Interleukin-27 ameliorates allergic asthma by alleviating the lung Th2 inflammatory environment

JIAMENG LU[1], XIAOQING JI[2], LIXIA WANG[3], FEI SUN[4], CHUANJUN HUANG[5], HAIYING PENG[4], YUNXIU JIANG[4], ZIHAN GUO[4], XINYI LIU[4], YANBO JI[2] and DEGAN LU[5]

[1]School of Microelectronics, Shandong University, Jinan, Shandong 250100; [2]Department of Nursing, The First Affiliated Hospital of Shandong First Medical University and Shandong Provincial Qianfoshan Hospital, Jinan, Shandong 250014; [3]Division of Disinfectant and Supply, Liaocheng People's Hospital, Liaocheng, Shandong 252000; [4]Graduate School of Shandong First Medical University, Jinan, Shandong 250000; [5]Department of Respiratory Medicine, The First Affiliated Hospital of Shandong First Medical University and Shandong Provincial Qianfoshan Hospital, Shandong Institute of Respiratory Diseases, Shandong Institute of Anesthesia and Respiratory Critical Medicine, Jinan, Shandong 250014, P.R. China

Received February 21, 2022; Accepted April 20, 2022

DOI: 10.3892/ijmm.2022.5142

Abstract. Interleukin (IL)-27 can inhibit the differentiation of Th2 cells and plays a role in the development of asthma. However, whether the therapeutic administration of IL-27 in a mouse model of asthma can inhibit allergic responses remains a matter of debate. Additionally, the mechanisms through which IL-27 ameliorates inflammatory responses in asthma are not yet fully understood. Thus, the aim of the present study was to examine the effects of IL-27 on asthma using a mouse model and to elucidate the underlying mechanisms. For this purpose, mice received an intranasal administration of IL-27 and the total and differential cell counts, levels of cytokines and type 1 regulatory T (Tr1) cells in the lungs were detected. The protein and mRNA levels of signal transducer and activator of transcription (STAT)1 and STAT3 were analyzed and airway remodeling was assessed. The results indicated that IL-27 did not ameliorate airway inflammation, airway hyperresponsiveness, and airway remolding when administrated therapeutically. Preventatively, the administration of IL-27 decreased the concentrations of Th2 cytokines and increased the number of Tr1 cells. The protein and mRNA levels of STAT1 and STAT3 were increased. Taken together, these findings demonstrate that the prophylactic administration of IL-27 ameliorates asthma by alleviating the lung Th2 inflammatory environment through the restoration of both the STAT1 and STAT3 pathways. IL-27 may thus prove to be useful as a novel agent for the prevention of asthma.

Correspondence to: Professor Degan Lu, Department of Respiratory Medicine, The First Affiliated Hospital of Shandong First Medical University and Shandong Provincial Qianfoshan Hospital, Shandong Institute of Respiratory Diseases, Shandong Institute of Anesthesia and Respiratory Critical Medicine, 16766 Jingshilu, Lixia, Jinan, Shandong 250014, P.R. China
E-mail: deganlu@126.com

Key words: asthma, interleukin-27, signal transducer and activator of transcription 1, signal transducer and activator of transcription 3, type 1 regulatory T-cells

Introduction

Asthma is a chronic inflammatory disease characterized by variable respiratory symptoms and airflow limitation (1). According to the Global Initiative for Asthma (GINA), ~300 million individuals worldwide suffer from asthma and the number is estimated to reach 400 million by the year 2025 (2). As a result, the socio-economic burden of this disease is increasing. Corticosteroids and bronchodilators continue to play a major role in the treatment of asthma (3,4); however, symptom control is poor in ~10% of patients (5). Furthermore, asthma attacks are common and are associated with an accelerated and permanent loss of lung function (6). Therefore, effective prevention measures and novel therapies are required for the more effective control of symptoms and for the reduction of asthma attacks in patients with asthma.

As a novel cytokine, interleukin (IL)-27 was identified as a new member of the IL-6/IL-12 family (7). IL-27 is a heterodimeric molecule that consists of EBV-induced gene 3 (EBi3) and p28 subunit, and is primarily produced from activated antigen-presenting cells and macrophages (7,8). By binding to its receptor [composed of WSX-1 (T-cell cytokine receptor) and gp130], IL-27 activates signal transducer and activator of transcription (STAT)1 (8,9) and thereby functions as a promoter of T-helper 1 (Th1) differentiation (10,11), and a suppressor of Th2 and Th17 differentiation (12,13). Group 2 innate lymphoid cells (ILC2) also play a crucial role in allergic inflammation and IL-27 suppresses the proliferation of ILC2 cells in lung tissue (14). Thus, IL-27 may be involved in allergic inflammatory diseases, including asthma.

Previous studies have found that the levels of IL-27 in serum are altered in patients with acute asthma and are associated with alterations in lung function (15,16). Jirmo *et al* (17) reported that IL-27 enhanced the secretion of IFN-γ, whereas it decreased that of IL-5 and IL-13, demonstrating its critical role in the control of allergic asthma. In a previous study by the authors, it was demonstrated that the preventative intranasal administration of IL-27 alleviated airway inflammation and remodeling in mouse models of asthma by restoring both the STAT1 and STAT3 pathways (18). However, the associated mechanisms have not yet been fully elucidated and required further investigation. As a distinct subset of T-cells, T regulatory type 1 (Tr1) cells are characterized by the ability to secrete high levels of IL-10 and the lack of the forkhead box P3 (Foxp3) expression (19,20). Tr1 cells play a crucial role in maintaining peripheral tolerance and preventing T-cell-mediated diseases (21). However, the effects of IL-27 on Tr1 cells have not yet been investigated in asthma, at least to the best of our knowledge. Based on the mouse model of ovalbumin (OVA)-induced airway inflammation, Yoshimoto *et al* (22) found that the intranasal administration of IL-27 reduced OVA-induced airway hyperresponsiveness (AHR) and inflammation. However, Su *et al* (23) reported that IL-27 alleviated airway inflammation and improved the pathological changes when it was preventatively administered, while IL-27 did not affect AHR and airway inflammation when delivered therapeutically. Therefore, whether the therapeutic administration of IL-27 can suppress airway inflammation, AHR, and airway remodeling in a mouse model of asthma remains unclear.

In the present study, the effects of the therapeutic intranasal administration of IL-27 on airway inflammation, AHR and airway remodeling were investigated in mouse models of OVA-induced asthma. Additionally, the inflammatory environment in the lungs of mice was evaluated based on the analysis of bronchoalveolar lavage (BAL) fluid (BALF) and lung tissue from mice which received IL-27 prophylactically. Finally, the main signaling pathways involved in the anti-inflammatory effects of IL-27 were examined.

Materials and methods

Animals. A total of 48 healthy female BALB/c mice (6-8 weeks old, weighing 12-14 g) were purchased from the Experimental Animal Center of Shandong University (Jinan, China). The animals were housed in pathogen-free and standard conditions (12-h light/dark cycle, room temperature of 22˚C and a relative humidity of 60%). The mice were randomly divided into three groups as follows: the phosphate-buffered saline (PBS) group (n=8), the OVA group (n=8) and the OVA and IL-27 group (OVA + IL-27, n=8). All animal procedures were conducted according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (24). Moreover, all protocols were approved by the Ethics Committee for Laboratory Animals Care and Use in First Affiliated Hospital of Shandong First Medical University, Jinan, China.

