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Research article

MicroRNA-155 is a critical regulator of regulatory T cells in OM-85 Broncho-Vaxom treated experimental models of allergic rhinitis

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Abstract: *Background:* Bacterial lysates could alleviate the clinical symptoms of allergic rhinitis (AR) and decrease the recurrent rate of AR through regulation of regulatory T cell (Treg) cells. However, the molecular regulatory mechanisms of bacterial lysates to Treg are still unclear. *Objective:* We aimed to investigate the importance of microRNA-155 (miR-155) to Treg cells function in OM-85 Broncho-Vaxom (OM-85 BV) treated experimental mouse models of AR. *Methods:* AR mouse models were established and treated by intranasal administration of OM-85 BV to investigate the role of bacteria lysate for Treg cell function. The proliferation of Treg cells in peripheral blood was examined. The mRNA levels of IL-10, transforming growth factor-β (TGF-β) were examined by real-time PCR. miR-155 mimics and inhibitor were used to verify the role of miR-155 for Treg cells function. *Results:* OM-85 BV, miR-155 mimics or their combination reduced total cells, lymphocytes, neutrophils and eosinophils in nasal lavage fluid of AR mouse models and improved allergic symptoms. OM-85 BV promoted the proliferation of Treg and the expression of Foxp3, IL-10 and TGF-β both *in vivo* and *in vitro*. The miR-155 enhanced the proliferation and

function of Treg. *Conclusions:* MiR-155 promotes Treg cells function in OM-85 BV bacteria lysate treated experimental models of AR and alleviate the upper airway allergic inflammation in AR mice.

Keywords: allergic rhinitis; bacteria lysate; OM-85 Broncho-Vaxom; regulatory T cell; miR-155

1. Introduction

Allergic rhinitis (AR) is a chronic inflammation which often occurs in childhood and adolescence. The prevalence of AR varying by country, ranging from 10–30% in adults and up to 40% in children [1]. AR is estimated to affect 9.8% of the child population in eight metropolitan cities in China [2]. The complex pathophysiology of AR is due to a complex interplay between various inflammatory cells, such as mast cells, basophils, lymphocytes, et al. [3]. The treatment options include antiallergic medicine and allergen immunotherapy (AIT), the latter one is the only way to change the allergic march [4,5]. However, the long course of AIT, high cost, uncertainty of effectiveness and potential adverse events limit the use of AIT. Developing effective new therapies has always been challenging. Hence, it is a new perspective to look for add-on therapeutic methods to treat AR from modulatory of the immune system.

Bacteria lysates are antigen mixtures consisting of inactivated antigens obtained from respiratory tract pathogens, which can be administrated by oral, intranasal or injection [6]. OM-85 Broncho-Vaxom (OM-85 BV) belongs to polyvalent chemical bacterial lysates (PCBLs) and is an immunomodulator comprising lyophilized bacteria lysates form the eight major strains which are responsible for recurrent respiratory tract infections. The hygiene hypothesis suggests a correlation between limited exposure to pathogens in childhood and the rapid increase in allergic disease observed over the past 50 years [7]. Bacteria lysates may shorten the duration and frequencies of respiratory infections since its first use in 1950s [6]. Treatment with bacteria lysates also induced a clinical improvements in patients with AR, asthma, et al. [8]. Reviews have concluded that the mechanism of action of bacteria lysates in treating AR was reducing Th2 response and restoring Th1/Th2 balance [6].

MicroRNAs (miRNAs) are small (18–22 nucleotides) non-coding highly conserved RNA molecules. The role of miRNA has also been revealed in several immunological and inflammatory diseases, including allergic inflammation [9]. The effect of miR-155 was confirmed in the regulation of Th2 activation as well as ILC2 and IL-33 signaling in response to allergen-induced airway inflammation [10,11]. We previously found that miR-181a and miR-155 were related to the proliferation and function of Treg cells in AR children [12].

