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IL-37 attenuates allergic process via STAT6/STAT3 pathways in murine allergic rhinitis

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ABSTRACT

Allergic rhinitis (AR) is a common upper airway allergic disease caused by allergens triggering a type 2 immune response. The imbalance of CD4+ T cell subsets is the essential immunological feature of AR, which is mainly characterized by the predominance of T helper (Th) 2 cells. Recent studies indicated that the anti-inflammatory factor interleukin (IL)-37 is involved in the immune regulation of AR. However, the mechanism of IL-37 acts on AR has not been fully elucidated. Thus, we sought to assess the protective role of IL-37 in AR and further explore the possible mechanism. An ovalbumin (OVA)-induced AR murine model was established. After IL-37 treatment, the allergic symptoms (sneezes and nasal rubbings), nasal mucosal infiltration with eosinophils, and serum IgE production were found significantly attenuated. For CD4+ T cell subsets, the proliferation and differentiation of Th2 and Th17 cells were restrained. The relevant effector cytokines of IL-4, IL-5, IL-6, and IL-17a protein expression and transcription factors GATA3 and RORγt mRNA levels were obviously decreased. However, IL-37 had no significant effect on Th1 and Treg response including in IFN-γ, IL-10, T-bet, and Foxp3 expression. Furthermore, IL-37 was found down-regulated the STAT6, STAT3, phospho-STAT6, and phospho-STAT3 expression. In conclusion, IL-37 alleviates allergic inflammation in AR possibly through repressing STAT6 and STAT3 signaling pathways.

1. Introduction

Allergic rhinitis (AR) has become a global public health disease, affecting 10 to 40% of the population, particularly in children [1,2]. Patients' quality of life and efficiency of work are severely affected. Moreover, it is closely associated with the occurrence and exacerbation of asthma. At present, therapeutic treatments only can improve the symptoms of AR [1]. The main reason is that its pathomechanism has not been fully elucidated.

AR results from inhaled harmless airborne allergens triggering an immune response disorder in susceptible subgroups. The immune response disorder is mediated by immunoglobulin (Ig) E and driven naive CD4+ T cells to become T helper (Th) 2 phenotype, eventually leading to eosinophilia. Furthermore, the imbalance of Th1/Th2 cells in AR is characterized by Th2 over-differentiation [3,4]. In the past few decades, research has tried to favor Th1 cells development to reverse the pathological process of AR but was not helpful [5,6]. Recent findings have found that Th17 cells and regulatory T (Treg) cells were also involved in the pathogenesis of AR [7]. These suggest that Th2 over-differentiation in AR is associated with CD4+ Th cell subsets imbalance, including Th1, Th2, Th17, and Treg cells.

Interleukin (IL)-37 is a new member of IL-1 families with extensive anti-inflammatory properties [8]. Similar to IL-18 in IL-1 families, caspase-1 processes the precursor IL-37 to mature and enter the nucleus for transcriptional regulation, and IL-37 activity is regulated of Smad3 [9]. In TLR ligands induced inflammation, IL-37 significantly suppresses pro-inflammatory factors (IL-18, IFN-γ, IL-4, IL-1β, TNF, and CpG) and relevant signaling pathways (FAK, cJun, p38-MAPK, STAT1, and p53) [10]. IL-37 also plays an anti-inflammatory role in autoimmune diseases, such as colitis [11], systemic lupus erythematosus [12], psoriasis [13], and asthma [14]. Additionally, we recently reported that the expression of IL-37 in PBMCs of AR patients was abnormally low. In vitro, IL-37 was found to significantly repressed naive CD4+ T cells into Th2 and Th17 cells [15]. Similar results were found

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from the experiment with HDM-induced AR mice, nasal application of recombinant human IL-37 (rhIL-37) could attenuate the local allergic symptoms and suppress the expression of Th2 and Th17 related cytokines in nasal mucosa [16]. However, it is not yet known whether non-local application of IL-37 has a similar inhibitory effect in AR mice. Moreover, the mechanism of IL-37 in AR remains to be fully elucidated.

A few recent studies on the mechanism of IL-37 modulated immunological diseases have indicated that the phosphorylation of signal transducer and activator of transcription (STATs) linked with IL-37 signaling pathway. In allergic asthma, IL-37 alleviates pulmonary eosinophilia and airway remodeling through down-regulated IL-4/IL-13-induced STAT6 activation and STAT3 phosphorylation [17,18]. STATs are a class of cytoplasmic proteins with both signal transduction and transcriptional activation, which play a vital role in regulating the differentiation of naive CD4+ T cells into distinct Th cells. In AR, IL-4 stimulates the activation of STAT6 promotes Th2 response, and the existence of STAT6 inhibited Th1 response. Besides, exposure to IL-6 induces Th17 cells development by activating STAT3, whereas exposure to IL-2 induces Treg cells development by activating STAT5 [19,20].