Experimental model of acute asthma. Mice were exposed to OVA as previously described (25,26) to establish a model of acute asthma. When IL-27 was administered in a preventative manner to the mice in the OVA + IL-27 group (Fig. 1A), the mice received 50 µl PBS and 50 ng IL-27 intranasally twice a day from day-6 to day 7. However, the mice received 50 µl PBS alone on the same days when IL-27 was delivered in a therapeutic manner in the OVA and PBS groups (Fig. 1B). The mice were then sensitized with intraperitoneal (i.p.) injections of 100 µg OVA (Sigma-Aldrich; Merck KGaA), 2 mg alum (Thermo Fisher Scientific, Inc.) and 100 µl sterile endotoxin-free PBS (Invitrogen; Thermo Fisher Scientific, Inc.) on days 0 and 7 in the OVA and OVA + IL-27 groups. The mice received PBS instead of OVA on the same days in the PBS group. In the OVA group, the mice were challenged with 50 µl PBS and 100 µg OVA intranasally from days 14 to 18 under light isoflurane (3%) anesthesia (27). In the OVA + IL-27 group, the mice were treated intranasally with 50 µl PBS and 1 µg IL-27 1 h prior to OVA sensitization and subsequent challenge (50 µl PBS and 100 µg OVA) on days 0, 7 and 14-18. The mice received PBS alone on the same days in the PBS group. Each group included 8 mice.

Experimental model of chronic asthma. To establish the phenotype in chronic murine models of allergic asthma, the mice were treated with OVA as previously described (28). Briefly, 20 µg OVA/1 mg Alum/200 µl PBS were delivered subcutaneously (s.c.) to mice in the OVA and OVA + IL-27 groups on days 0, 7, 14 and 21, while 1 mg alum/200 µl PBS was administered to the mice in the PBS group. On days 26, 27 and 28, and on the following 4 weeks (twice a week), the mice were challenged intranasally with 20 mg of either OVA in 50 µl PBS (OVA and OVA + IL-27 group) or 50 µl PBS alone (PBS group) following anesthesia (27). In the OVA + IL-27 group, the mice received 50 µl PBS and 20 ng IL-27 intranasally 1 h before the OVA challenge on days 0, 7, 26-28, 35, 38, 42, 49, 52, 56 and 59 (please refer to the protocol scheme illustrated in Fig. 2A). Each group included 8 mice.

Measurement of airway responsiveness. AHR is one of the characteristic features of asthma (29). In the present study, AHR was assessed using invasive techniques 24 h following the final OVA challenge, as previously described (30). In brief, the mice were anesthetized with pentobarbital sodium (50 mg/kg, i.p. injection) and their responses to nociceptive stimulation and movement were assessed in order to determine the depth of anesthesia (27). A tracheostomy was performed and the mice were connected to the flexiVent system (SCIREQ Scientific Respiratory Equipment, Inc.). The mice were then mechanically ventilated according to the following parameters: tidal volume, 5 ml/kg; breathing rate, 150 breaths/min; positive end-expiratory pressure, 3 cmH_2O (31). Subsequently, the mice were challenged with aerosol saline followed by increasing concentrations of acetyl-β-methylcholine chloride (methacholine; 0, 5, 10 and 20 mg/ml; Sigma-Aldrich; Merck KGaA) for 10 sec at each dose. Airway resistance and lung compliance were presented as a percentage change relative to baseline levels (saline challenge).

BAL and inflammatory cell analysis in BALF. At 24 h after the final OVA challenge and immediately following the AHR assessments, the animals were euthanized by cervical dislocation and the verification of death was based on a combination of criteria, including the absence of breathing, pulse, corneal reflex

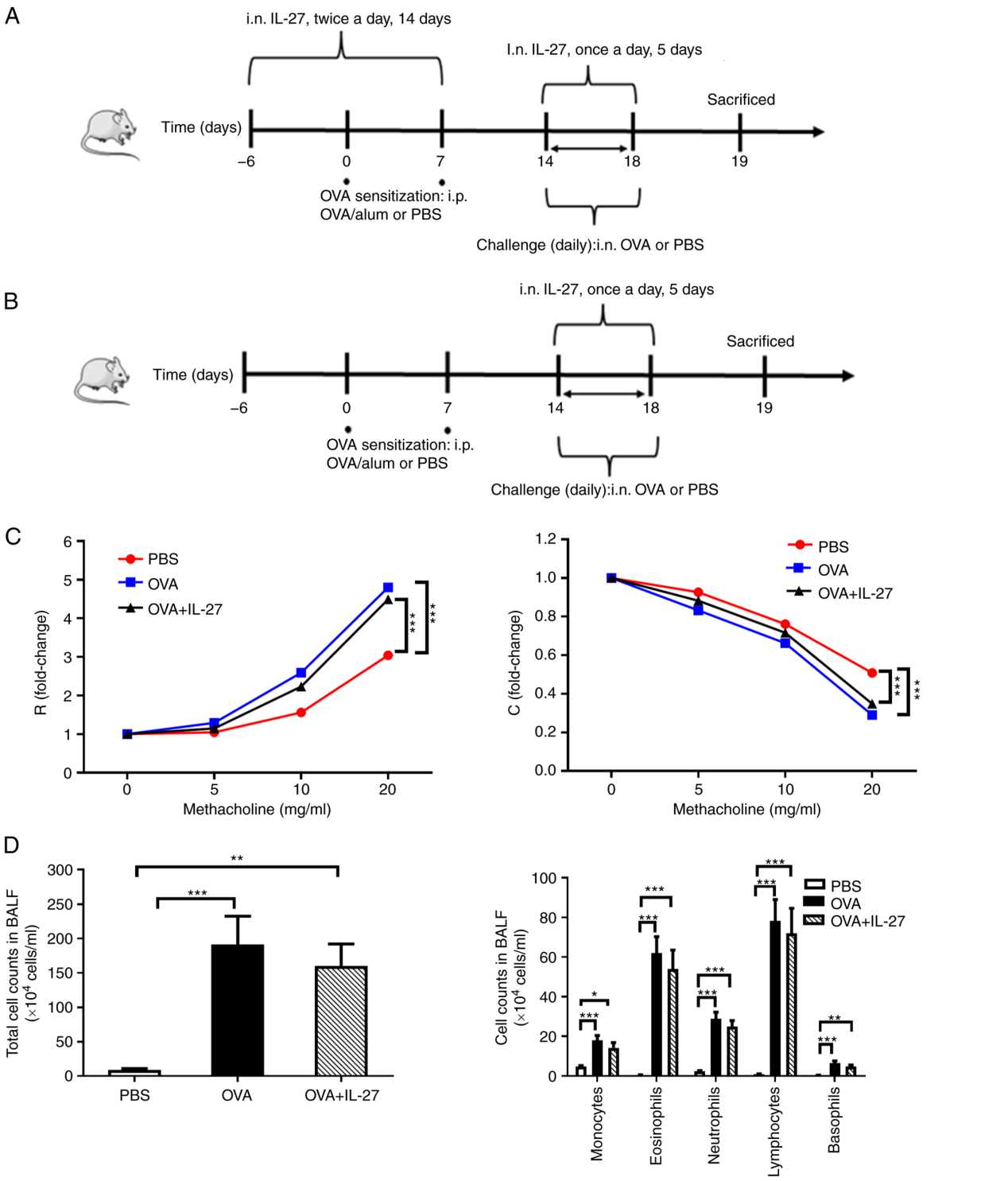

Figure 1. Intranasal administration of IL-27 following sensitization with OVA cannot alleviate airway inflammation and AHR in an acute model of experimental allergic asthma. (A and B) Protocol of OVA-induced allergic asthma and administration of IL-27. (C) AHR was invasively measured by lung resistance and compliance in response to increasing concentrations of methacholine. Treatment with IL-27 did not improve lung resistance and lung compliance. (D) Total cell number and the differential cell counts in the BALF samples. The total cell number and the differential cell counts exhibited no significant difference between the OVA + IL-27 group and OVA group. The data are expressed as the mean ± SEM of three independent experiments, with 8 animals per group. *P<0.05, **P<0.01, ***P<0.001. IL-27, interleukin 27; OVA, ovalbumin; AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; i.n., intranasal.