Lee's and Fu's study proved that OM-85 BV treatment enhances Treg cell proliferation so as to prevent allergic inflammation in an asthmatic mouse model [13,14]. However, the regulation of OM-85 BV in AR was not reported. In this study, we aimed to use an animal model with AR to investigate the underlying mechanisms of OM-85 BV in regulating Treg cell function. Uncovering the underlying mechanisms of bacteria lysates will allow the development of antiallergic medicine to maintain or improve efficacy for AR.

2. Materials and methods

2.1. Animals

BALB/C mice (6–8-weeks old) were bred under pathogen-free conditions at the Guangdong Medical Laboratory Animal Center. All procedures were performed according to protocols approved by Animal Ethical and Welfare Committee. The housing condition was in a 12 h light/dark cycle and a temperature of about 22 ℃. All the animals were monitored daily and euthanized with a lethal dose of anesthetic through the intraperitoneal route.

2.2. Induction of allergic airway inflammation and administration of OM-85 BV and miR-155

OM-85 BV (3.5 mg) dissolved in PBS (corresponding to 40 mL of OM-85 concentrate, 20 mL/nostril) was delivered via gastrogavage for 10 consecutive days, followed by no treatment for the next 20 days. The 30-day treatment was regarded as a single course and three courses were given.

The induction of AR mice included two phases: Sensitization and challenge, which were reported previously [13]. Briefly, mice were sensitized intraperitoneally with 40 μg of ovalbumin (OVA) (Sigma) solution on Days 0, 7 and 14. From Day 21, 1% OVA was given nasally three times for one week for challenge. Control group were provided with PBS instead of OVA. The number of sneezes and nasal rubs within the ten minutes following the final OVA intranasal provocation on day 27 was used to measure the severity of the nasal symptoms. One milliliter of cold PBS was placed into the nasal cavity and collected using a tube. After being collected, the samples were centrifuged and Giemsa dye was applied to the cell pellet for counting inflammatory cells.

The mice were divided into six groups according to different treatments: (1) PBS/PBS mice which were sensitized (Days 0, 7 and 14) and challenged with PBS (Days 21–27); (2) OVA sensitized (Days 0, 7 and 14) and OVA challenged (Days 21–27) mice; (3) OVA sensitized (Days 0, 7 and 14) and OVA challenged (Days 21–27) mice + OM-85 BV (3 months' course before induction of allergy); (4) OVA sensitized (Days 0, 7 and 14) and OVA challenged (Days 21–27) mice + OM-85 BV (3 months' course before induction of allergy) + miR-155 mimics (2 hours before each OVA challenge); (5) OVA sensitized (Days 0, 7 and 14) and OVA challenged (Days 21–27) mice + OM-85 BV (3 months' course before induction of allergy) + miR-155 inhibitors (2 hours before each OVA challenge); (6) OVA sensitized (Days 0, 7 and 14) and OVA challenged (Days 21–27) mice + miR-155 mimics (2 hours before each OVA challenge); and (7) OVA sensitized (Days 0, 7 and 14) and OVA challenged (Days 21–27) mice + miR-155 inhibitors (2 hours before each OVA challenge). For miRNA interfere experiment, at the respective time, mice were infected intranasally with miR-155 mimics or inhibitors in 50 mL of saline solution 2 hours before each OVA challenge. The mice were weighed daily and randomly allocated 5 per cage for each group.

2.3. Flow cytometry

Blood was obtained from mice through cardiac puncture and anticoagulated with acid citrate-dextorse as described in our previous studies [12]. Percentage of blood Treg cells ($CD4^+CD25^+$ $Foxp3^+$) in $CD4^+$ T cells was identified and quantified by flow cytometry. In brief, CD4+ T cells were isolated from peripheral blood mononuclear cells (PBMCs) by Whole Blood CD4 MicroBeads (Miltenyi Biotec, Germany) according to the manufacturer's recommendations. Then, the cells were centrifuged and stained with 20 μl of CD25 (FITC-conjugated; Becton

Dickinson, USA), 10 µl of Foxp3 (PE-conjugated; Becton Dickinson, USA), and permeabilized solution. Using the CellQuest program from Becton Dickinson Biosciences in the USA, FACS Calibur flow cytometry was used for the flow cytometric analysis.

