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Inhibition of dendritic cell autophagy alleviates the progression of allergic rhinitis by inhibiting Th1/Th2/Th17 immune imbalance and inflammation

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Summary. Introduction. Immune imbalance is a fundamental immunological feature of allergic rhinitis (AR). The autophagy in $CD11c^+$ dendritic cells (DCs), the strongest antigen-presenting cells, was reported to induce the occurrence of AR by facilitating $CD4^+$ T cell immune imbalance and subsequent inflammation. Our study was designed to confirm that inhibition of DC autophagy can alleviate the progression of AR by inhibiting the T cell immune imbalance.

Methods. The AR mouse model was established by using ovalbumin (OVA). OVA-induced mouse models were then injected intraperitoneally with the autophagy inhibitor Baf-A1. Levels of OVA-specific IgE, PGD2, ECP, LTC4, and Th1/Th2/Th17 cell-related cytokines in serum or nasal lavage fluid (NLF) were examined using the corresponding commercial ELISA kits. Morphological changes in the nasal mucosa were observed by HE staining. Nasal mucosa tissues were collected for western blotting to assess the expression of autophagy markers (LC3, P62, and Beclin 1) in each group of mice.

Results. Baf-A1 treatment alleviated the allergic symptoms, mitigated inflammatory immune cell infiltration in the nasal mucosa, decreased IgE, LTC4, ECP, and PGD2 levels in both serum and NLF, impaired $CD11c^+$ DC autophagy, and restored Th1/Th2/Th17 cytokine imbalance in OVA-induced AR mice. Furthermore, Baf-A1 treatment also reversed the immune imbalance of $CD4^+$ T cell subtypes and attenuated Th1/Th2/Th17 cytokine imbalance *in vitro*.

Conclusion. Inhibition of $CD11c^+$ DC autophagy suppressed the immune imbalance of $CD4^+$ T cell subsets and attenuated the subsequent inflammatory response, thereby ameliorating the progression of AR.

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Introduction

Allergic rhinitis (AR) is an IgE-mediated allergic inflammatory reaction of the nasal mucosa, whose clinical symptoms include nasal pruritus, nasal congestion, sneezing, and rhinorrhea (Skoner, 2001). The prevalence of AR has rapidly increased in recent years, reaching approximately 10% of the global population (Tharpe and Kemp, 2015). AR does not pose a fatal threat to human life, however, it severely impairs the quality of life and work productivity among patients, which makes it an important health concern (Hong et al., 2014). A variety of drugs have been applied in the clinical treatment of AR, such as antihistamines, mast cell stabilizers, nasal decongestants, leukotriene receptor antagonists, and glucocorticosteroids, in which glucocorticosteroids are believed the most effective (Ridolo et al., 2016; Klimek et al., 2019). However, many AR patients are still faced with failed treatments, unsatisfactory results, or recrudescence (Drazdauskaitė et al., 2020). Thus, the development of more innovative and effective remedies for AR treatment is urgently required.

In the pathogenesis of AR, allergens are ingested by antigen-presenting cells (APCs) after contact with the body (Liu et al., 2022). Processed antigens form complexes with major histocompatibility complex (MHC) molecules and are presented on the cell surface for recognition by helper T cells (Th) to induce immune imbalance (Kawamoto et al., 2016). Attenuated Th1 cell responses and excessive Th2 and Th17 cell responses are always involved in this process, thereby facilitating the allergic inflammatory response (Elkord, 2006). Dendritic cells (DCs), as the strongest antigen-presenting cells in

the body, are strategically located in the skin and mucosal system (Froidure et al., 2016). They are responsible for recognizing and capturing invading foreign pathogens and antigens, as well as eliciting immune responses by activating effector T cells or inducing tolerance through regulatory T cells (Schuurhuis et al., 2006). DCs in contact with allergens are trigger points for allergic responses, playing an essential role in the pathogenesis of AR (Hammad and Lambrecht, 2008).

Autophagy is an important mechanism for maintaining cellular homeostasis, which can remove misfolded or aggregated proteins and damaged or aged organelles in cells (Tesseraud et al., 2021). Autophagy is involved in various fundamental biological processes including differentiation, development, immunity, and aging (Deng et al., 2022). Under normal growth conditions, cells can carry out a lower level of autophagy, namely basal autophagy, to maintain the homeostasis of the body's internal environment under physiological conditions (Racanelli et al., 2018). However, under various extracellular or intracellular stimuli (starvation, hypoxia, high temperature, or injury), the autophagy level will be rapidly upregulated (Wei et al., 2022). Under the above conditions, some autophagy genes (such as LC3II and Beclin 1) participate in inducing the formation of autophagosomes, accompanied by enhanced conversion of LC3I to LC3II and the degradation of p62 (Wei et al., 2022). Increasing research has demonstrated that autophagy is associated with the pathogenesis of respiratory allergic inflammation, such as asthma and AR (Liu et al., 2016). Patients with AR had more autophagosomes and higher LC3II and Beclin-1 expression in the upper airways than healthy people (Li and Li, 2019).

Autophagy can regulate adaptive immunity by modulating antigen presentation and lymphocyte development and plays an active role in host defense by eliminating pathogens, regulating cytokine signaling, and inducing innate immunity (Levine et al., 2011). Numerous studies have shown the vital role of autophagy in the immunomodulatory mechanism of respiratory diseases (Dowling and Macian, 2018). Previously, the association between autophagy and immune imbalance in the development of AR has been reported. For example, baicalin was demonstrated to relieve the symptoms of AR by inhibiting autophagy and regulating Treg/Th17 balance (Li et al., 2020). The inhibition of IL-33 activates autophagy and suppresses the degranulation and release of inflammatory factors in mast cells, thereby alleviating the development of AR (Nian et al., 2020). However, whether the autophagy in DCs is implicated in the immune imbalance in the pathogenesis of AR has rarely been investigated. Therefore, our study intended to confirm the role of DC autophagy in the Th1/Th2/Th17 cell immune imbalance and inflammation in the mouse model of AR by using the autophagy inhibitor Baf-A1.