and response to a firm toe pinch. After the left main bronchus was clamped, BAL was immediately performed in the right lung and BALF was subsequently collected and processed, as previously described (12,32). The BALF was centrifuged (80 x g for 10 min at 4˚C) and the cell pellets were resuspended in 1 ml PBS-EDTA (Sigma-Aldrich; Merck KGaA). The total number of BALF cells and BALF differential cell counts were determined on cytospin slide preparations which were stained with Wright-Giemsa (Beyotime Institute of Biotechnology) at room temperature for 8 min. A total of 200 cells per slide were counted per sample under a light microscope (DP73; Olympus corporation) at x400 magnification (33).

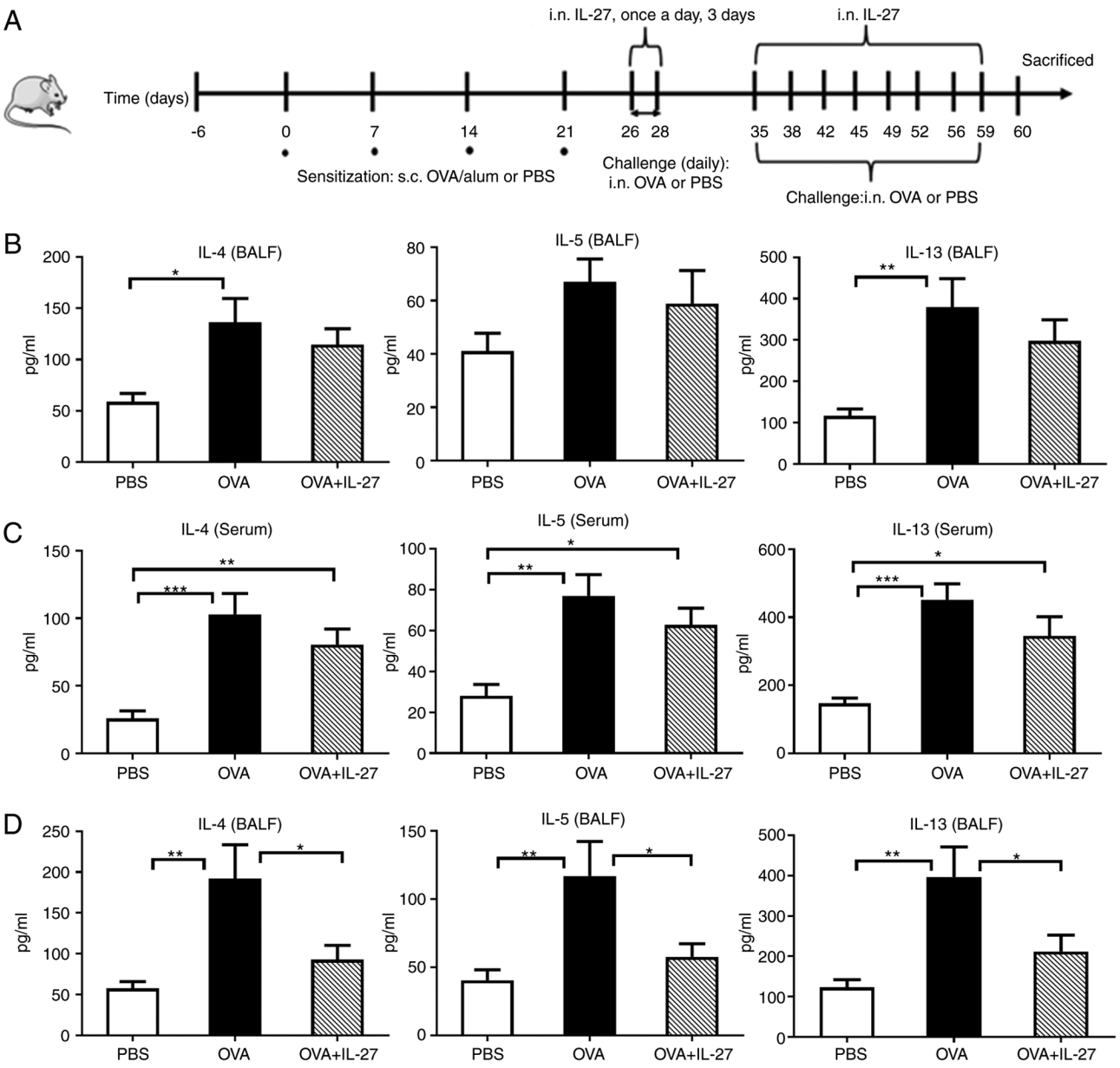

Figure 2. Levels of IL-4, IL-5 and IL-13 in BALF and serum samples. (A) Protocol of OVA-induced allergic asthma and administration of IL-27. (B and C) The levels of IL-4, IL-5 and IL-13 were measured using ELISA when IL-27 was administered in a therapeutic manner. The concentrations of IL-4, IL-5 and IL-13 exhibited no significant differences in BALF and serum between the OVA + IL-27 group and OVA group. (D) The levels of IL-4, IL-5 and IL-13 in the BALF were measured using ELISA when IL-27 was used in a preventive manner. The levels of IL-4, IL-5 and IL-13 were lower in the OVA + IL-27 group compared with the OVA group. The data are expressed as the mean ± SEM of three independent experiments, with 8 animals per group. *P<0.05, **P<0.01, ***P<0.001. BALF, bronchoalveolar lavage fluid; ELISA, enzyme linked immunosorbent assay.

Enzyme-linked immunosorbent assay (ELISA). IL-4, IL-5 and IL-13 are typical allergic asthma-associated cytokines which initiate and promote airway inflammation, mucus overproduction, immunoglobulin E (IgE) synthesis and fibrosis (34,35). Therefore, in the present study, ELISA kits (R&D Systems, Inc.) were used to evaluate the levels of IL-4 (cat. no. M4000B), IL-5 (cat. no. M5000) and IL-13 (cat. no. dY413) in the serum and in the supernatant of BALF according to a standard protocol as previously described (36).

Cell isolation for flow cytometry. Cell isolation from the lungs was performed as previously described (37,38). In brief, the right lung was excised after the ligation of the left main bronchus. The whole lungs were minced, homogenized and subsequently incubated with shaking for 30 min at 37˚C with the collagenase type IV solution (cat. no. C8160; Beijing Solarbio Science & Technology Co., Ltd.). The supernatant was removed and then filtered through a nylon mesh with pore size 48 μm (cat. no. YA0691; Beijing Solarbio Science & Technology Co., Ltd.), and the resulting cells were washed in complete medium [CM; RPMI-1640 + 10% heat-inactivated fetal bovine serum + 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer + 10 mM non-essential amino acids + 10 mM sodium pyruvate + 10 U/ml penicillin/streptomycin; all from Sigma-Aldrich]. Subsequently, the isolated cells were enriched by density gradient centrifugation at 800 x g for 30 min at 4˚C (Percoll, Sigma-Aldrich; Merck KGaA) and they were re-suspended in CM for further analysis.