A total of 1×10^6 cells/mL CD4+CD25+Treg cells were sorted by FACS Aria III (BD Biosciences, San Jose, USA) and cultured in 96-well flat-bottomed plates. Sixteen hours before the culturing process was terminated, one microgram of [3H]-thymidine was added to each well, and the incorporation of isotopes was measured using a liquid scintillation counter (Pharmacia-LKB, Germany). The counts per minute (cpm) of triplicates or quadruplicates were expressed as mean ± standard deviation for the respective results.

2.4. Real-time polymerase chain reaction (RT-PCR)

The cDNA was prepared from the extracted total RNA using the Trizol (Invitrogen). The quality of the cDNA was confirmed using endogenous beta-actin gene amplification by TaqMan-specific primers and probes. The target gene mRNA levels were normalized by GAPDH using comparative Ct values and $2^{-\Delta Ct}$ was used to calculate gene expression. The primer sequences for Foxp3, IL-10 and TGF-β, GAPDH (Invitrogen) were as follows: Foxp3: 5'-CTACGCCACGCTCATCCGCTGG-3', 5'-GTAGGGTTGGAACACCTGCTGGG-3'; IL-10: 5'-GGTTGTCGTCTCATTCTGAAAGA-3', 5'-GGTAGAGGACCCAAGTTCGTTAAGA-3'; TGF-β: 5'-CCCAGCATCTGCAAAGCTC-3', 5'-GTCAATGTACAGCTGCCGCA-3'; GAPDH: 5'-TCCCCATCACCATCTTCCAGG-3', 5'-GATGACCCTTTTGGCTCCC-3', respectively.

2.5. Statistical analysis

The data were analyzed by GraphPad Prism 8 statistical software package. One-way ANOVA and Kruskal–Wallis were performed for multiple comparisons (with Dunn's multiple comparison test as post hoc). The significance level was set at $P < 0.05$.

2.6. Ethics approval of research

The animal experiments were performed according to protocols approved by Animal Ethical and Welfare Committee and were approved by Ethics Committee of Guangzhou Women and Children's Medical Center. The associated permit number is [2020] No.26901.

3. Results

3.1. OM-85 BV treatment reduced nasal allergy inflammation

Following OVA sensitization and challenge mice, there was a substantial rise in the allergic symptoms, total cells, lymphocytes, neutrophils, and eosinophils in nasal lavage fluid (Figure 1). The mice treated with OM-85 BV, miR-155 mimics or their combination showed reduced total cells, lymphocytes, neutrophils, and eosinophils in nasal lavage fluid, along with improved symptoms, while miR-155 inhibitors can promote allergic inflammations (Figure 1).