Materials and methods

Animals

C57BL/6 female mice (age, 4-6 weeks; weight, 20-25g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Mice were raised in a specific pathogen-free barrier (SPF) room (temperature, 18-22°C; humidity, 50-60%) with food and water *ad libitum* at the Animal Experimental Center of Wuhan Fourth Hospital. Animal protocols were approved by the Ethical Review Committee of Wuhan Myhalic Biotechnology (Approval number: 202203031).

Establishment of AR mouse model

Eighteen mice were fed adaptively in the SPF laboratory for 1 week and were then randomly assigned to sham, AR, and AR+Baf-A1 groups, with 6 mice in each group. The mice in AR and AR+Baf-A1 groups were intraperitoneally injected with 50 μg OVA (Sigma-Aldrich) and 1 mg aluminum hydroxide (Sigma-Aldrich) for basic sensitization on days 1, 7, and 14 (Dong et al., 2020). Mice in the sham group were administered with an equivalent volume of saline at the same time. Then, mice in the AR group received an intranasal challenge with 10% OVA once a day from day 15 to 28 (Xu et al., 2019). Baf-A1 was dissolved in DMSO and diluted to 0.1 mg/mL with normal saline. Mice in the AR+Baf-A1 group were injected intraperitoneally with Baf-A1 solution at a dose of 1 mg/kg 1h before each OVA administration from day 15 to 28. The sham mice were intraperitoneally injected with normal saline once daily from day 15 to 27, the experimental flowchart is shown in Figure 1A.

Allergic symptom examination and scoring

After the last nasal drip, sneezing and nasal rubbing events in 10 min were recorded in each group of mice blindly. Furthermore, the features of a runny nose as well as the number of sneezes and nasal rubs in each group of mice were observed within 30 min. Allergic symptoms were scored as previously described (Jiao et al., 2019).

Nasal lavage fluid (NLF) collection and cell counting

At 24h after the last nasal drip, mice were sacrificed under anesthesia. Part of the trachea was removed, and an 18-gauge catheter was inserted into the trachea and up the airway into the nasopharynx. Next, the nasal passages were perfused with 1 mL of pre-chilled phosphate-buffered saline (PBS, Gibco). NLF was collected, and 150 μL NLF samples were centrifuged onto glass slides using a Cytopro Cytocentrifuge Series 2 cytospin apparatus (ELITech Group, Puteaux, France). Neutrophils, lymphocytes, eosinophils, and leukocytes in

NLF were stained with a Diff-Quick staining kit (Solarbio) and observed using a Nicolet Continuum Infrared Microscope (Thermo Fisher Scientific).

ELISA

The blood samples were centrifuged to obtain the serum. NLF was also centrifuged to collect the supernatant for cytokine measurement. The levels of IgE, ECP, PGD2, LTC4, IL-2, IFN-γ, IL-4, IL-6, IL-10, and IL-17 in serum or NLF were detected by corresponding commercial ELISA kits (eBioscience, San Diego, CA, USA) following the manufacturer's protocols.

Morphological observation of nasal mucosa

Mice were sacrificed 24h after the last nasal drip. The complete nasal bone was excised, fixed in 10% paraformaldehyde, and then transferred to a 10% EDTA decalcification solution. After 3-4 weeks, the completely softened bone was embedded in paraffin and sectioned for hematoxylin and eosin (HE) staining. Morphological changes in the nasal mucosa were observed and the eosinophil infiltration status was assessed.

Western blotting

Mouse nasal mucosal tissues were placed in cold high-efficiency tissue lysate radioimmunoprecipitation assay (RIPA) buffer and homogenized with an electric homogenizer. The supernatant of the tissue lysate was collected by centrifugation. An equivalent amount of protein was separated in a 10% SDS-PAGE gel and transferred to the PVDF membrane (Millipore). The PVDF membrane was placed in 5% skimmed milk for 1 h. After washing three times with 0.1% TBST, the membrane was incubated with primary antibodies (CST) targeting LC3B (#43566S, 1:1000), P62 (#5114S, 1:1000), Beclin 1 (#3495S, 1:1000), and GAPDH (#2118S, 1:1000) at 4°C overnight. Then, the blots were washed thrice with 0.1% TBST and incubated with HRP-labeled secondary antibody (#7074S, 1:1000; CST) at 37°C for 1h. Finally, an ECL chemiluminescence solution was added to the membrane for visualization. The intensities of protein bands were quantified by using ImageJ software. The protein level was normalized to that of GAPDH.

Isolation and culture of mouse bone marrow-derived dendritic cells (BMDCs)

C57BL/6 mice were euthanized through cervical dislocation, and the tibial and femoral bones were stripped out under aseptic conditions. The bone marrow cells were extracted and washed with PBS, and red blood cells were removed using ACK Lysing Buffer (Thermo Fisher Scientific). The primary bone marrow cells were added into the culture medium, and cultured at a density of 1×10^6 cells/ml in 6-well plates. Then, 20 ng/ml recombinant mouse granulocyte-monocyte colony-stimulating factor (rmGM-CSF) and 20 ng/ml recombinant mouse IL-4 (Sigma-Aldrich) were added to each well, followed by incubation at 37°C in a 5% CO_2 atmosphere. On day 3, the plate was gently shaken, and most suspended cells were absorbed. An equal amount of medium was added to the cells, supplemented with the corresponding cytokines. Half the cell medium was replaced every other day. On day 7, the suspended and loosely adherent dendritic cells were collected.

Treatment of BMDCs

The *in vitro* experiment contains four groups: the control group (BMDCs without OVA treatment), the OVA-L group (BMDCs with 100 μg/mL OVA treatment), the OVA-H group (BMDCs with 500 μg/mL OVA treatment), and the OVA-H+Baf-A1 group (BMDCs with 500 μg/mL OVA and 5 nM Baf-A1 treatment). OVA or the autophagy inhibitor Baf-A1 was added on day 6. The related assays were performed on day 7.