Flow cytometry. The antibodies used in the present study included the following: fluorescein isothiocyanate (FITC)-labeled anti-mouse CD4 (1:100; cat. no. 100510), APC-labeled anti-mouse CD49b (1:100; cat. no. 103516) and PerCP/Cyanine 5.5 anti-mouse CD223 (LAG-3) (1:100; cat. no. 125212) (all purchased from BioLegend). The cells were stained in fluorescence-activated cell sorting (FACS) buffer at 4˚C in the dark for 30 min with mixed the antibodies. After washing, 2% formaldehyde was added to the system at 4˚C for 30 min and the cells were analyzed using a flow cytometer (FACs Verse, BD Biosciences) and FlowJo software (version 10.4, Tree Star, Inc.) (39).

Reverse transcription-quantitative PCR (RT-qPCR). After the BAL was collected, the left lungs were immediately excised and total RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), as per the manufacturer's protocol. The RNA concentration was determined using a Nanodrop™ Nd-1000 spectrophotometer (Thermo Fisher Scientific, Inc.). The synthesis of complementary DNA (cDNA) was conducted using a Superscript III First-Strand Synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.) at 50˚C for 50 min. qPCR was then performed using TB Green Premix Ex Taq II (Tli RNaseH Plus; cat. no. RR820A, Takara Bio, Inc.) according to the manufacturer's instructions. The thermocycling conditions comprised an initial denaturation at 94˚C for 5 min, 40 cycles of 10 sec at 94˚C and 20 sec at 60˚C, and a final extension of 30 sec at 72˚C. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the reference control gene. An ABI 7000 PCR instrument (Thermo Fisher Scientific, Inc.) was used to quantify the mRNA. The relative abundance of the mRNA transcripts was determined using the $2^{-\Delta\Delta Cq}$ method (40). Each sample was analyzed in triplicate and at least three duplicate wells were used for each group. The sequences of primer and the expected size of the PCR products are presented in Table SI.

Western blot analysis. Western blot analysis was conducted as previously described (41). In brief, the mouse lung tissues were lysed in RIPA buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% TritonX-100, 1% sodium deoxycholate and 0.1% SDS; Beyotime Institute of Biotechnology) supplemented with protease inhibitor cocktail (Sigma-Aldrich; Merck KGaA). The bicinchoninic acid (BCA) protein assay kit (cat. no. 23225; Pierce; Thermo Fisher Scientific, Inc.) was used to determine protein concentration. Proteins were separated on 10% acrylamide SDS-PAGE gels. A total of 30 μg protein was loaded per lane. Following electrophoresis, the isolated proteins were transferred onto PVDF membranes (EMD Millipore). After blocking with 5% (w/v) dried skimmed milk at 37˚C for 1 h, the membranes were then incubated overnight at 4˚C with primary antibodies (all 1:500) against STAT1 (cat. no. sc-464), phosphorylated (p)-STAT1 (cat. no. sc-8394), STAT3 (cat. no. sc-482), p-STAT3 (cat. no. sc-8059) and β-tubulin (cat. no. sc-47778; all from Santa Cruz Biotechnology, Inc.), separately. Following five washes with PBST (PBS and 0.15% Tween-20), the membranes were incubated with goat anti-rabbit IgG-HRP secondary antibody (cat. no. sc-2004; 1:5,000; Santa Cruz Biotechnology, Inc.) at 37˚C for 45 min. β-tubulin was used as an internal control. The protein lanes were detected using ECL detection reagents kit (GE Healthcare; Cytiva) as per the manufacturer's instructions. Densitometry was performed using ImageJ software (v1.8.0; National Institutes of Health).

Lung histological analysis. The left lungs were excised and were subsequently prepared for histological analysis, as previously described (42). The lung tissue samples were fixed in 4% paraformaldehyde at room temperature for 24 h. The samples were dehydrated, cleared and embedded in paraffin for sectioning. The lung sections (5-μm-thick) were processed and stained with hematoxylin and eosin (H&E), Masson's trichrome and periodic-acid schiff (PAS) stain following a standard procedure (43). For H&E staining, the sections were immersed in 1% hematoxylin (Yuanmu Biotechnology Co., Ltd.) for 5 min and washed in distilled water. A 1% hydrochloric acid-alcohol solution was used to clear the residual dye, and 0.5% eosin was then used to stain the sections (Huihong Reagent Co., Ltd, China) at room temperature for 3 min. The histology sections were evaluated and scored independently by two experienced pathologists who were blinded to the identity of the experimental groups. The H&E-stained lung sections were used mainly to assess thickening of the basal membrane and hyperplasia of the airway smooth muscle cells. In total, three bronchioles that were 150-200 μm in inner diameter were selected and examined. The perimeter of the basement membrane (Pbm), the total area of the airway wall (Wat) and the area of smooth muscle (Wam) were measured by morphometric analysis (Image-Pro Plus 6.0; Media cybernetics, Inc.) and the ratios of Wat to Pbm (Wat/Pbm) and Wam to Pbm (Wam/Pbm) were calculated (43).

Masson's trichrome-stained slides were used to determine subepithelial fibrosis around the airways of the mice. The sections were stained in Biebrich scarlet-acid fuchsin solution (Thermo Fisher Scientific, Inc.) at room temperature for 10 min and washed in distilled water. Subsequently, the sections were differentiated in phosphomolybdic-phosphotungstic acid solution (Thermo Fisher Scientific, Inc.) for 10 min and then transferred directly to aniline blue solution (Thermo Fisher Scientific, Inc.) and stained at room temperature for 5 min. In total, three sections in which the epithelial basement membranes of the bronchioles were 1.0-2.5 mm and the diameter of bronchial wall was >250 μm were selected. The area of collagen fiber (stained in blue) beneath the basement membrane and Pbm were measured using Image-Pro Plus 6.0 software and the results are expressed as the fibrotic area per unit length (area of Masson-positive/Pbm) (44).

To evaluate the degree of goblet cell hyperplasia, the lung sections were stained with PAS. Sections were immersed in PAS solution (Thermo Fisher Scientific, Inc.) for 10 min and washed four times with distilled water. They were then immersed in Schiff's solution (Thermo Fisher Scientific, Inc.) for 15 min and successively in hematoxylin for 3 min (both at room temperature), followed by washing with distilled water. The bronchioles with 1.0-2.5 mm long epithelial basement membrane were selected and the area of goblet cells (PAS-positive stained) was measured using Image-Pro Plus 6.0 software. Subsequently, the mean score of the area of PAS-positive staining divided by Pbm was calculated (45).

To assess the degree of myofibroblast activation and angiogenesis, the lung sections were heated, blocked with 3%

hydrogen peroxide (Thermo Fisher Scientific, Inc.) for 15 min at room temperature, and then incubated with primary antibody α-smooth muscle actin (α-SMA) (1:200; cat. no. sc-15320; Santa Cruz Biotechnology, Inc.) and CD31 (1:100; cat. no. sc-28188; Santa Cruz Biotechnology, Inc.) at 4˚C for 12 h, respectively. After rinsing with PBS, the sections were incubated with secondary antibody from the MaxVision™ HRP-Polymer anti-mouse/rabbit IHC kit (Maixin Group China Co. Ltd.) at 37˚C for 60 min. The peribronchial a-SMA-positive and CD31-positive areas in the sub-mucosa were measured using Image-Pro Plus 6.0 software and the results are expressed as the area of α-SMA and CD31-positive staining per mm of Pbm of bronchi (46). All the sections were observed using a BX51 microscopic imaging system (Olympus Corporation).