Based on these previous studies and our recent research, we suspected that IL-37 might improve the imbalance of CD4+ Th cells via STATs signaling pathway in AR. Hence, an ovalbumin (OVA)-induced AR murine model was established. Firstly, we planned to confirm the role of systemic application of IL-37 in AR. Secondly, we planned to detect the effect of IL-37 on the differentiation and function of CD4+ T cell subsets. Finally, we planned to explore the possible mechanism involved in AR through STATs signaling pathways.

2. Materials and methods

2.1. Animals

Male BALB/c mice (6–8 weeks) were purchased from the Center of Experimental Animals of Chongqing Medical University (Chongqing, China). The mice were housed in a specific pathogen-free (SPF) condition. The Institutional Animal Care Committee at Chongqing Medical University approved the experimental procedures.

2.2. AR mouse models and IL-37 interventions

Mice were randomly divided into three groups and each with ten mice. The procedures for sensitization, challenge, and IL-37 treatment protocol are summarized in Fig. 1A. The OVA-induced AR mice were sensitized on days 1, 3, 5, 7, 9, 11, and 13 via an intraperitoneal (i.p.) administration of 1 mg/mL of OVA (Grade V; Sigma-Aldrich, St. Louis, MO, USA) and 20 mg/mL of aluminum hydroxide (Sigma-Aldrich) in sterile saline (0.1 mL/mouse). After sensitization, from days 20 to 30, mice were challenged using intranasal (i.n.) administration daily of 60 mg/mL of OVA in sterile saline (20 μL/mouse). For IL-37 intervention experiment, reference to described previously [21], we changed the administration route, 1 μg rhIL-37 (R&D Systems, Minneapolis, MN, USA) was i.p. administrated 30 min before every OVA challenge and a continuous i.p. for 5 days from days 26 to 30. The control mice were sensitized and challenged with sterile saline instead of OVA.

2.3. Evaluation of allergic symptoms

Ten min after last challenge on day 30, the number of sneezes and nasal rubbings were recorded by two observers blinded to the experimental groups.

2.4. Histologic analysis

Within 24 h after the last challenge on day 30, all mice were decease. The partly nasal mucosa was removed and fixed with 4% paraformaldehyde. And then dehydrated, embedded in paraffin, and cut into 4–5 μm sections. The sections were stained with hematoxylin and eosin (H&E). The number of eosinophils was counted, which observed in 4 randomly selected fields at a magnification of 400×.

2.5. ELISA

The concentrations of total IgE, IFN-γ, IL-4, IL-5, IL-6, IL-10, and IL-17A in serum were determined by mouse ELISA kits (Neobioscience, Shenzhen, China).

2.6. Flow cytometry

Mononuclear cells obtained from murine spleens by Ficoll-Hypaque density-gradient centrifugation. Suspended the cells in RPMI-1640 medium (with 10% FBS) at 1×10^6 cells/mL. For intracellular cytokines staining, to analyze Th1 (CD4+ IFN-γ+), Th2 (CD4+ IL-4+), and Th17 (CD4+ IL-17a+), added 1 μL of BD GolgiPlug (BD Pharmingen, Franklin Lakes, NJ, USA) for every 1 mL of cell medium (1×10^6 cells/mL) and mixed thoroughly. Placed culture in a 37 °C humidified 5% CO_2 incubator for 4–6 h. Followed activation, stained with anti-mouse CD4-FITC Abs (BD Pharmingen) at 4 °C for 30 min. Next, the cells fixed and permeabilized using a fix/perm solution (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions. After that incubated with anti-mouse IFN-γ-PerCP-Cy5.5, IL-4-PE-Cy7, IL-17a-PE Abs (BD Pharmingen) at 4 °C for 30 min. For nuclear transcription factor staining, to analyze Treg (CD4+CD25+Foxp3+), 1×10^6 cells were simultaneously stained with anti-mouse CD4-FITC and CD25-APC Abs (BD Pharmingen) at 4 °C for 30 min, and then cells fixed and permeabilized using a Transcription Factor Buffer Set RUO (BD Pharmingen) according to the manufacturer's instructions. After that incubated with anti-mouse Foxp3-PE Abs (BD Pharmingen) at 4 °C for 40–50 min. Flow cytometry was performed using a BD Aira III flow cytometer (BD Biosciences Inc., USA). Data were performed using FlowJo software (ver. 10.0.7; Tree Star Inc., USA).

2.7. Quantitative real-time PCR (RT-PCR) analysis

The total RNA was isolated from nasal mucosa using RNAiso Plus (Takara Biotechnology, Dalian, China) and then 1 μg RNA was reversed transcribed into cDNA using the PrimeScript RT reagent kit (TaKaRa). The RT-PCR analysis was performed on CFX96 Real Time PCR Machine (Bio-Rad, Hercules, CA) using SYBR Premix Ex Taq™ II (TaKaRa). All procedures according to the manufacturer's instructions. The sequences of the RT-PCR primers were seen for Table 1. The relative expression of each target gene was measured using the cycle threshold ($2^{-\Delta\Delta CT}$) method and normalized to the internal control GAPDH.