Coculture of lymphocytes with BMDCs

After the mice were sacrificed, the spleens were harvested and ground, erythrocytes were lysed, and lymphocytes were collected. On day 7, the lymphocytes were cocultured with BMDCs that received the above different treatments. Before coculture, BMDCs were washed to avoid the presence of IL-4 and GM-CSF in the culture medium. After coculture for 48h, the relevant experiments were conducted.

Flow cytometric analysis

To assess the influence of BMDC autophagy on Th1, Th2, and Th17 cells, lymphocytes were cocultured for 48h with BMDCs that had received different treatments. Through centrifugation, cells and cell supernatants were harvested. Then, a total of 1×10^6 cells were prepared into a single-cell suspension, which was added to the flow cytometric antibodies (BD Biosciences) including BUV395-conjugated anti-T-BET (#568109), BB700-conjugated anti-GATA3 (#56642), and BV650-conjugated anti-RORγT (#563424), followed by 30 min incubation at 4°C in the dark. The single-cell suspension was filtered into a new EP tube with a 200-mesh filter after being washed twice with PBS. In addition, the cell coculture supernatant was collected, and the levels of Th1/Th2/Th17 cell-related cytokines in the supernatant were measured with the corresponding commercial ELISA kits.

RT-qPCR

Total RNA from lymphocytes was extracted using Trizol (Aidlab, 252250AX, Beijing, China), and 1 μg of total RNA was reverse transcribed to cDNA using

HiScript Reverse Transcriptase (VAZYME, Nanjing, China). Real-time PCR was conducted using FastStart Universal SYBR Green Master (Rox; Roche, Basel, Switzerland, Germany) and the following primers: T-bet, 5'-CCACCTGTTGTGGTCCAAGTTC-3' (forward) and 5'-CCACAAACATCTGTAATGCTTG-3' (reverse); GATA3, 5'-CCTCTGGAGGAGGAACGCTAAT-3' (forward) and 5'-GTTTCGGGTCTGGATGCCTTCT-3' (reverse); RORγt, 5'-GTGGAGTTTGCCAAGCGG CTTT (forward) and 5'-CCTGCACATTCTGACTAG GACG-3' (reverse); GAPDH, 5'-CATCACTGCCACCC AGAAGACTG (forward) and 5'-ATGCCAGTGAGCTT CCCGTTCAG-3' (reverse). The relative expression of mRNA was calculated based on the $2^{-\Delta\Delta Ct}$ formula.

Statistical analysis

All data collected from three independent experiments were analyzed using GraphPad Prism 7.0. For data consistent with a normal distribution, the Student's t-test or one-way analysis of variance was used for comparisons between two groups or among multiple groups. All data are presented as the mean ± standard deviation. $P<0.05$ was considered statistically significant.

Results

Baf-A1 treatment alleviates the symptoms of AR in mice

C57BL/6 mice were first injected with OVA to establish an AR mouse model and then injected with Baf-A1 to investigate the effects of dendritic cell autophagy inhibitor on AR in mice. As shown in Figure 1B,C, AR mice showed an increased frequency of sneezing and nasal rubbing (important symptoms of AR) within 10 min of the last nasal OVA administration. However, Baf-A1 injection considerably reduced sneezing and nasal rubbing events. The allergic symptom scores were higher in the AR group than in the sham group but were decreased in the AR+ Baf-A1 group (Fig. 1D). These data indicate that the autophagy inhibitor significantly ameliorates the symptoms of AR in mice.

Baf-A1 treatment mitigates inflammatory immune cell infiltration in AR mice

The pathogenesis of AR is related to the infiltration of inflammatory immune cells, which was then examined in each group of mice. As shown by HE staining, AR mice presented enhanced inflammatory immune infiltration and injury in nasal mucosa, which however was attenuated after Baf-A1 injection (Fig. 2A). Furthermore, leukocyte, eosinophil, lymphocyte, and neutrophil numbers in the NLF of AR mice were markedly higher than in that of sham mice but were lower than in that of AR mice injected with Baf-A1 (Fig. 2B-E). These data imply that the inflammatory immune cell infiltration in AR mice can be mitigated by the autophagy inhibitor.

Baf-A1 treatment decreases IgE, LTC4, ECP, and PGD2 levels in AR mice

IgE is a crucial antibody that mediates type I allergy. We discovered that the OVA-specific IgE level in serum was considerably upregulated in AR mice, which was restored by the subsequent administration of Baf-A1 (Fig. 3A). During the development of AR, LTC4 and ECP are vital for mast cells and eosinophils. Herein, AR mice displayed much higher serum levels of LTC4 and ECP than sham mice, which were decreased by Baf-A1 (Fig. 3B,C). Additionally, the levels of OVA-specific IgE, LTC4, and PGD2 (an inflammatory cytokine during AR) in the NLF of AR mice were significantly higher than in that of sham mice but were remarkably lower than in that of AR mice injected with Baf-A1 (Fig. 3D-F). Overall, the autophagy inhibitor reduces IgE and inflammatory cytokine levels in AR mice.

Baf-A1 treatment impairs autophagy in the nasal mucosa of AR mice

Next, the influence of Baf-A1 on autophagy in AR mice was assessed by measuring autophagy markers through western blotting. A significantly enhanced LC3II/LC3I ratio, upregulated Beclin 1 expression, and downregulated P62 expression can be observed in the