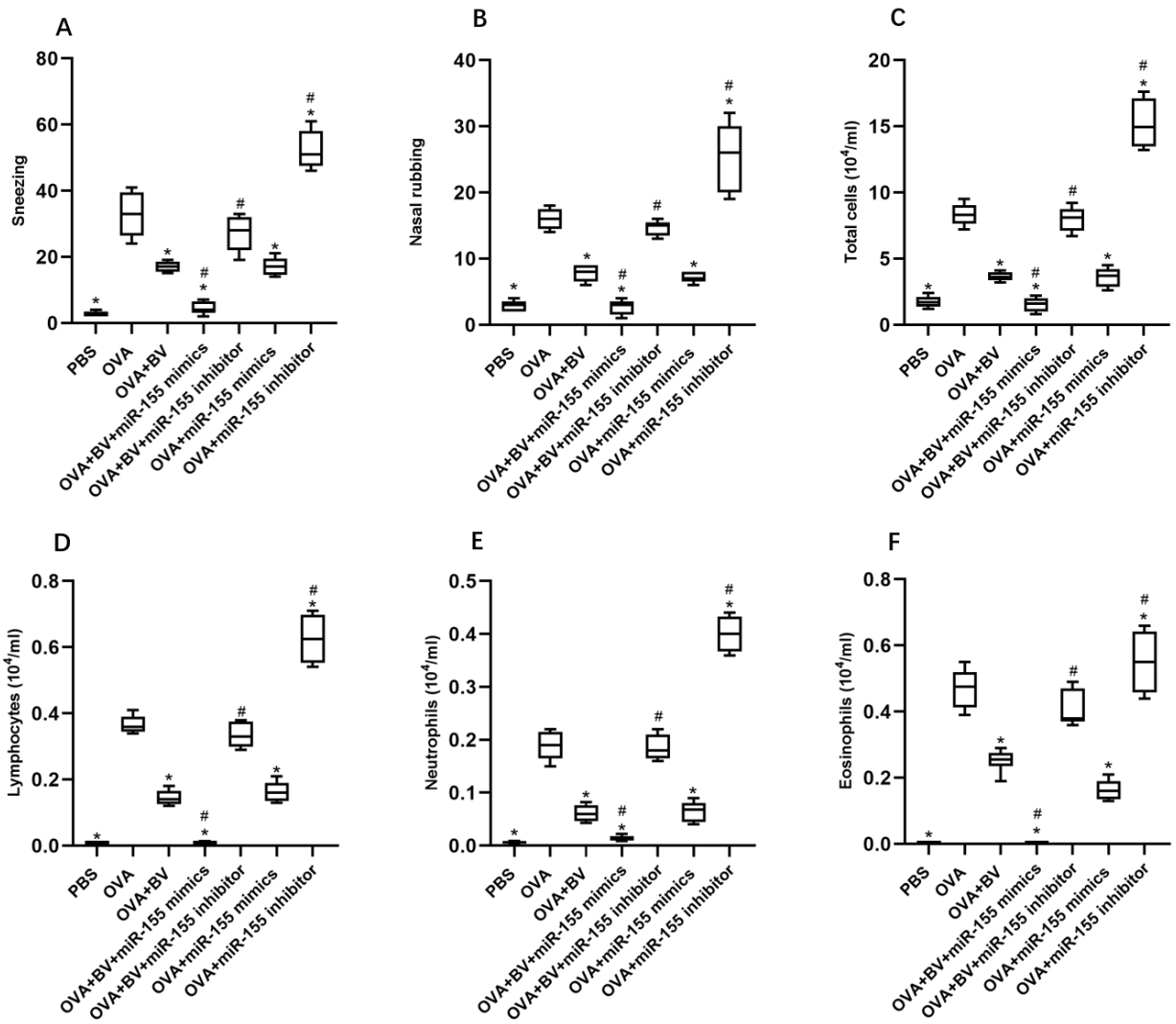

Figure 1. The allergic symptoms and inflammatory cells of OVA induced mice model with allergic rhinitis treated by BV. (A,B) Typical allergic symptoms after BV or miR-155 treatments. (C–F) The total cells, lymphocytes, neutrophils and eosinophils in nasal lavage fluid of allergic mice after different treatment. * Compared with OVA group, $P < 0.05$. # Compared with OVA+BV group, $P < 0.05$. At least 3 of independent experiments were performed. BV: OM-85 Broncho-Vaxom; OVA: Ovalbumin.

3.2. OM-85 BV treatment promote Treg proliferation and function

As shown in Figure 2, the percentage of Treg cells in $CD4^+$ T cells of peripheral blood of OVA-induced AR mice was significantly decreased than control mice (Figure 1A,B). Not only was the proliferation influenced but the function of Tregs were also influenced. The mRNA level of Foxp3, IL-10 and TGF-β was also decreased in AR group (Figure 2C–F). With administration of OM-85 BV or miR-155 mimics, the frequency of Treg cells and the Foxp3, IL-10 and TGF-β mRNA level all were restored (Figure 2). OM-85 BV and miR-155 mimics presented with synergistic effects, while miR-155 inhibitor decreased the proliferation and function of Tregs.