2.8. Western blotting

Protein extracted from mouse nasal mucosa and the protein concentrations were determined by bicinchoninic acid (BCA) reagent kit (Dingguo, Beijing, China). Primary antibodies against phospho-STAT6 (Tyr641) (1:1000) (Cell Signaling Technology, Beverly, MA, USA), total-STAT6 (1:1000) (Abcam, Cambridge, MA, USA), phospho-STAT3 (Tyr705) (1:1000) (Cell Signaling Technology), total-STAT3 (1:1000) (Cell Signaling Technology), and GAPDH (1:1000) (Proteintech, USA) were incubated. Blots were detected with the ECL detection system (Pierce/Thermo Fisher Scientific, USA).

2.9. Statistical analysis

All data from three independent experiments were expressed as mean ± SEM. A one-way ANOVA was used for multiple comparisons among 3 groups followed by a Bonferroni post hoc test. P value < 0.05 was considered as significant difference. Statistical analyses were

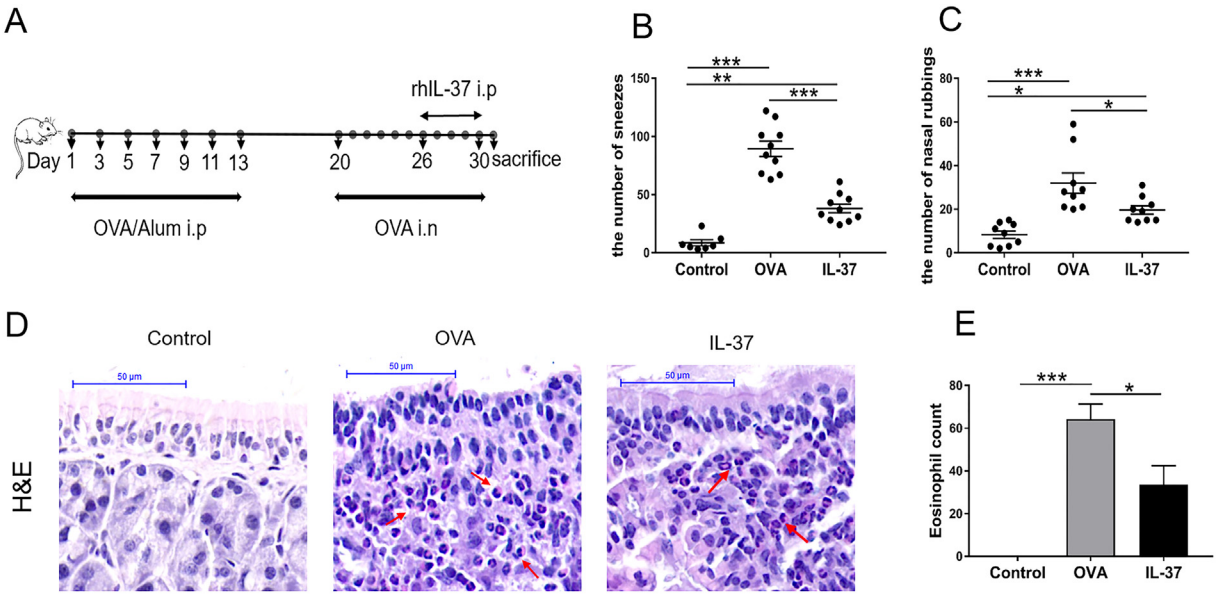

Fig. 1. Systemic administration of IL-37 attenuates ovalbumin (OVA)-induced allergic symptoms and nasal eosinophil infiltration. (A) OVA and rhIL-37 administration protocol. The number of (B) sneezes and (C) nasal rubbings of each treated mouse was recorded. (D) Representative photomicrographs of the nasal mucosa sections from control, OVA, and IL-37 mice were stained with H&E. The arrows indicate the eosinophils. (Original magnification, 400×). (E) The number of eosinophils was counted in nasal mucosa. Data are expressed as mean ± SEM and are representative of 3 independent experiments. Sneezes: n = 10, except for control group where n = 7; nasal rubbings: n = 9 per group; n = 4 per group for histopathology. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 1
Primer sequences used for RT-PCR.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
T-bet	AGCAAGGACGGCGAATGTT	GGGTGGACATATAAGCGGTTC
GATA3	CTCGGCCATTCGTACATGGAA	GGATACCTCTGCACCGTAGC
RORγt	GACCCACACCTCACAAATTGA	AGTAGGCCACATTACACTGCT
Foxp3	CACCCAGGAAAGACAGCAACC	GCCTTGCCTTTCTCATCCAG
STAT3	GCTGCTGGGCTTGTTTAGTG	AGGTTGACAAGACACAATGGAG
STAT6	CTCTGTGGGGCCTAATTTCCA	GCATCTGAACCGACCAGGAAC
GAPDH	TGTCTCCTGCGACTTCAACA	AGCCGTATTCATTGTCATACCAG

performed using the GraphPad Prism software (ver. 7.0; GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Systemic application IL-37 alleviates allergic symptoms in AR mice

We first assessed the effect of IL-37 on AR symptoms in mice by systemic administration. Within 10 min after the last challenge, the number of sneezes and nasal rubbings of each mouse was counted. As showed in Fig. 1B and C, compared with control mice, OVA-induced mice and IL-37-treated mice significantly increased the number of sneezes and rubbings. However, in OVA-induced mice, both sneezes and nasal rubbings were observed more frequently than those in IL-37-treated mice. Therefore, the data showed that systemic administration of IL-37 alleviated the allergic symptoms in AR mice.