Statistical analysis. Data are presented as the mean ± SEM. Statistical analysis was performed using PRISM version 6 (GraphPad, Inc.). Comparisons between multiple groups were performed using one-way ANOVA with the Bonferroni post-test. A value of $P<0.05$ was considered to indicate a statistically significant difference.

Results

Therapeutic administration of IL-27 does not improve airway inflammation, AHR and airway remodeling in a mouse model of OVA asthma. To investigate whether IL-27 exerts an inhibitory effect on airway inflammation and AHR, IL-27 was administered to mice with acute asthma in the final 5 consecutive days of the experiment (Fig. 1B). As shown in Fig. 1C, IL-27 was unable to alleviate AHR, a hallmark of asthma ($R_{OVA + IL-27}$ vs. R_{OVA}: 4.49 vs. 4.80, t=2.22, P>0.05; $C_{OVA + IL-27}$ vs. C_{OVA}: 0.31 vs. 0.33, t=2.78, P>0.05). Furthermore, IL-27 did not decrease the numbers of total cells [OVA + IL-27 vs. OVA ($x10^4$ cells/ml): 191.40 vs. 160.00, t=0.74, P>0.05] or those of differential cells [OVA + IL-27 vs. OVA ($x10^4$ cells/ml) for monocytes: 14.19 vs. 18.06, t=1.31; eosinophils: 54.03 vs. 61.95, t=0.77; neutrophils: 24.79 vs. 28.79, t=1.04; lymphocytes: 71.94 vs. 78.23, t=0.46; basophils: 4.78 vs. 6.48, t=1.53; all P>0.05] in BALF (Fig. 1D). It also did not significantly decrease the levels of Th2 cytokines in BALF and serum (OVA + IL-27 vs. OVA), such as IL-4 [BALF (pg/ml): 114.60 vs. 136.20, t=0.91; serum (pg/ml): 80.58 vs. 102.90, t=1.37, both P>0.05], IL-5 [BALF (pg/ml): 58.87 vs. 61.11, t=0.61; serum (pg/ml): 62.80 vs. 72.07, t=1.22, both P>0.05] and IL-13 [BALF (pg/ml): 297.70.6 vs. 380.00, t=1.16; serum (pg/ml): 345.40 vs. 451.90, t=1.75, both P>0.05] (Fig. 2B and C). Moreover, the effects of the therapeutic administration of IL-27 on airway remodeling were assessed in mice with chronic asthma using different staining methods. As depicted in Fig. 3, no significant differences were found between the OVA and OVA + IL-27 groups. Taken together, these results demonstrated that IL-27 did not effectively suppress airway inflammation, AHR and airway remodeling in mice with OVA-sensitized allergic asthma when administrated in a therapeutic manner.

Preventive administration of IL-27 alleviates the lung Th2 inflammatory environment. To examine whether the effects of IL-27 on inflammation and AHR resulted from the suppression of the inflammatory environment when IL-27 was prophylactically administrated, the concentrations of Th2 cytokines (IL-4, IL-5 and IL-13) were examined in BALF samples obtained from mice. As shown in Fig. 2D, compared with the OVA group, the intranasal administration of IL-27 led to a significant reduction in the levels of the inflammatory cytokines (OVA + IL-27 vs. OVA, pg/ml) IL-4 (92.81 vs. 191.70, t=2.63, P<0.05), IL-5 (57.68 vs. 117.00, t=3.64, P<0.05) and IL-13 (212.10 vs. 397.00, t=3.68, P<0.05) in BALF. These results demonstrated that the preventative administration of IL-27 ameliorated the lung Th2 inflammatory environment which would result in an improvement of allergic asthma in the experimental model.

Preventive administration of IL-27 promotes the generation of Tr1 cells in lung tissue. There is emerging evidence to indicate that Tr1 cells play a role in inhibiting the production of the pro-inflammatory cytokines, IL-5 and IL-13, and are instrumental in the prevention of tissue inflammation (47,48). Therefore, in the present study, Tr1 cells in the lung tissue were evaluated by means of FACS analysis. As shown in the right column of Fig. 4A and B, the proportion of Tr1 cells in the OVA group was lower than that in the PBS group, and the administration of IL-27 increased the proportion of Tr1 cells in the OVA + IL-27 group (Fig. 4C and D). These results suggested that the preventative administration of IL-27 promoted the generation of Tr1 cells in mouse lung tissue, which led to the the decreased production of Th2 cytokines.

Preventative administration of IL-27 prior to the OVA challenge upregulates both the STAT1 and STAT3 pathways. The possible signaling pathways involved in the effects of IL-27 were subsequently investigated. The STAT1 pathway is critical for Th1 differentiation (49,50). Studies have found that the blockade of IL-6, the activator of STAT3, leads to diminished Th2 responses in mice (51,52). Therefore, whether the ameliorating effects of IL-27 on the lung Th2 inflammatory environment are associated with the STAT1 and STAT3 pathways was further examined. As shown in Fig. 5A, compared with the OVA group, the mRNA expression levels of STAT1 exhibited no significant changes, while those of STAT3 were significantly upregulated in the OVA+ IL-27 group. As for the total levels of STAT1 and STAT3 proteins, no significant differences were found between the OVA+ IL-27 and the OVA group. However, obvious differences were observed in the phosphorylation levels of STAT1 and STAT3 between the OVA+ IL-27 and the OVA group. Moreover, similar trends were observed in the ratios of the p-STAT1/3 and to STAT1/3 (Fig. 5B and C). These results indicated that IL-27 inhibited Th2 cell differentiation and ameliorated the lung Th2 inflammatory environment by reversing the impairment of the STAT1 and STAT3 pathways.

Discussion

The findings of the present study provide novel contributions to the current knowledge of the association between IL-27 and allergic asthma. First, the therapeutic administration of IL-27 does not attenuate airway inflammation, AHR and airway remodeling in a mouse model of acute and chronic

