. Subsequently, adenovirus-infected or uninfected DCs were injected into asthmatic mice. Our data revealed that uninfected DCs and the DCs infected with adenovirus vector have no effect on the airway hyper-responsiveness of asthmatic mice. However, the airway hyper-responsiveness of asthmatic mice was notably increased by the

March1-overexpressed DCs treatment (Fig. 4A). Besides, uninfected DCs and the DCs infected with adenovirus vector were also found have no influence on the histomorphology of lung tissues of asthmatic mice and the inflammatory response in lung tissues. Whereas, March1-overexpressed DCs could obviously induce injury and inflammatory response in the lung tissues of asthmatic mice (Fig. 4B and C). The expression of OX40L in lung tissues has no significantly difference among asthmatic mouse model, uninfected DCs-treated and adenovirus vector-infected DCs-treated OVA mouse model. Nevertheless, March1-overexpressed DCs promoted OX40L expression in the lung tissues of asthmatic mouse model (Supplementary Fig. 1). Consistently, the uninfected DCs and the DCs infected with adenovirus vector have no influence on the ratio of Th1/Th2 cells in the peripheral blood of asthmatic mice, and on the levels of IFN- γ and IL-4 in serum. March1-overexpressed DCs significantly downregulated the Th1/Th2 cell ratio and increased the concentration of serum IL-4, but has no influence on the concentration of serum IFN- γ (Fig. 4D-G). Overall, March1-overexpressed DCs declined Th1/Th2 cell ratio and induced lung injury in asthmatic mice.