3.2. IL-37 reduces nasal eosinophil infiltration in mice

Since eosinophils release inflammatory mediators causing allergic symptoms [22,23], we investigated these pathological changes of nasal mucosa in mice by histologic analysis. H&E stain was used to analyze the eosinophils (Fig. 1C and D). The OVA-induced mice showed histopathologic features of AR, including cilia shedding, numerous eosinophils, and monocytes assembled in the nasal mucosa. The number of eosinophil positive cells was much higher in OVA-induced mice than in control mice. By contrast, IL-37-treated mice were getting fewer eosinophils than those in OVA-induced mice. The IL-37 displayed an anti-inflammatory capacity to reduced inflammatory cell infiltration with eosinophils in the nasal mucosa.

3.3. IL-37 decreases serum total IgE levels in mice

The increase of serum IgE level is the key feature of AR, and IgE response is positively correlated with the severity of AR [4,24]. To investigate the effect of IL-37 on total IgE level, we measured the level of serum total IgE by ELISA kit. As seen in Fig. 2, the total IgE levels of OVA-induced mice were much higher than those in control mice. After IL-37 treatment, the mice had significantly reduced the level of total IgE. The experiment showed that IL-37 regulated the expression of total IgE in mice with AR.

3.4. IL-37 treatment effects on CD4+ Th cell subsets

CD4+ Th cell subsets imbalance, particularly Th2 cell polarization,

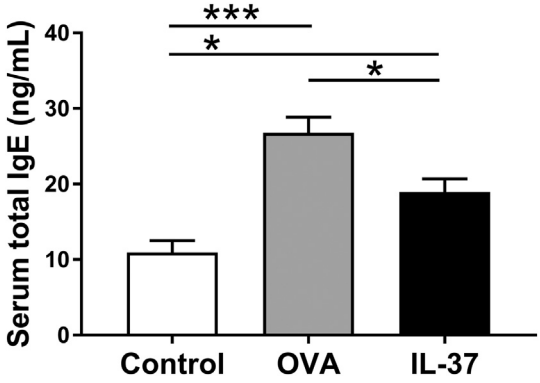

Fig. 2. IL-37 decreases the total IgE levels in serum. Serum total IgE levels of each treated mice were measured by ELISA Kit. Data are expressed as mean ± SEM. Representative findings from 7 mice in each treatment group are shown. *$P < 0.05$; ***$P < 0.001$.