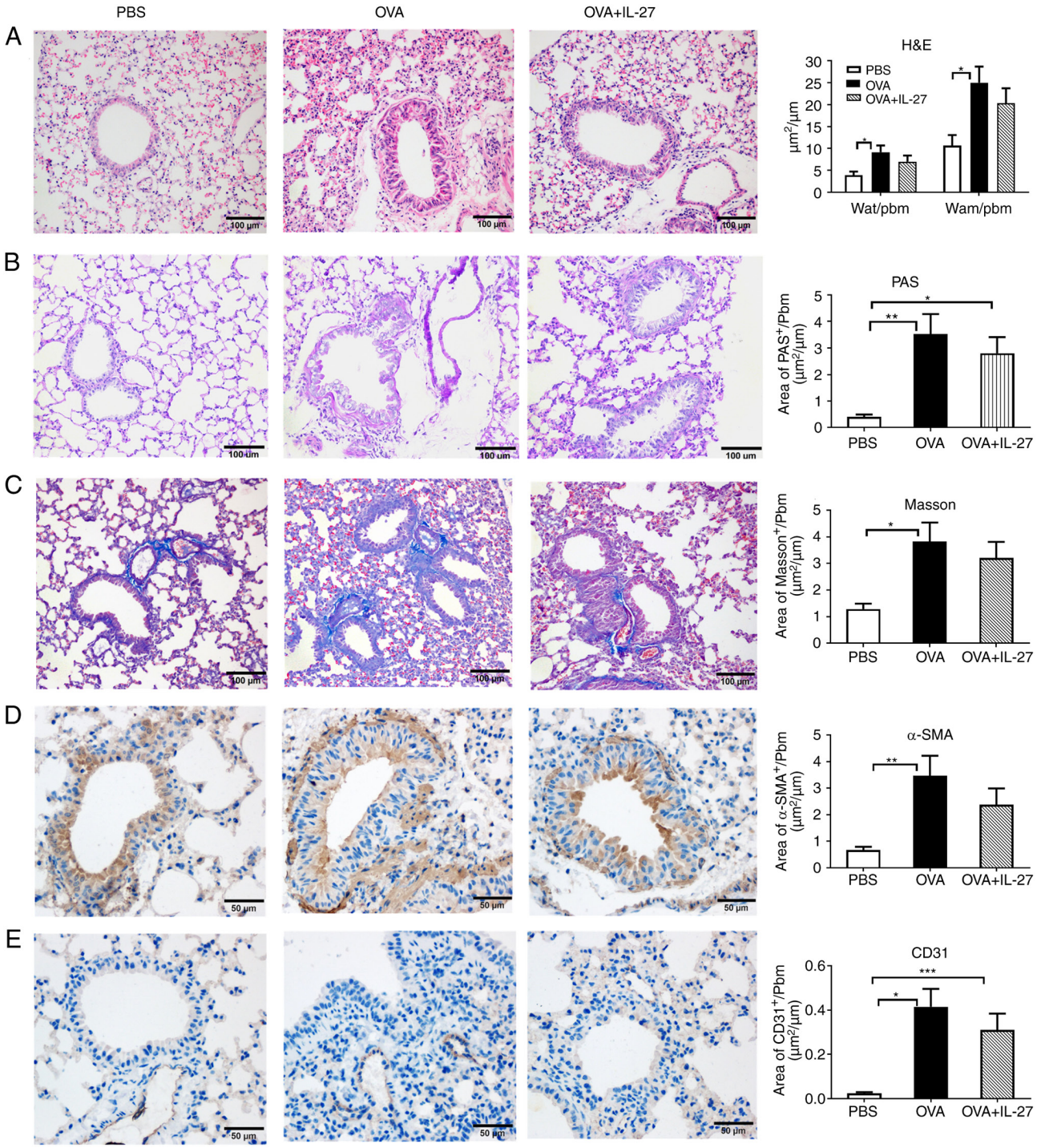

Figure 3. Therapeutic administration of IL-27 does not ameliorate OVA-induced airway remodeling in a chronic experimental model of asthma. (A) Representative photomicrographs of H&E-stained lung sections from each group (magnification, x200). No significant difference was observed in basal membrane thickening and the hyperplasia of airway smooth muscle cells between the OVA + IL-27 group and the OVA group. (B) Representative photomicrographs of PAS-stained lung sections from each group (magnification, x200). No significant difference was found in airway mucus production between the OVA + IL-27 group and the OVA group. (C) Representative photomicrographs of Masson's trichrome-stained sections from each group (magnification, x200). No obvious difference was found in subepithelial fibrosis between the OVA + IL-27 group and the OVA group. (D) Representative photomicrographs of α-SMA-immunostained sections from each group (magnification, x400). No significant difference was found in the peribronchial α-SMA-immunostained area between the OVA + IL-27 group and the OVA group. (E) Representative photomicrographs of CD31-immunostained sections from each group (magnification, x400). There was no significant difference in the peribronchial CD31-immunostained area between the OVA + IL-27 group and the OVA group. The data are expressed as mean ± SEM of three independent experiments with n=8 per group. *P<0.05, **P<0.01, ***P<0.001. IL-27, interleukin 27; OVA, ovalbumin; H&E, hematoxylin and eosin; PAS, periodic-acid Schiff; α-SMA, α-smooth muscle actin; Pbm, perimeter of basement membrane; Wat, total area of airway wall; Wam, area of smooth muscle.

asthma. Second, IL-27 increased the proportion of Tr1 cells in the lung tissues of asthmatic mice, which partially indicated that IL-27 alleviated the lung Th2 inflammatory environment and sequentially ameliorated OVA-induced