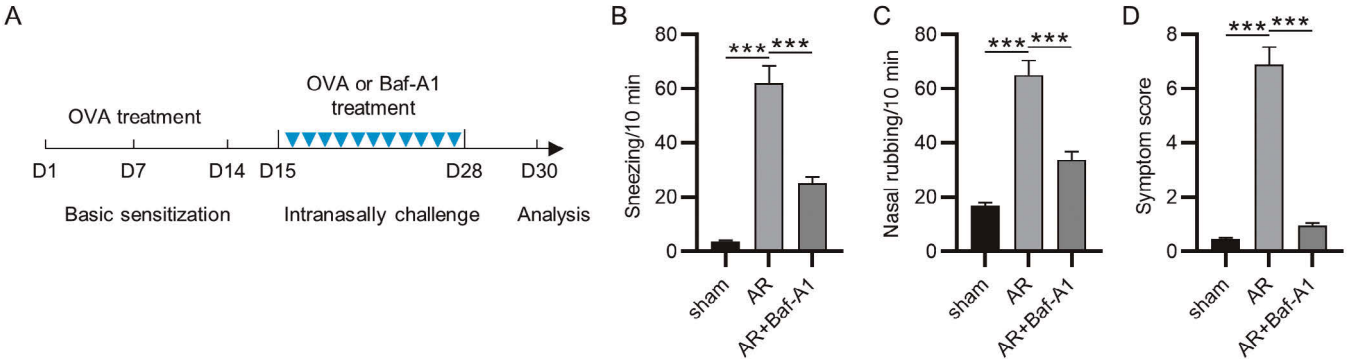

Fig. 1. Baf-A1 treatment alleviates the symptoms of AR in mice. **A.** Flow chart of the experimental design. **B, C.** Sneezing and nasal rubbing events within 10 min after the last nasal OVA administration were recorded. **D.** The allergic symptom scores. n=6 in each group. ***$P<0.001$.

AR group, which were all recovered in the AR+Baf-A1 group (Fig. 4A-D). This indicated that the autophagy inhibitor Baf-A1 restored the activated autophagy reactions in AR mice.

Baf-A1 treatment restores Th1/Th2/Th17 cytokine imbalance in AR mice

The Th1/Th2/Th17 cytokine imbalance is a significant factor that contributes to the occurrence of AR. Therefore, the serum levels of Th1-, Th2-, and Th-17-related cytokines in each group of mice were evaluated. Compared with the sham mice, AR mice demonstrated decreased levels of IFN-γ and IL-2 (Th1-related cytokines), as well as increased levels of IL-4 and IL-6 (Th2-related cytokines) and IL-10 and IL-17 (Th17-related cytokines). Nevertheless, the changes in these cytokine levels in AR mice were reversed by Baf-A1 treatment (Fig. 5A-F). In summary, the autophagy inhibitor improves the Th1/Th2/Th17 cytokine imbalance in AR mice.

Baf-A1 treatment reverses the immune imbalance of CD4⁺ T cell subtypes

From an immunological point of view, the main pathological feature of AR is that Th1 cells are suppressed, and Th2 and Th17 cells are overactive. In our study, compared with the control group, the number of Th1 cells ($CD4^+$ T-bet^+) gradually decreased in OVA-L and OVA-H groups (Fig. 6A,B), while the number of Th2 cells ($CD4^+$ $GATA3^+$) and Th17 cells ($CD4^+$ $ROR\gamma T^+$) gradually increased (Fig. 6C-F). This indicates that OVA-induced BMDC autophagy leads to Th1/Th2/Th17 cell imbalance. However, the addition of the autophagy inhibitor Baf-A1 to the high-concentration OVA group markedly increased Th1 cell number (Fig. 6A,B) but decreased Th2 and Th17 cell numbers (Fig. 6C-F). Moreover, as shown by RT-qPCR analysis, the key transcriptional factors of Th1/Th2/Th17 cells showed the same trends among these groups. Compared with the control group, T-bet expression was gradually decreased while GATA3 and RORγt expression were gradually increased in OVA-L and OVA-H groups. Nevertheless, Baf-A1 significantly upregulated T-bet levels, as well as downregulated GATA3 and RORγt levels (Fig. 6C-F). Taken together, the autophagy inhibitor can effectively reverse the BMDC autophagy-induced imbalance of $CD4^+$ T cell subtypes.