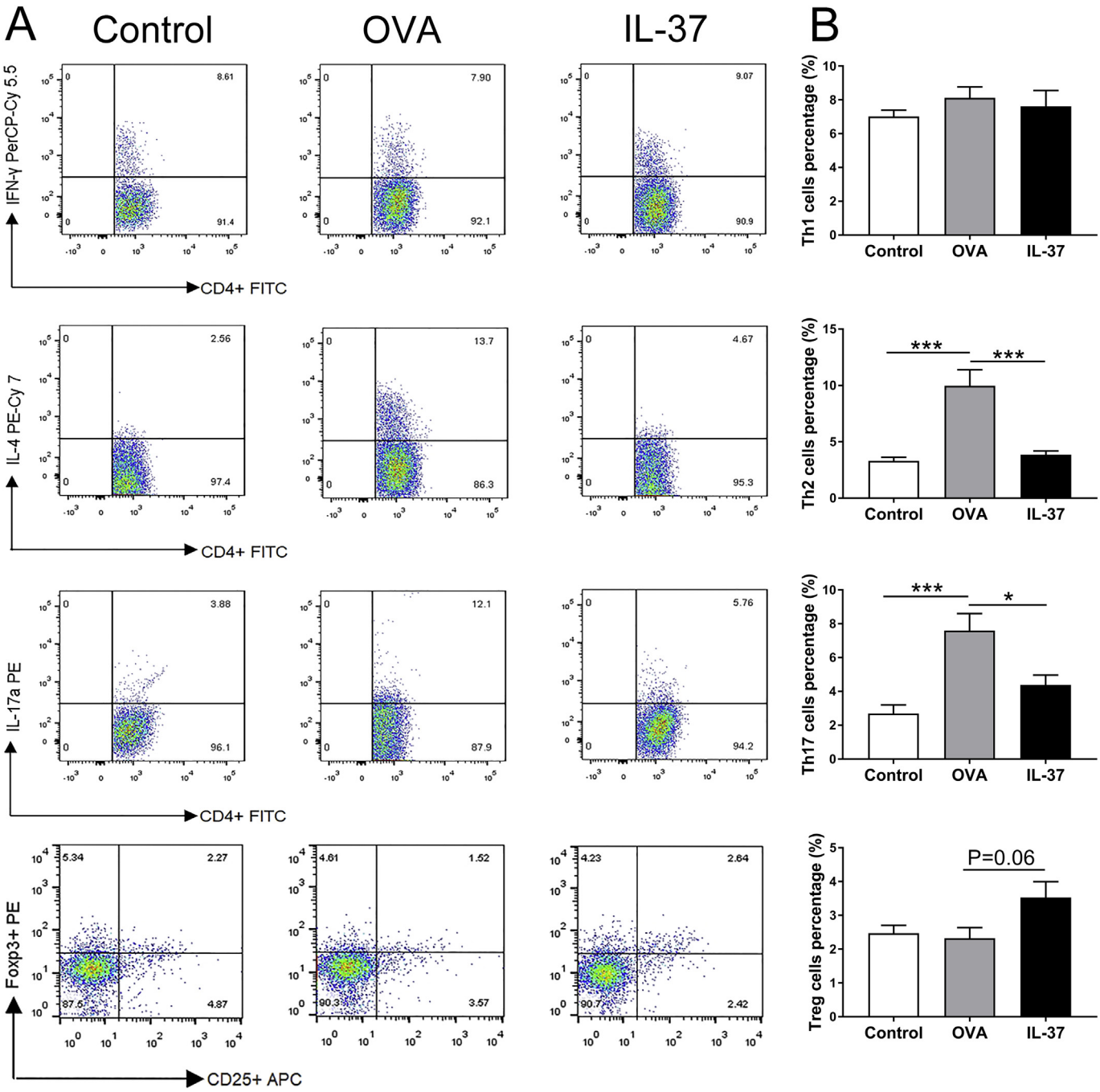

Fig. 3. IL-37 influences on the differentiation and proliferation of Th2 and Th17 cells in CD4+ T cells. The proportions of Th1, Th2, Th17, and Treg cells in splenic CD4+ T cells were measured using flow cytometry. Gating of CD4+ T cell populations. (A) Scatter plots display intracellular expression of IFN-γ, IL-4, IL-17a and Foxp3 in CD4+ T cells. The upper right quadrant represents the proportion of each target factor in CD4+ T cells. (B) Show the statistics of percentages of CD4+ T cell subsets. Data are expressed as mean ± SEM and are representative of 3 independent experiments performed in triplicate. n = 10 per group. $^{*}P < 0.05$; $^{***}P < 0.001$.

contributes to the trigger and maintain allergic response [3,25]. To investigate whether IL-37 affects the differentiation of CD4+ T cell subsets including Th1, Th2, Th17, and Treg, we utilized flow cytometry to measure the proportions of Th1, Th2, Th17, and Treg cells in splenic CD4+ T cells (Fig. 3A and B). For Th2 and Th17 cells, OVA-induced mice increased the proportions of CD4+ IL-4+ and CD4+ IL-17a+ T cells. These were distinctly higher than those of control mice. However, the proportions of CD4+ IL-4+ and CD4+ IL-17a+ T cells in IL-37-treated mice had decreased compared with those measured in OVA-treated mice. For Th1 cells, the proportions of CD4+ IFN-γ+ T cells showed no significant difference among differently treated mice. For Treg cells, the proportion of CD4+CD25+Foxp3+ T cells in OVA-induced mice were less than that measured in other treated mice. Moreover, IL-37-treated mice expressed higher CD4+CD25+Foxp3+ T cell proportion compared with that observed in other treated mice. However, the data analyses showed that IL-37 treatment had no significant promotion on the proportion of Treg cells in OVA-induced mice. In summary, these results indicated that IL-37 inhibited the proliferation and differentiation of Th2 and Th17 cells, but did not of Th1 and Treg cells in AR models.