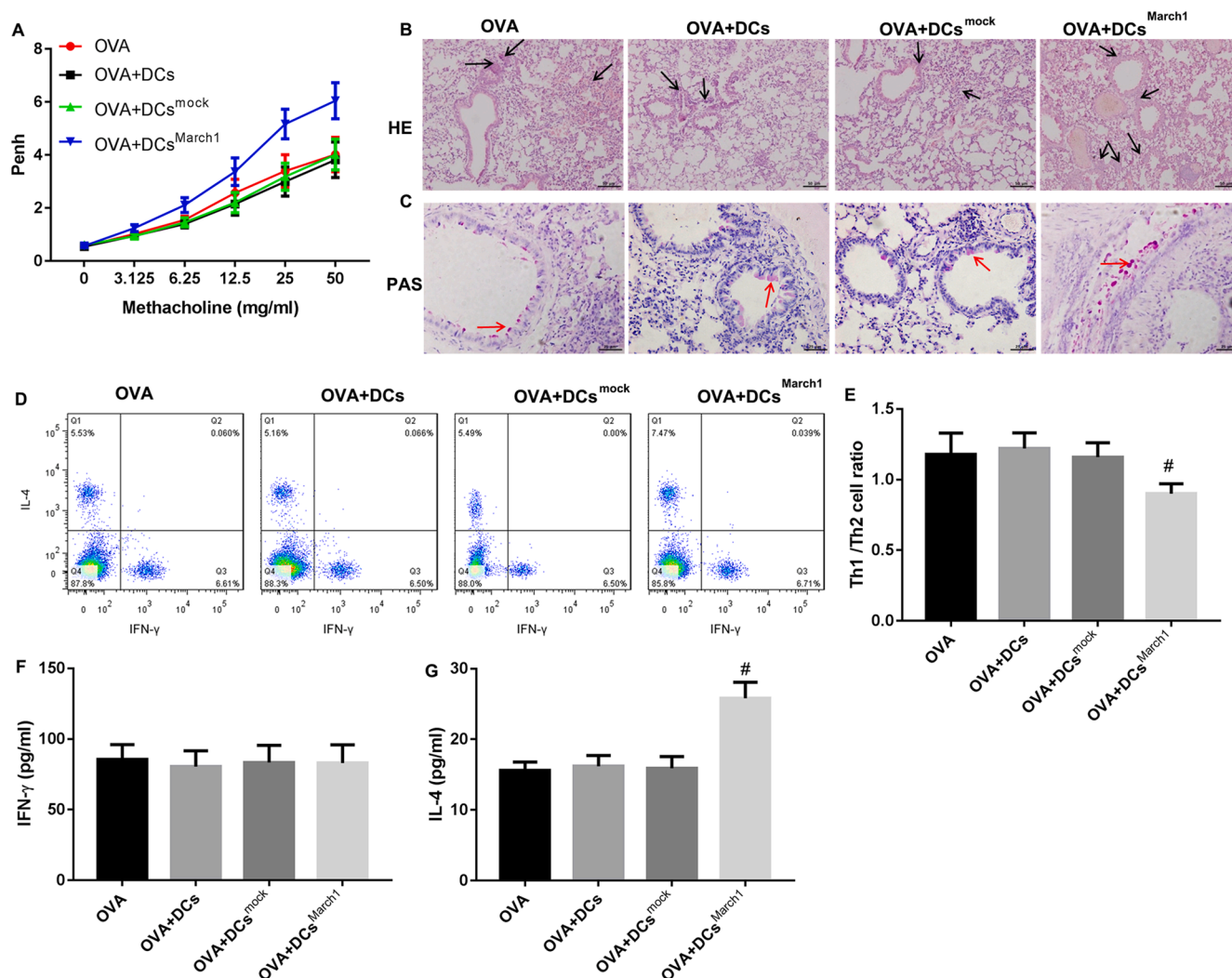


Fig. 4. Effect of March1-overexpressed DCs on Th1 and Th2 differentiation in asthmatic mice. Uninfected DCs, the DCs infected with adenovirus vector, and the DCs infected with adenovirus expressing March1 were injected into the mice through tail vein. Then, OVA was used to induce asthmatic mice model. At 24 h after the last OVA challenge, (A) airway hyper-responsiveness of all mice were measured. (B and C) H&E staining and PAS staining were performed to detect the pathological changes in the lung tissues of mice. Black arrow indicates infiltrated inflammatory cells, and red arrow indicates goblet cells. (D and E) Flow cytometry was utilized to analyze the ratio of Th1/Th2 cells in the peripheral blood of mice. (F and G) The concentrations of serum IFN- γ and IL-4 were measured by using ELISA assay. # $P < 0.05$ compared with OVA + DCs^{mock} group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

March family belongs to E3 ubiquitin ligase. March1 is a member of March family. It was demonstrated that March1 plays a crucial role in immune response. March1 regulated T cells co-stimulatory and antigen presentation function of DCs through suppressing the expression of MHC II and CD86 [12]. Co-stimulatory molecules like DC86 are necessary for DCs activating T cells. March1 could regulate the antigen presentation function of DCs through inducing the ubiquitination and degradation of CD86 [13]. In recent years, some studies suggested that March1 may participate in the development of asthma. For instance, Kishta et al. proved that March1 involves in the regulation of lung inflammation in OVA-induced asthmatic mice [11]. Nevertheless, the role and action mechanism of March1 in asthma still is not clear. In our present study, our results showed that March1 is increased in the DCs from asthmatic children, asthmatic mice model and HDMs-treated DCs. Furthermore, our data identified that highly expressed March1 in DCs promotes asthma development through decreasing the ratio of Th1/Th2 cells by promoting OX40L expression.

CD4⁺T cells are an important class of immune cells. Th1 and Th2 cells together regulate the immune balance. Th1 cells secrete IFN- γ and other pro-inflammatory cytokines, but Th2 cells secrete IL-4 and other anti-inflammatory cytokines. Upon the balance of Th1 cells and Th2 cells was broken, inflammatory response will be activated in body [14]. Existing evidence have demonstrated that Th2 cells-mediated immune response is a crucial starting factor for the pathogenesis of asthma [15]. High percentage of Th2 cells and levels of Th2 cells-mediated immune responses were found in both asthmatic mice and clinical patient samples. Imbalanced Th1/Th2 ratio can eventually cause the inflammation in airway and aggravate asthma attacks [16]. Recently, Huang et al. reported that *Helminthostachys zeylanica* water extract could effectively improve the airway hyper-responsiveness, inflammation response and lung injury of asthmatic mice through declining the percentage of Th2 cells and reducing the releasing of Th2 cells-related cytokines [17]. In our present study, the data showed that March1 notably promotes the production of pro-inflammatory cytokines (IL-1 β , IL-6 and TNF- α) and suppresses the production of anti-inflammatory cytokine (IL-12) in HDMs-treated DCs. Moreover, overexpression of March1 in HDMs-stimulated DCs markedly decreases the ratio of Th1/Th2 cells, and decreasing of March1 upregulates Th1/Th2 cell ratio. The experiment *in vivo* showed that intravenous injection of March1-overexpressed DCs into asthmatic mice lead to the aggravation of lung injury and down-regulation of Th1/Th2 cell ratio.

OX40L, also named as CD252, is one of the members of TNFR/TNF superfamily. OX40L also plays an important role in inflammatory response, and involves the pathogenesis of many disorders, including asthma, through interacting with OX40 [18,19]. Cheng et al. proved that OX40L is highly expressed, Th1 cell-related IFN- γ is lowly expressed and Th2 cell-related IL-4 is highly expressed in the plasma of OVA-induced asthmatic mice compared with control mice [20]. Huang et al. indicated that overexpression of OX40L in DCs could effectively facilitate the proliferation of CD4⁺T cells and contribute Th2 and Th17 cells differentiation in OVA-induced asthmatic mice through targeting p38/MAPK and PI3K/AKT signaling [8]. More and more research suggested that OX40L plays a crucial role in the pathogenesis of asthma [21]. In our present study, we found that March1 promotes the expression of OX40L in DCs. Pre-treated March1-overexpressed DCs with anti-OX40L mAb could rescue DCs-induced downregulation of Th1/Th2 cell ratio.

5. Conclusions

In conclusion, highly expressed March1 in DCs facilitates asthma development through declining the ratio of Th1/Th2 cells by promoting the expression of OX40L in DCs. Our data identified firstly that March1 regulates asthma development by targeting OX40L. Our results might provide a new target for asthma treatment.

CRedit authorship contribution statement

Wenjing Gu: Conceptualization, Funding acquisition, Investigation. **Suyu Guo:** Project administration, Investigation, Methodology. **Jiahui Zhang:** Investigation, Data curation, Formal analysis. **Xinxing Zhang:** Investigation, Resources, Software. **Zhichao Sun:** Investigation, Validation, Visualization. **Zhengrong Chen:** Investigation, Writing – original draft. **Li Huang:** Investigation, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2021.108444>.

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