Baf-A1 treatment attenuates Th1/Th2/Th17 cytokine imbalance

To probe the effects of BMDC autophagy on the

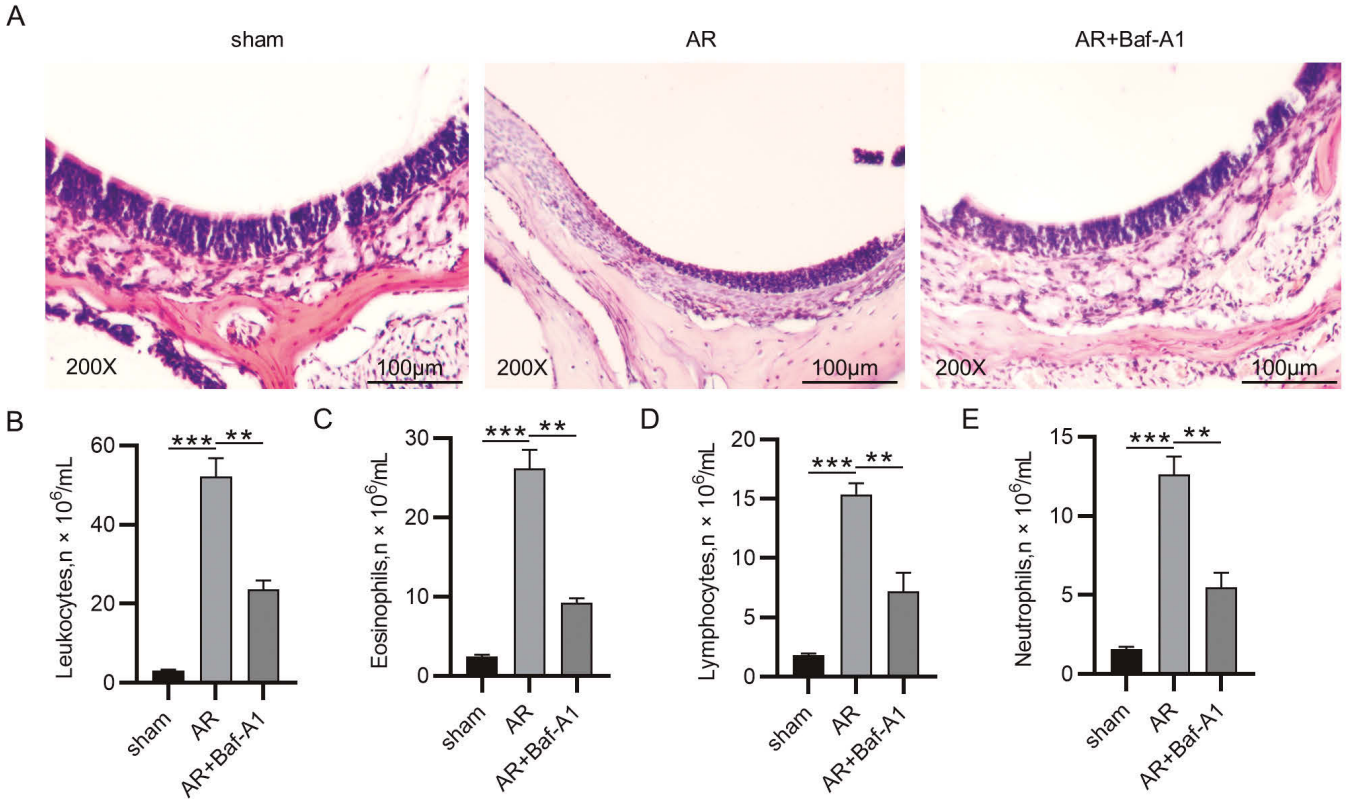

Fig. 2. Baf-A1 treatment decreases inflammatory cell infiltration in AR mice. **A.** HE staining showed the inflammatory immune cell infiltration in the nasal mucosa of mice. Scale bar: 100 μm. (B-E) Leukocyte, eosinophil, lymphocyte, and neutrophil numbers in the NLF of mice. $^{**}P<0.01$, $^{***}P<0.001$.

inflammation in AR, the levels of Th1 (IFN-γ and IL-2), Th2 (IL-4, IL-6, and IL-10), and Th17 (IL-17) cytokines were detected. Compared with the control group, IFN-γ and IL-2 contents in the supernatant of lymphocyte cocultures after adding OVA-stimulated BMDC were significantly decreased, while IL-4, IL-6, IL-10, and IL-17 contents were markedly increased (Fig. 7A-F). These data imply that Th1/Th2/Th17 cytokine imbalance can be promoted by OVA-induced BMDC autophagy. Nevertheless, compared with those in the supernatant of lymphocyte cocultures with high or low concentration OVA-stimulated BMDC, IFN-γ, and IL-2 contents in the coculture supernatant after addition of Baf-A1 were elevated, while IL-4, IL-6, IL-10, and IL-17 contents were considerably reduced (Fig. 7A-F). These findings confirm that the inhibition of BMDC autophagy

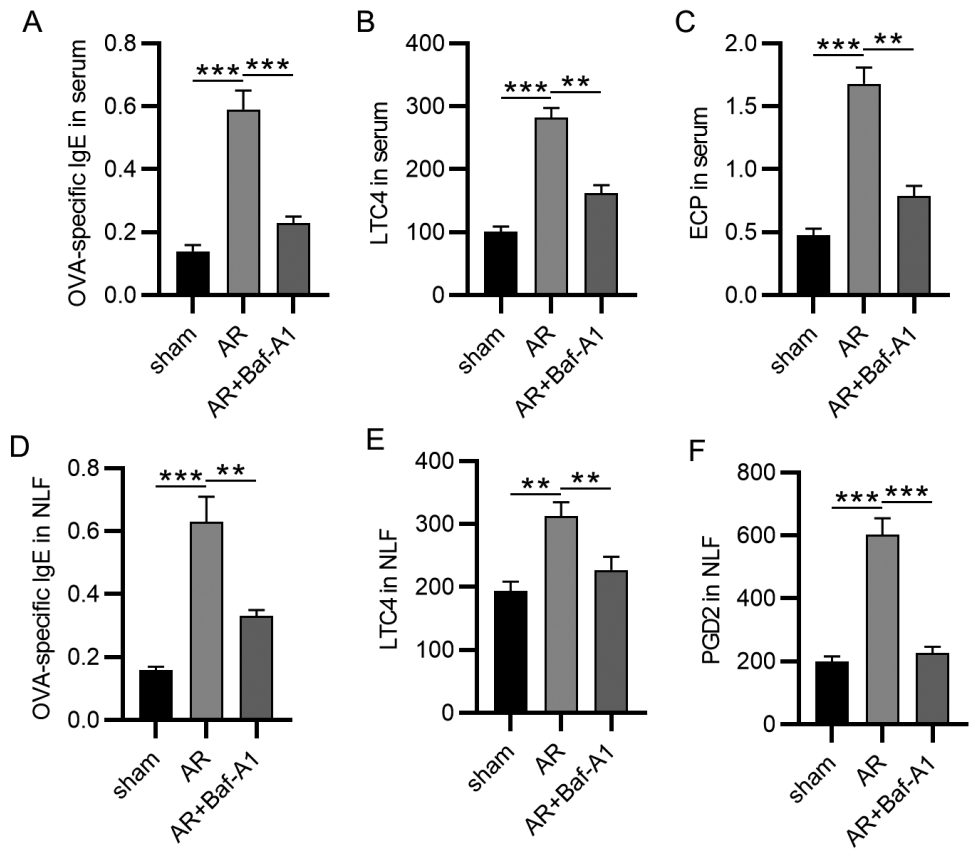

Fig. 3. Baf-A1 treatment decreases IgE, LTC4, ECP, and PGD2 levels in AR mice. The levels of OVA-specific IgE, LTC4, and ECP in the serum **(A-C)**, and OVA-specific IgE, LTC4, and PGD2 in the NLF of AR mice **(D-F)** were assessed by using the corresponding ELISA kits. n=6 in each group. ***P*<0.01, ****P*<0.001.