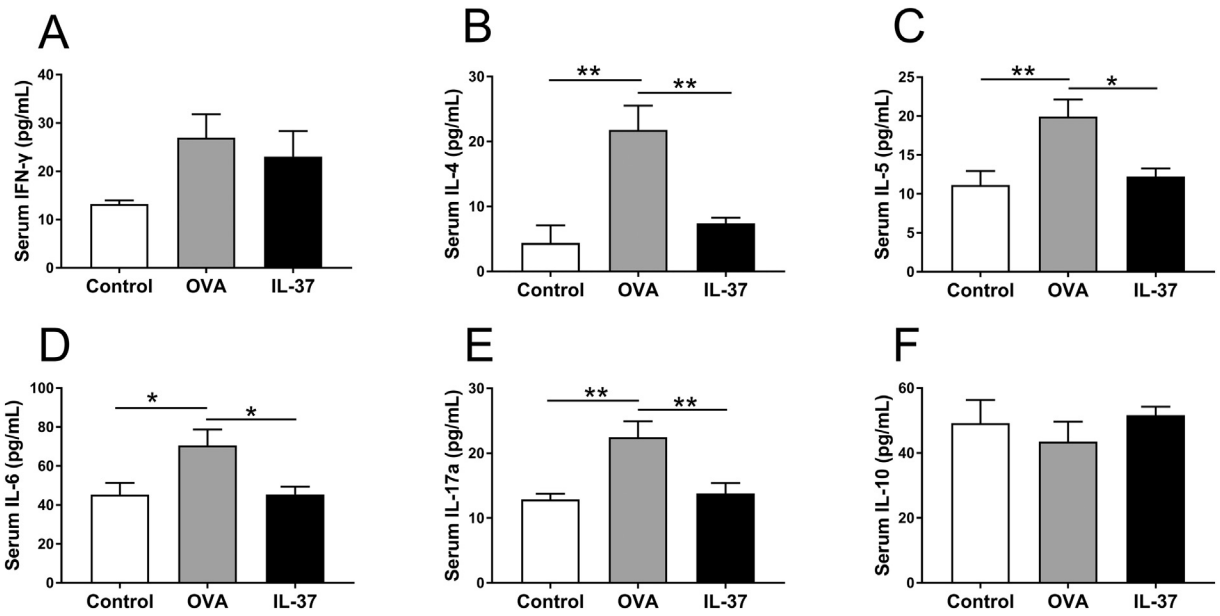

Fig. 4. IL-37 represses Th2 and Th17 cell related effector cytokines in serum. The protein expressions of (A) IFN-γ, (B) IL-4, (C) IL-5, (D) IL-6, (E) IL-17a, and (F) IL-10 in serum were measured by ELISA Kits. Data are expressed as mean ± SEM and are representative of 3 independent experiments that were carried out in triplicate. n = 6 per group for IL-4; n = 8 for IL-5 and IL-6; n = 10 for IFN-γ, IL-17a, and IL-10. $^{*}P < 0.05$; $^{**}P < 0.01$.

3.5. IL-37 treatment effects on Th cells effector cytokines

IFN-γ as the Th1 effector cytokine; IL-4, IL-5, and IL-6 as the Th2 effector cytokines; IL-17a as the Th17 effector cytokine; and IL-10 as the Treg effector cytokine. Exposed to different cytokines would influence the direction of naive CD4+ T cell differentiation and amplify the specific T responses [3,5]. We therefore examined the protein levels of Th1, Th2, Th17, and Treg effector cytokines in serum. As showed in Fig. 4, the protein levels of Th2 and Th17 effector cytokines were all significantly higher in OVA-induced mice than those observed in IL-37-treated mice, and similar results were obtained in control mice (Fig. 4B–E). However, Th1 and Treg effector cytokines had no statistically significant differences among differently treated mice (Fig. 4A and F). These data demonstrated that IL-37 mainly affects effector cytokines relevant for Th2 and Th17 cells. To sum up, IL-37 could affect the function and activation of Th2 and Th17 cells in AR mice.

3.6. IL-37 treatment effects on Th cell-related gene expression

To further confirm the underlying effects of IL-37 improves CD4+ T cells imbalance in AR, we measured the mRNA levels of relevant transcription factors for T cells in the nasal mucosa. As showed in Fig. 5B and C, GATA3 regulates Th2 and RORγt regulates Th17, the mRNA expression levels of GATA3 and RORγt were all down-regulated in IL-37-treated mice. Additionally, T-bet regulates Th1 and Foxp3 regulates Treg. There was no significant mRNA expression difference between T-bet and Foxp3 among differently treated mice (Fig. 5A and D). Compared data, the transcription levels of Th subsets were consistent with the protein levels of Th effector cytokines in differently treated mice. Thus, these results showed that IL-37 mainly affected Th2 and Th17 relevant transcription factors.

3.7. IL-37 regulates Th2 and Th17 response via STAT6 and STAT3 signaling pathways

Previous data confirmed that IL-37 mainly suppresses naive CD4+ T cells into Th2 and Th17 cells both in protein and mRNA levels. STAT6 and STAT3 act on transcriptional regulators GATA3 and RORγt, which directly up-regulate GATA3 and RORγt expression [19]. Accordingly, we evaluated the effect of IL-37 on STAT6 and STAT3 mRNA levels in the murine nasal mucosa. Fig. 6A and B shows that the mRNA levels of STAT6 and STAT3 significantly decreased in IL-37-treated mice compared with detected in OVA-induced mice. Furthermore, we suspected IL-37 may inhibit the phosphorylation of STAT6 and STAT3. To confirm the possibility, we performed western blotting on protein prepared from nasal mucosa in each treatment group (Fig. 6C). We found that IL-37 treatment had an inhibitory effect on STAT6 and STAT3 phosphorylation. Combined with these data, we speculated that IL-37 could suppress naive CD4+ T cells into Th2 and Th17 through STAT6 and STAT3 signals.

4. Discussion

Here, in a murine AR model induced by OVA, systemic application of IL-37 attenuated the ongoing allergic inflammatory response in vivo. Allergic symptoms, nasal mucosal eosinophilia, and serum total IgE production were found evidently alleviated in IL-37-treated AR mice. Subsequently, our results showed that IL-37 alters the immune imbalance of CD4+ Th subsets by suppressing Th2 and Th17 response. Furthermore, we provided data that IL-37 regulates Th2 and Th17 response are associated with STAT6 and STAT3 signaling pathways.