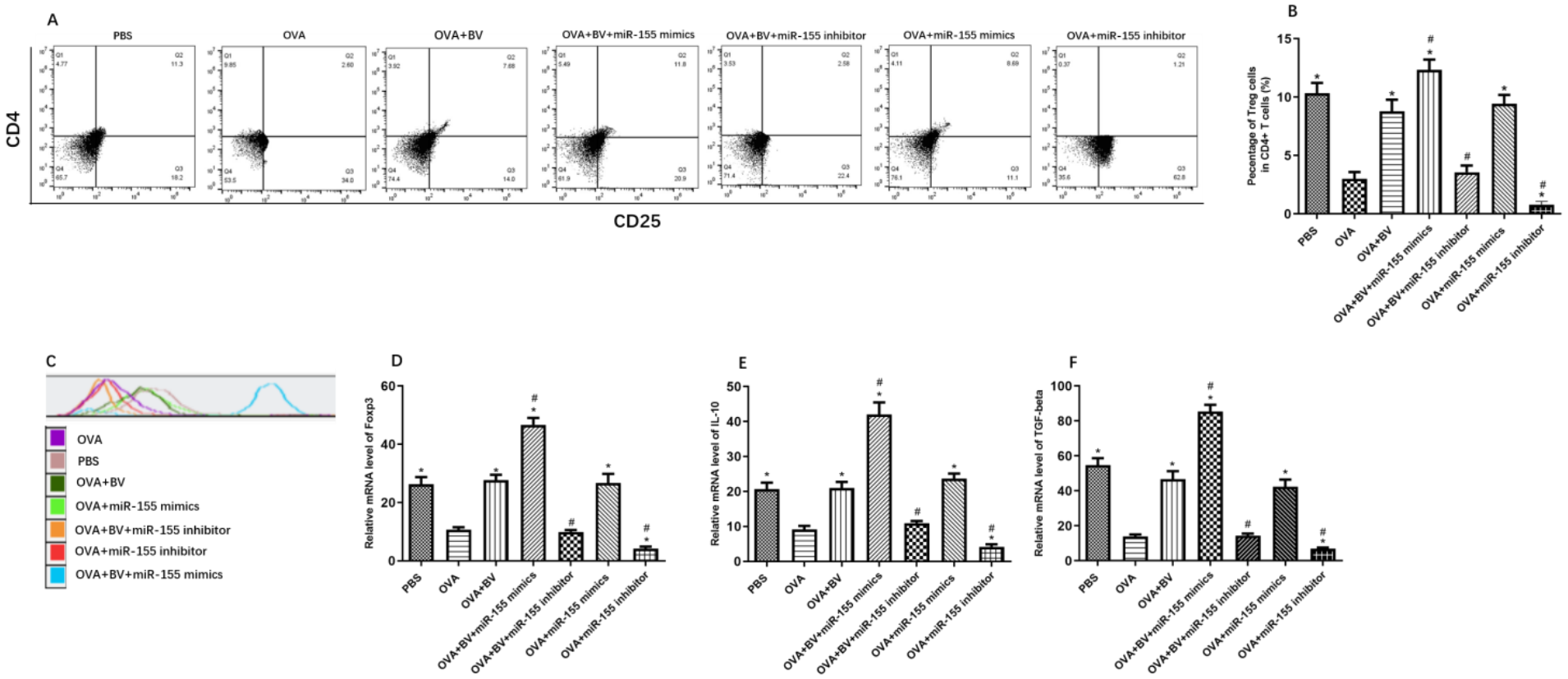

Figure 2. The regulation of proliferation and function of peripheral regulatory T cell populations in mice model treated with BV. (A) Flow cytometry image of Treg cells. (B) Percentage of Treg cells in CD4+T cells in OVA-induced AR with different treatments. (C) Foxp3 expression by Treg cells detected by flow cytometry. (D–F) Relative mRNA level of Foxp3, IL-10 and TGF-β in peripheral blood. * Compared with OVA group, $P < 0.05$. # Compared with OVA+BV group, $P < 0.05$. At least 3 of independent experiments were performed. BV: OM-85 Broncho-Vaxom. OVA: Ovalbumin.