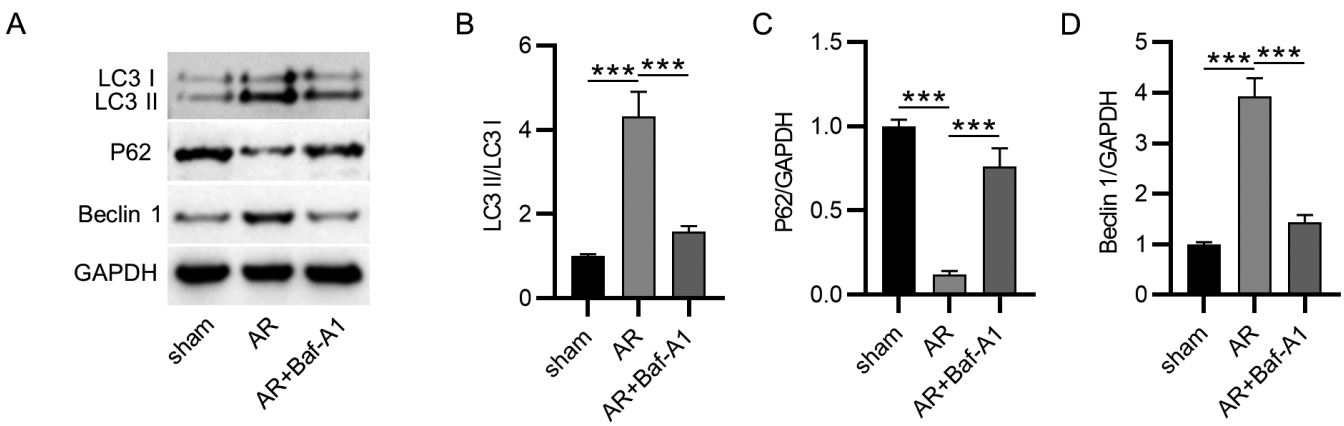

Fig. 4. Baf-A1 treatment impairs CD11c+ DC autophagy in the nasal mucosa of AR mice. **A-D.** The expression of autophagy markers in the nasal mucosal tissues of mice was detected by western blotting. n=6 in each group. ***P*<0.01, ****P*<0.001.

alleviates Th1/Th2/Th17 cytokine imbalance.

Discussion

During the past several years, advanced progress has been achieved in AR diagnosis and treatment, however, the results remain unsatisfactory, and the recurrence rate is still high (Shamji et al., 2022). DCs play an essential role in the pathogenesis of AR as primary antigen-presenting cells responsible for the initiation and maintenance of the T-cell response (Kaczynska et al., 2022). A previous study reported that allergens can induce the autophagy of DCs, which promotes immune imbalance and thereby aggravates the progression of AR (He et al., 2022). In our research, the OVA-induced mouse model was established as previously described and then administrated with the autophagy inhibitor Bal-A1 to confirm the effects of DC autophagy inhibition against AR. We discovered that Bal-A1 alleviated the allergic symptoms, mitigated inflammatory immune cell infiltration, decreased IgE and inflammatory cytokine levels, and improved the Th1/Th2/Th17 cytokine imbalance in AR mice. Furthermore, Baf-A1 reversed the BMDC autophagy-induced Th1/Th2/Th17 cell imbalance and the subsequent inflammatory response. Therefore, we concluded that the inhibition of DC autophagy suppressed the immune imbalance and inflammation in AR.

Autophagy widely exists in eukaryotic cells, with the functions of phagocytosing pathogens, regulating inflammatory response, reducing apoptosis, and maintaining the stability of the body's internal environment (Deretic and Kroemer, 2022). Many studies have disclosed the involvement of autophagy in the progression of AR. For example, Wang et al. discovered that miR-338e-3p ameliorated nasal symptoms in PM2.5-exacerbated AR rat models by inhibiting autophagy (Wang et al., 2021). Zhang et al. disclosed that autophagy-associated proteins showed markedly elevated expression in the nasal mucosa of AR mice, and the autophagy inhibitor suppressed autophagy status and thus alleviated the nasal symptoms of AR mice (Zhang et al., 2019). Previous research confirmed that autophagy influences the presentation of cell epitopes to affect the nature and intensity of the T-cell response, which allows autophagy as a vital regulator of T-cell activation (Oral et al., 2017). The autophagy of DCs was proven to modulate T-cell responses via various pathways, including T-cell-related metabolism, production of related cytokines, inflammatory signal transduction, and MHC antigen presentation (Merkley et al., 2018). Therefore, autophagy may participate in AR occurrence and progression by controlling the T cell-mediated immune response. LC3B and P62 are typical autophagy