Allergens trigger the activation of IgE bind to the high-affinity IgE receptor FcεRI on the surface of eosinophils. Then chemical mediators are released into surrounding tissues causing a series of clinical allergic symptoms [22–24]. Thus, we first evaluated the effect of the systemic application of IL-37 in AR. An OVA-induced mouse model of allergic rhinitis was used. After IL-37 treatment, allergic symptoms, serum IgE levels, and eosinophil infiltration were found significantly diminished. These data suggest that systemic application of IL-37 abates the hallmarks of experimental AR.

The imbalance of Th1/Th2/Th17/Treg cells is the essential immunological feature of AR. The excessive differentiation and activation of Th2 and Th17 cells can result in issues such as declined quantity and function of Th1 and Treg cells [3]. Therefore, in order to learn about the role of IL-37 on the immune regulation network, we investigated the effect of IL-37 on restraining CD4+ Th cell subsets. Th cell proportions in CD4+ T cells, Th effector cytokines protein levels and transcription factors mRNA levels were analyzed. Our results are partially similar to

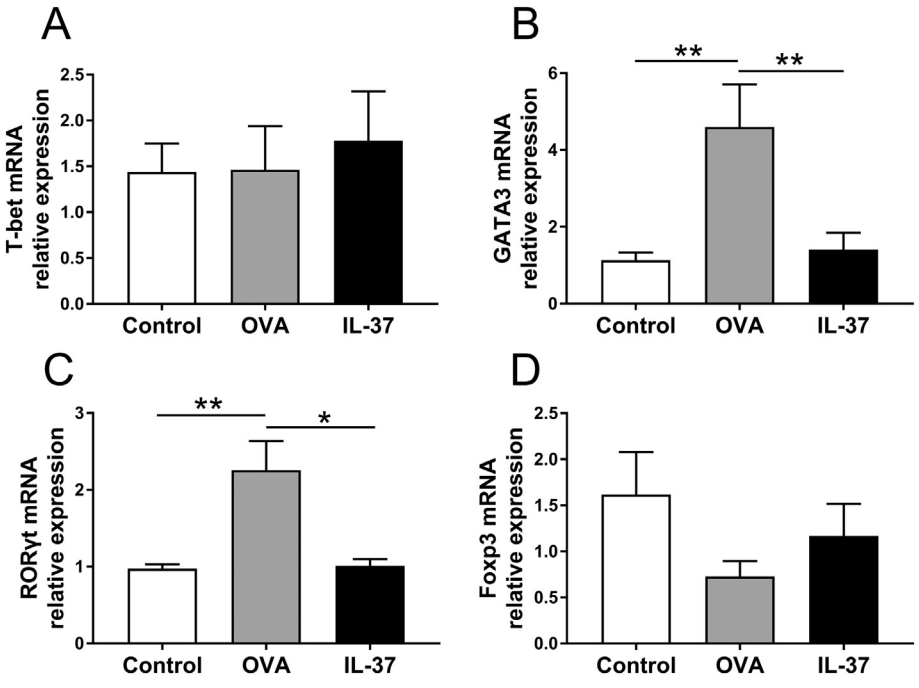

Fig. 5. IL-37 inhibits Th2 and Th17 related transcription factor gene expression. RT-PCR analyses of (A) T-bet, (B) GATA3, (C) RORγt, and (D) Foxp3 mRNA expression in nasal mucosa extracts of mice treated as indicated. Data are expressed as mean ± SEM and are representative of 3 independent experiments performed in triplicate, n = 5 for T-bet and RORγt; n = 8 for GATA3 and Foxp3. $*P < 0.05$; $**P < 0.01$.