3.3. OM-85 BV cooperated with miR-155 to upregulate Treg function in mice with OVA-induced AR

The proliferation of Treg cells has been increased by OM-85 BV or miR-155 mimics treatment (Figure 3A), as well as the expression of Foxp3 (Figure 3B,C), IL-10 (Figure 3D) and TGF-β (Figure 3E). Moreover, as shown in Figure 3, these effects had been enhanced with the combination of OM-85 BV and miR-155 mimics. Moreover, all effects were reversed to the basal level by the use of miR-155 inhibitors.

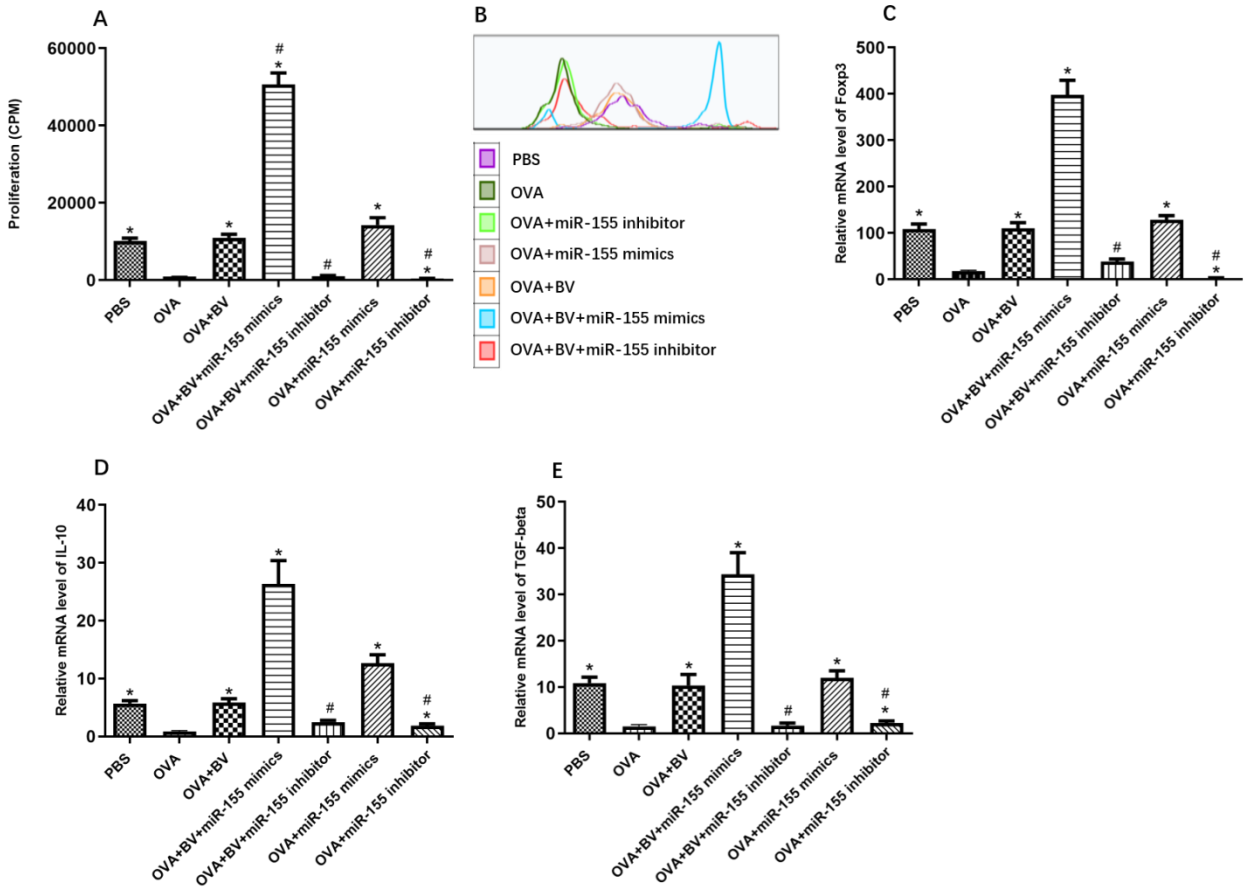

Figure 3. OM-85 BV cooperated with miR-155 mimics to enhance the proliferation and function of peripheral regulatory T cell. (A) Proliferation of Treg cells in OM-85 BV-treated AR and the coeffect with miR-155 mimics/inhibitors. (B) Foxp3 expression by Treg cells detected by flow cytometry. (C–F) Relative mRNA level of Foxp3, IL-10 and TGF-β expressed by Tregs. * Compared with OVA group, $P < 0.05$. # Compared with OVA+BV group, $P < 0.05$. At least 3 of independent experiments were performed. BV: OM-85 Broncho-Vaxom; OVA: Ovalbumin.

4. Discussion

We found that OM-85 BV intranasal treatment provided a protective effect against OVA-induced AR and OM-85 BV cooperated with miR-155 to upregulate Treg function in allergic airway mouse model.

For many decades, extensive studies have demonstrated that OM-85 BV was effective in protecting against virus infection and allergic inflammation in human cells and animal models. With regard to the allergic inflammation, bacterial lysates can prevent atopic dermatitis in children [15], seasonal and perennial AR in children and adults [16–18], and preschool wheezing and childhood asthma [6].

With regard to the mechanism of OM-85 BV in AR, Han's study showed that short-term treatment with OM-85 BV decreased Th2 cytokines and IgE expression, while the Th1 response was up-regulated significantly in mice [19]. Consistently, Meng's research found that the ratio of IFN-γ/IL-4 and IL-10 levels of peripheral blood were upregulated in the OM-85 BV-treated children, whereas IL-4 was reduced in the OM-85 BV -treated group [18]. Moreover, they found that the expression of IL-4 and IL-13 in nasal lavage were down-regulated significantly while the expression of INF-γ was significantly upregulated in the OM-85 BV group [18].