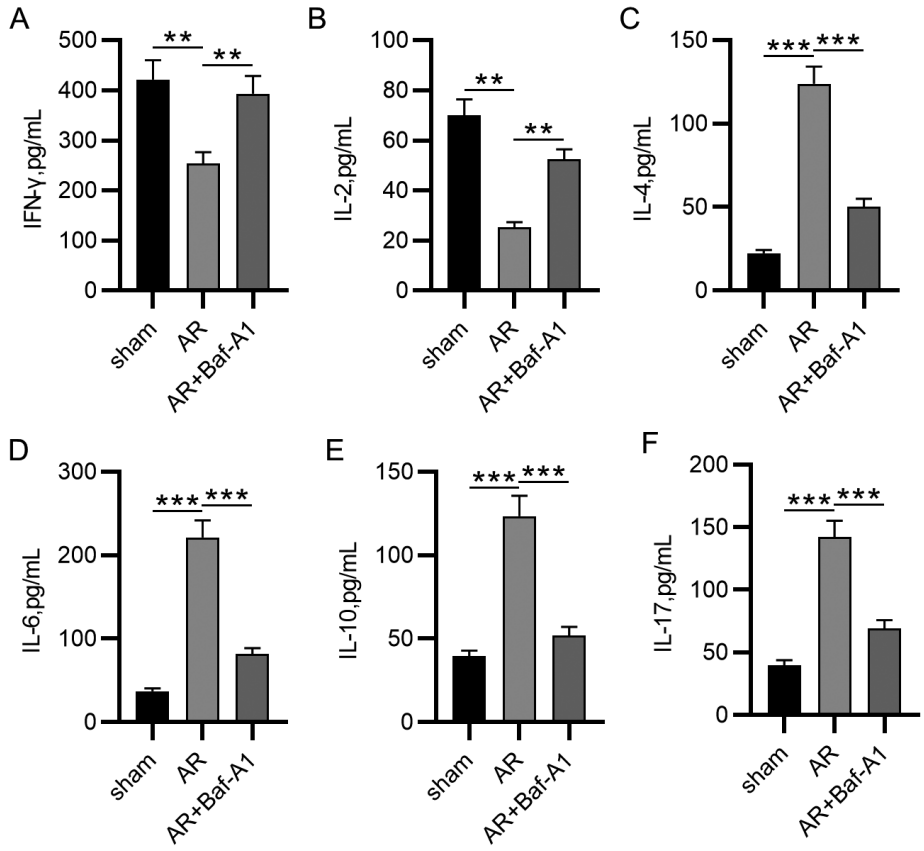

Fig. 5. Baf-A1 treatment restores Th1/Th2/Th17 cytokine imbalance in AR mice. The serum levels of IFN-γ and IL-2 (Th1-related cytokines) **(A, B)**, IL-4 and IL-6 (Th2-related cytokines) **(C, D)**, and IL-10 and IL-17 (Th17-related cytokines) **(E, F)** in mice were detected by using ELISA kits. n=6 in each group. ***P*<0.01, ****P*<0.001.

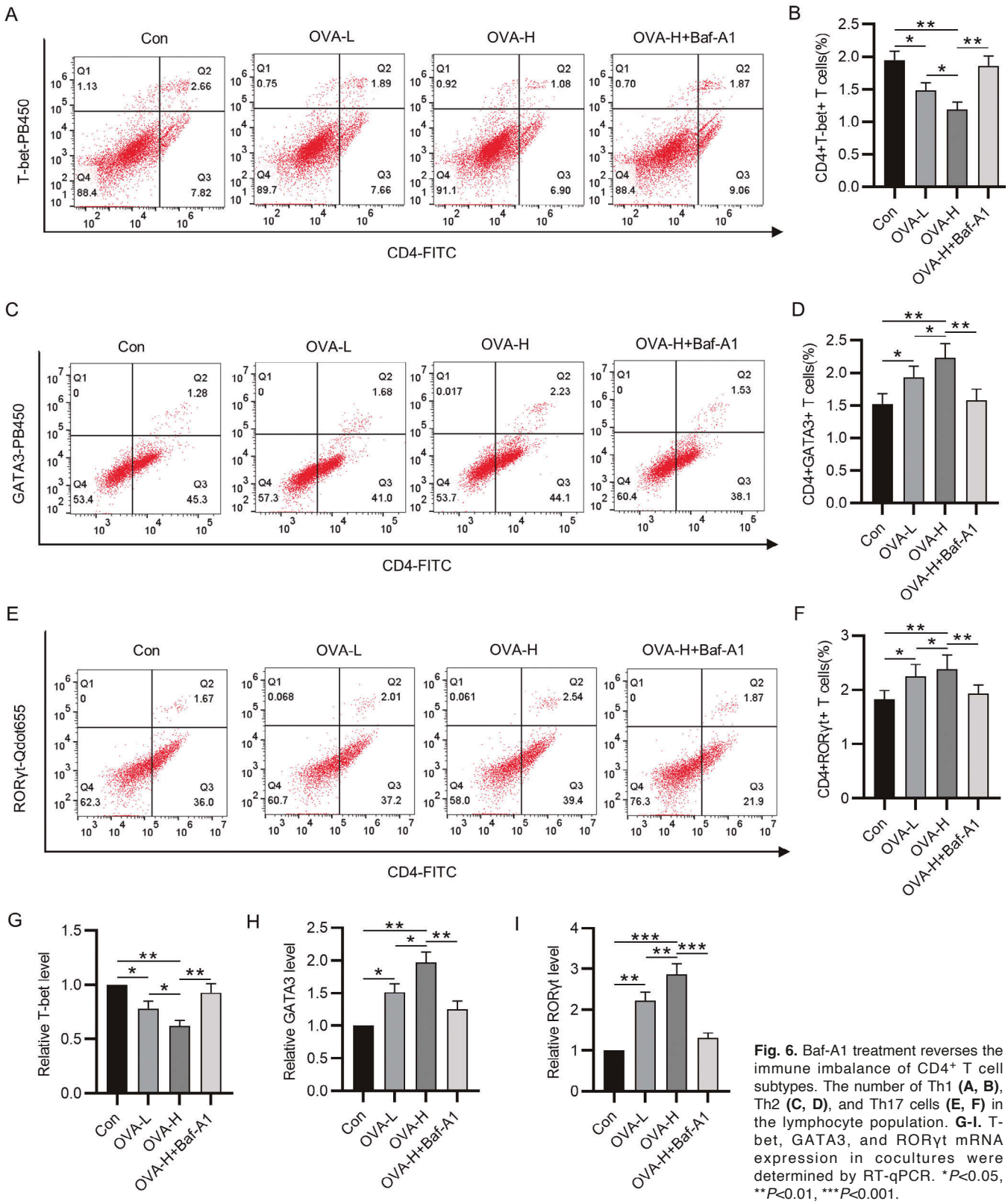

Fig. 6. Baf-A1 treatment reverses the immune imbalance of CD4$^+$ T cell subtypes. The number of Th1 **(A, B)**, Th2 **(C, D)**, and Th17 cells **(E, F)** in the lymphocyte population. **G-I.** T-bet, GATA3, and RORγt mRNA expression in cocultures were determined by RT-qPCR. **P*<0.05, ***P*<0.01, ****P*<0.001.

markers (Bresciani et al., 2018). When autophagy occurs, LC3-I can be converted to LC3-II, which binds to autophagosome-localized p62 to form a complex, resulting in the degradation of p62 (Bresciani et al., 2018). Moreover, Beclin1 is an essential molecule for the initiation of autophagy, which mediates the localization of autophagy-related genes and proteins to autophagic vesicles, thereby facilitating the occurrence of autophagy (Maejima et al., 2016). In our study, we first discovered that autophagy inhibitor Bal-A1 treatment remarkably alleviated allergic symptoms, mitigated inflammatory immune cell infiltration, and reduced the levels of OVA-specific IgE and inflammatory cytokines in AR mice. We also detected the key autophagy markers in the nasal mucosa of AR mice. The results showed that the LC3II/LC3I ratio and Beclin 1 expression were upregulated while P62 expression was downregulated in AR mice, which were reversed by the autophagy inhibitor Baf-A1. These data demonstrate that the activation of autophagy is associated with the progression of AR.