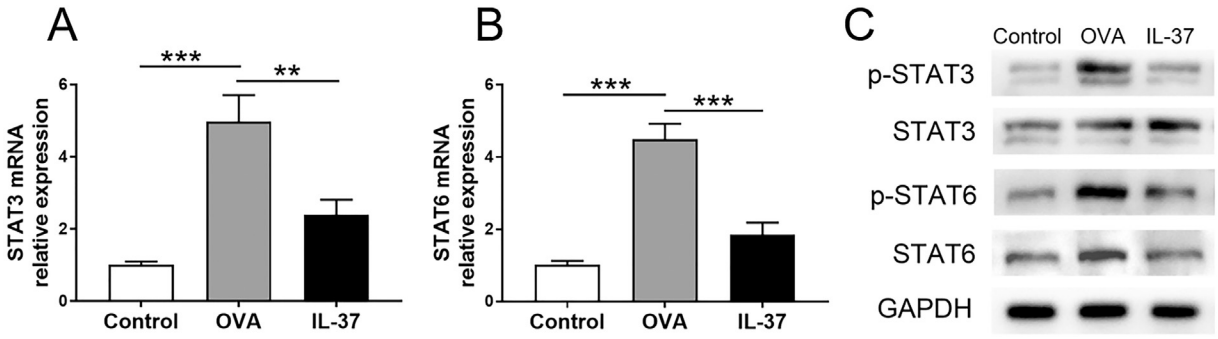

Fig. 6. IL-37 reduces nasal mucosal STAT6, p-STAT6, STAT3, and p-STAT3 expression. (A) STAT3 and (B) STAT6 mRNA expression in nasal mucosa were measured by RT-PCR. (C) STAT3, p-STAT3, STAT6, and p-STAT6 protein expression in nasal mucosa were measured by western blotting. mRNA and protein extracts of mice treated as indicated. Data are expressed as mean ± SEM and are representative of 3 independent experiments run in triplicate, n = 6 per group. $**P < 0.01$; $***P < 0.001$.

Do Hyun Kim's [16] study. In his study, after local stimulation with IL-37, the protein expression of IL-4, IL-17a, GATA3, and RORγt in the nasal mucosa which analyzed by western blotting were decreased. Notably, we further found the proportions of Th2 and Th17 in CD4 + T cells were both decreased. For Th1 and Treg response (the proportions of Th1 and Treg; IFN-γ and IL-10 protein; T-bet and Foxp3 mRNA) were not any significantly changed by IL-37 treatment. However, the *P* value of Treg proportions in CD4 + T cells between OVA-induced mice and IL-37-treated mice are very close to 0.05 ($P = 0.06$). We suspect that differences among those with the proportions of Treg were not significant because of the small sample size (n = 10 per group). Besides, IL-10 is considered to be an anti-inflammatory factor in AR [26]. However, inconsistent with Do Hyun Kim's study, IL-37 treatment appeared not to elevate IL-10 protein levels in AR murine models. As we reported previously, IL-37 has no effect on IL-10 in AR patients [15]. Meanwhile, Nold et al. [8], McNamee et al. [11], and Lunding et al. [21] also showed that IL-37 treatment do not effect on IL-10 expression in mice of LPS-induced shock, colitis, and asthma models. We suspect that this difference is due to IL-10 also secreted by other lymphocytes, such as, B cells [27], monocytes [28], and macrophages [29], which might obscure the inhibition of IL-37 on IL-10. Hence, it is recommended to further explore the effect of IL-37 influences on other lymphocytes. Taken together, we provided data that IL-37 directly suppressed Th2 and Th17 response in AR mice models.

So far, the exact mechanism for IL-37 works on Th2 and Th17 cells in AR has not been elucidated. In an allergic response, Th2 cells activation depends on abnormal STAT6 signal transduction pathway. More notably, IL-4 stimulation is required for STAT6 activation. Activated STAT6 induces naive CD4+ T cells into Th2 cells by up-regulating GATA3 [20]. Thus, we further explored whether IL-37 affects the activation of STAT6 signaling in OVA-induced mice. Our data displayed that STAT6 phosphorylation and IL-4 levels were both decreased in IL-37-treated mice. These data indicate that IL-37 may down-regulate GATA3 expression by inhibiting the IL-4/STAT6 signaling pathway, ultimately impairing the proliferation, differentiation, and activation of Th2 cells in AR. The similar relationship also exists among IL-37, STAT3, and Th17 cells. IL-6 stimulation is required for STAT3 activation [19,30,31]. In our study, IL-6 levels were significantly reduced in IL-37-treated mice. Therefore, data also suggest that IL-37 may down-regulate RORγt expression by suppressing the IL-6/STAT3 signaling pathway, leading to weakening the proliferation, differentiation, and activation of Th17 cells in AR. Of note, some reports showed that STAT3 also involves in Th2 response in allergic response [32–34]. In murine allergic asthma, lack of STAT3 did not form Th2 inflammatory

[34,35]. These indicated that IL-37 also suppress Th2 response through the IL-6/STAT3 pathway. According to our data suggest that IL-37 may suppress Th2 and Th17 response via IL-4/STAT6 and IL-6/STAT3 signaling pathways.

At present, an issue that is not addressed in this study is which type of Th cells might be the precise target cell affected by IL-37. Besides, our research on the molecular mechanisms of the interaction among IL-37 and STAT6/STAT3 remains to be fully elucidated. Next, we may further explore whether these effects are direct or indirect through in vivo and in vitro experiments, such as using transgenic mice, Co-IP, et cetera.

In conclusion, our findings provide evidence of the anti-inflammatory role of IL-37 in an OVA-induced murine AR model. Firstly, our study shows that local and systemic allergic responses in AR mice were both repressed. Subsequently, our study qualifies that IL-37 improves the imbalance of CD4+ T cell subsets. Finally, our study displays that STAT6 and STAT3 are involved in the anti-inflammatory activity of IL-37. Therefore, the potency of IL-37 with its anti-inflammatory effects could make it an attractive candidate for AR immunotherapy. In particular, IL-37 may become an important target molecule for AR prevention.

Conflict of interest

None.

Acknowledgments

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