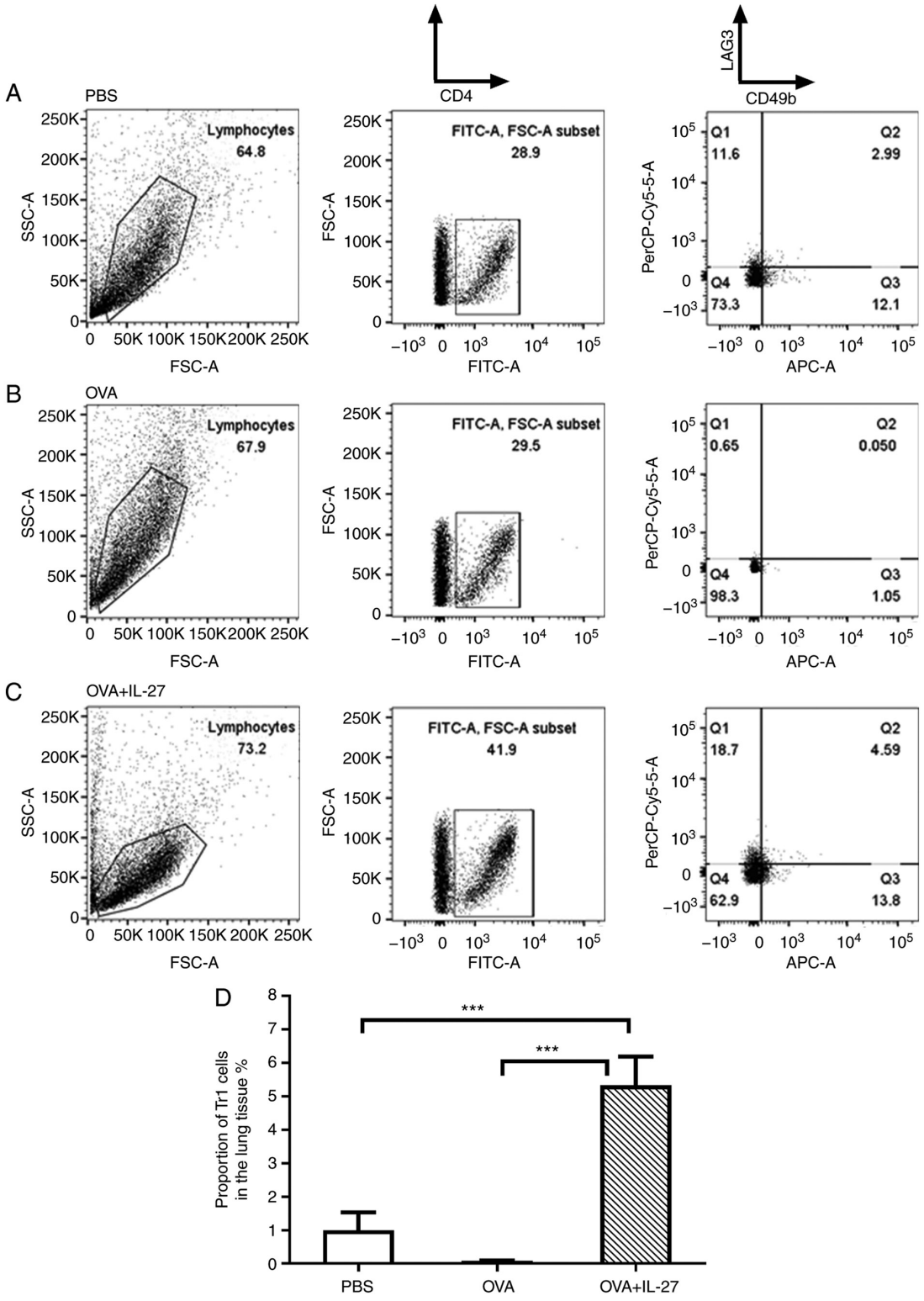

Figure 4. Preventative administration of IL-27 before the OVA challenge upregulates Tr1 cells in mouse lung tissue. (A) FACS analysis of Tr1 cells in the lung tissue of PBS group. (B) FACS analysis of Tr1 cells in the lung tissue of OVA group. (C) FACS analysis of Tr1 cells in the lung tissue of OVA + IL-27 group. (D) The proportion of Tr1 cells in the mouse lung tissue analyzed using flow cytometry. The proportion of Tr1 cells in the OVA + IL-27 group was significantly higher than that in the OVA group (P<0.001). The data are expressed as the mean ± SEM of three independent experiments, with 8 animals per group. ***P<0.001. FACS, fluorescence-activated cell sorting; IL-27, interleukin-27; OVA, ovalbumin; Tr1, type 1 regulatory T-cells.

allergic asthma. To the best of our knowledge, this is first study to evaluate the effects of IL-27 on Tr1 cells in a model of acute asthma. Finally, IL-27 alleviated airway inflammation and airway remodeling mainly through the STAT1 and STAT3 signaling pathways.

The findings that IL-27 exerted no inhibitory effect on asthmatic models of mice when used following sensitization with OVA are in accordance with those of a recent study by Su *et al* (23), who reported that the therapeutic administration of IL-27 did not suppress allergic asthma. However,

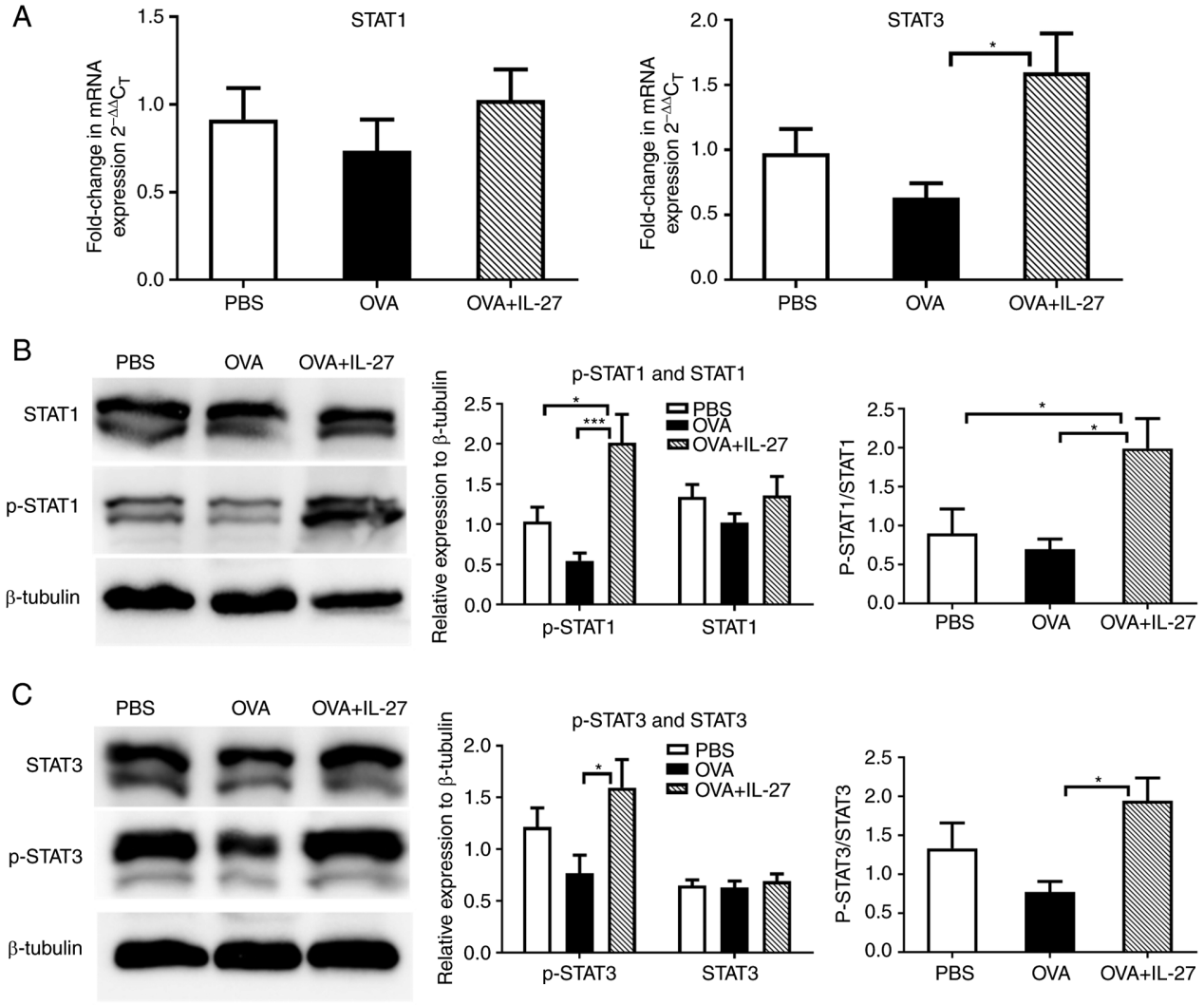

Figure 5. Preventative administration of IL-27 upregulates the STAT1 and STAT3 pathways in the acute model of asthma. (A) Analysis of STAT1 and STAT3 mRNA expression levels in the lung tissues of mice. (B) The protein expression levels of p-STAT1, STAT1 and their ratios in mouse lung tissues were determined using western blot analysis. (C) The protein expression levels of p-STAT3, STAT3 and their ratios in mouse lung tissues were determined using western blot analysis. The graphs display a summary of three independent experiments. Data are expressed as the mean ± SEM. *P<0.05 and ***P<0.001. IL-27, interleukin 27; OVA, ovalbumin; STAT, signal transducer and activator of transcription; p, phosphorylated.

the results of the present study are contradictory to those of a previous study by Yoshimoto *et al* (22), who revealed that IL-27 diminished AHR and airway inflammation when IL-27 was administered at the time of the OVA challenge. The reason for these contradictory results is unclear; however, it may be partly be ascribed to the different protocols used for the establishment of murine models of allergic asthma. Mice were challenged with 50 μg of OVA for 3 days in the study by Yoshimoto *et al* (22), whereas the mice were stimulated with 100 μg OVA for 5 days in the present study. In addition, Yoshimoto *et al* (22) selected an asthma model of 10 days, whereas the model used herein involved a period of 19 days. These differences may affect the severity of Th2-mediated airway inflammation and may result in the differences observed in the interventional effects of IL-27 on airway inflammation in the mouse models of allergic asthma. Th2 cells secrete IL-4 and play a fundamental role in the pathogenesis of allergic asthma. IL-4 from differentiated Th2 cells can upregulate the mRNA expression of suppressor of cytokine signaling 3 and impair IL-27-induced STAT1 phosphorylation. Consequently, established Th2 cells are resistant to reprogramming into Th1 cells (53). Th17 cells can produce IL-17, which can lead to neutrophilic airway inflammation (54). Airway neutrophilia is associated with asthma which is refractory to corticosteroids (55). IL-27 suppresses *de novo* Th17 development; however, it has a minimal effect on committed Th17 cells (56). When viewed in combination, these findings demonstrate that whether IL-27 plays an anti-inflammatory role in asthma is dependent upon the polarization of inflammatory cells in the context of an inflammatory environment.