Imbalanced differentiation of $CD4^+$ T cells is a fundamental immunological feature of AR, manifested by excessive differentiation of Th2 and Th17 cells and insufficient differentiation of Th1 cells (Yu et al., 2017). Th1 cells secrete cytokines, such as IL-2 and IFN-γ, participating in cellular immunity and delayed hypersensitivity inflammatory response, and playing a vital role in autoimmune disorders and host resistance to intracellular pathogenic infection (Ohmi et al., 1999). Th2 cells secrete IL-4, IL-6, and other cytokines to assist the differentiation of B cells into antibody-secreting cells and are involved in humoral immune responses (Zhong et al., 2016). Th2 cells play pivotal roles in allergic and infectious diseases, antagonizing extracellular pathogens, B-cell differentiation, and asthma (Muro et al., 2018). Th17 cells mobilize, recruit, and activate neutrophils by secreting IL-17 cytokines to mediate inflammatory responses, autoimmunity, tumor, and transplant rejection (Okada and Khoury, 2012). In this study, murine lymphocytes were isolated and then cocultured with OVA-stimulated BMDCs, and the differentiation of the $CD4^+$ T cell subtypes Th1, Th2, and Th17 was evaluated. The results showed that the Th1 cell number was decreased while Th2 and Th17 cell numbers were increased after coculture. T-bet, GATA3, and RORγt are the specific transcription factors of Th1, Th2, and Th17 cells, respectively (Kim et al., 2014). Concordant with the changes in the number of $CD4^+$ T cell subtypes, the T-bet expression level was decreased while GATA3 and RORγt expression levels were increased after coculture. However, treatment with autophagy inhibitor Baf-A1 restored such Th1/Th2/Th17 cell imbalance, which was consistent with the results in

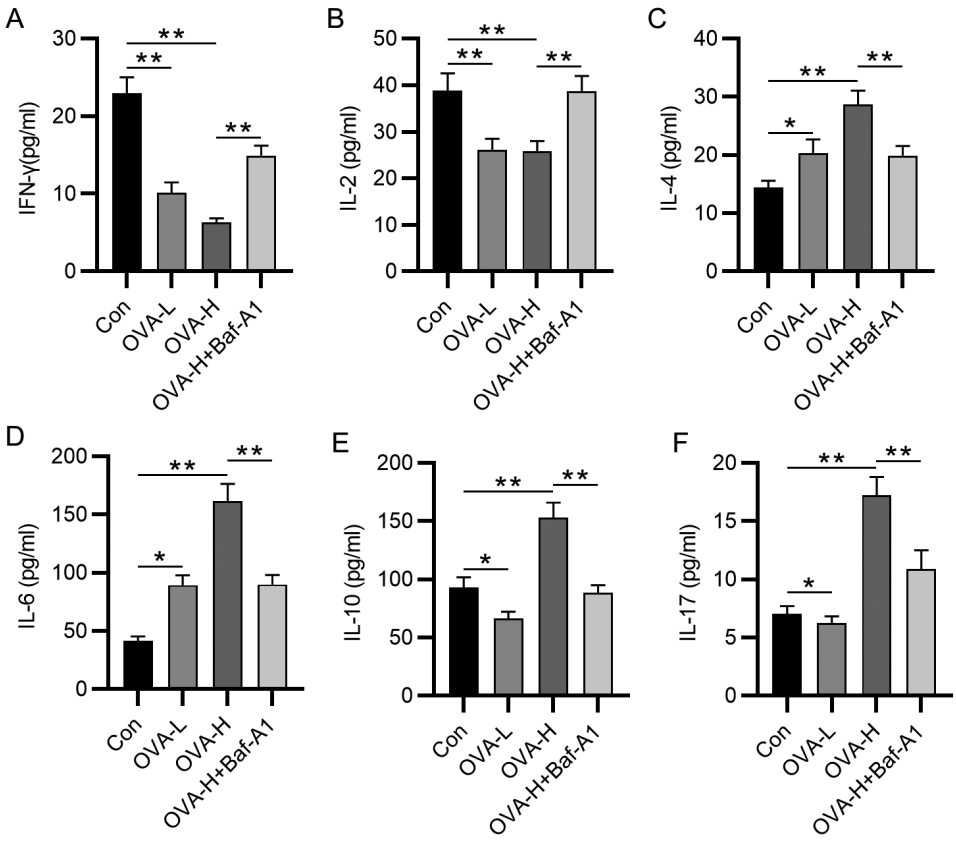

Fig. 7. Baf-A1 treatment attenuates Th1/Th2/Th17 cytokine imbalance. Levels of IFN-γ and IL-2 (Th1-related cytokines) **(A, B)**, IL-4 and IL-6 (Th2-related cytokines) **(C, D)**, and IL-10 and IL-17 (Th17-related cytokines) **(E, F)** in the supernatant of cocultures of lymphocytes and OVA-stimulated BMDCs. **P*<0.05, ***P*<0.01.

the previous study, where the autophagy inhibitor 3-MA reversed the Th1/Th2/Th9/Th17/Treg/Tfh cell immune imbalance caused by OVA-induced BMDC autophagy in AR development.

Overall, our study confirmed, for the first time, that inhibiting $CD11c^+$ DC autophagy improved the development of AR by ameliorating Th1/Th2/Th17 cell imbalance and alleviating the subsequent inflammatory response. Thus, the inhibition of DC autophagy may be a novel therapeutic target for treating AR.

Ethics approval. Animal protocols were approved by the Ethical Review Committee of Wuhan Myhalic Biotechnology (Approval number: 202203031).

Conflict of interest. The authors declared no conflict of interest.

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