Our data found that both OM-85 BV and miR-155 mimics can inhibit OVA induced allergic inflammation, while miR-155 inhibitors reversed OVA induced allergic inflammation, suggesting the important roles of miR-155 in allergic inflammation. Several studies have proven the regulation of miR-155 in Treg proliferation rather than its function. For example, Kohlhaas found that miR-155 was involved in Treg development by targeting Foxp3, but it did not control Treg function [20]. Schjenken also reported that miR-155 is necessary for expansion of regulatory T cell [21]. Consistently, we also showed that intranasal administration of OM-85 BV may restored the impaired proliferation and function of Tregs, while these effects can be amplified or inhibited by miR-155 mimics and miR-155 inhibitors, respectively. These results suggested that OM-85 BV may exert its effect on Treg through miR-155. Consistently, Fu's study found that oral administration of OM-85 BV inhibited mucus production, Th2 cytokine release and GSK3β expression while promoted Foxp3 production in asthmatic mice [14]. Interestingly, we also found that OM-85 BV and miR-155 had comparable effect in inhibition OVA induced allergic inflammation, while the effect of OM-85 BV was reduced significantly when miR-155 inhibitors was added. These phenomena suggested that the effect of miR-155 was independent of OM-85 BV, but their interaction mechanism needed further investigation.

5. Conclusions

In summary, nasal administration of miR-155 promoted Treg cells function in OM-85 BV treated experimental models of AR and alleviate the upper airway allergic inflammation in AR mice. This may indicate that OM-85 BV can be considered as a potential protective bacterial product against AR.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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