As type 2 cytokine-producing cells, ILC2 cells are increasingly recognized as a key controller of type 2 inflammation and play an essential role in the pathophysiology of asthma (14,57). ILC2 cell numbers are increased in patients with allergic rhinitis, chronic rhinosinusitis with nasal polyps and asthma (58,59). ILC2 cell-derived cytokines, such as IL-4, IL-5 and IL-13 are associated with the initiation and amplification of airway inflammation by promoting the activation

and survival of eosinophils, B cells, mast cells, macrophages and epithelial cells during the Th2 immune response (60). As a negative regulator, IL-27 inhibits the proliferation and type 2 cytokine production of ILC2s from mice and humans through the activation of STAT1 (60,61). IL-27-deficient mice exhibit an increased ILC2 cell fraction and an enhanced Th2 immune response following papain-induced lung inflammation (57). Therefore, IL-27 is a fundamental environmental cytokine for the regulation of ILC2 cells. Th2 cytokines may be partially produced by ILC2 cells and are involved in orchestrating the allergic inflammatory responses in the Th2 inflammatory environment.

Tr1 cells, a type of induced regulatory T-cells (Tregs), are derived from the peripheral lymphoid tissue. They are negative for Foxp3 and GATA-3, but double positive for LAG-3 and CD49b (62). By secreting high levels of the immunosuppressive cytokine, IL-10, and TGF-β, Tr1 cells can suppress immune and autoimmune responses in autoimmune diseases (63-65). Thus, Tr1 cells play a crucial role in maintaining the peripheral immune tolerance. Allergic individuals have lower levels of antigen-specific Tr1 cells in peripheral blood in comparison to healthy ones (66). The adoptive transfer of Tr1 cells has been shown to suppress the development of inflammation in a mouse model of inflammatory bowel disease and asthma (23,67). Allergen immunotherapy (AIT) is the only etiological therapy and provides a specific and potentially curative approach for the treatment of allergic asthma. Tr1 cells downregulate allergen-specific immune responses and play crucial roles in AIT (68). Matsuda *et al* (62) reported that subcutaneous immunotherapy was associated with an increased number of Tr1 cells in peripheral blood mononuclear cells. It has been demonstrated that Tr1 cells are instrumental for the inhibition of atopic diseases, including allergic asthma (62).

The mechanisms through which Tr1 cells suppress the development of allergies may include the following aspects: first, the production of IL-10 and TGF-β increases in Tr1 cells during autoimmune responses (63,69). IL-10 is a secreted cytokine that tempers inflammatory responses by antagonizing the activation of antigen-presenting cells (APCs) (70,71). TGF-β maintains tolerance by regulating the development of lymphocytes and the chemotaxis and survival of lymphocytes and other immune cells (72). Second, Tr1 cells express certain inhibitory receptors, such as cytotoxic T-lymphocyte antigen 4 and programmed cell death protein 1, which play inhibitory roles in T-cell function (19,73). Tr1 cells also express the ectoenzymes (CD39/CD73) and subsequently produce higher levels of adenosine and prostaglandin E2, which leads to the suppression of effector T-cells (74). Third, Tr1 cells kill myeloid APCs and regulate T-cell responses by a granzyme B- and perforin-dependent mechanism (19,75). Overall, Tr1 cells directly and indirectly affect the functions of different immune cells and molecules in an antagonistic manner to mitigate immune responses.

The present study found a significantly higher number of CD4(+)CD49b(+)LAG3(+) T-cells (Tr1) in the lungs of mice from the IL-27 + OVA group compared with those of mice from the OVA group, suggesting that IL-27 promoted the generation of Tr1 cells in the mouse model of asthma (Fig. 4). The present study also found that the levels of IL-4, IL-5 and IL-13 in BALF and serum were lower in mice from the IL-27 + OVA group compared with those from the OVA group (Fig. 2C), indicating the decreased production of Th2 cytokines. Studies have revealed that IL-27 promotes the development of Tr1 cells in addition to antagonizing the differentiation of Th2 and Th17 cells (10,63,76,77). Moreover, Tr1 cell-derived IL-10 can suppress differentiation of Th2 cells and decrease the cytokine production of eosinophils, basophils and mast cells (63). The balance between Tr1 and Th2 cells plays a critical role in the development of allergic and normal immune response against allergens (78). In a previous study by the authors, it was found that when administrated preventatively, IL-27 attenuated airway inflammation and remodeling in mouse models of asthma (18). Thus, IL-27 helps to improve allergic asthma by alleviating the Th2 inflammatory environment.

Some molecular pathways have been involved in the expansion and differentiation of Tr1 cells by IL-27. Activation with IL-27 induces the expression of three key factors: the transcription factor, c-musculoaponeurotic-fibrosarcoma (c-Maf), the cytokine IL-21, and the receptor for inducible co-stimulator (ICOS) (79). The ligand-activated transcription factor aryl hydrocarbon receptor binds to c-Maf, which leads to the transactivation of the IL-10 and IL-21 promoters following Tr1 cell induction and IL-10 production (47). IL-21 is produced by Tr1 cells due to the IL-27-driven c-Maf expression and functions as an autocrine growth factor for Tr1 cells (79). ICOS further promotes the differentiation of IL-27-driven Tr1 cells by interacting with ICOS ligand and subsequently inducing c-Maf expression (80,81). Previous studies have revealed that the signaling pathways of STAT1 and STAT3 activated by IL-27 are key to the transcriptional output (82,83). Interferon regulatory factor 1 (IRF1) is downstream of STAT1 and basic leucine zipper transcriptional factor ATF-like (BATF) is dependent on STAT3. It has been substantiated that IRF1 and BATF play a major role in preparing the chromatin landscape during IL-27-induced Tr1 development (84). In this process, IRF1 specifically upregulates the expression of the IL-10 gene and BATF serves as a pioneer factor. Therefore, the two transcription factors may function as drivers in the earliest steps during the expansion of Tr1 cells (84).

It has been reported that Janus kinase (JAK)1, JAK2 and tyrosine kinase-2 can be activated by IL-27 in naive $CD4^+$ T-cells (83). As downstream signaling pathways of JAKs, STAT1-6 can also be activated by IL-27 in bronchial epithelial cells and intestinal epithelial cells (82,85). A previous *in vitro* study demonstrated that the activation of STAT1 and STAT3 is required for the differentiation of Tr1-like cells elicited by IL-27 (86). In addition, this induction is associated with the expression of IL-10 and IFN-γ (87). Harb *et al* (88) found that the upregulation of Notch4 receptor in Tregs in asthmatic lung tissue is indispensable for allergens to potentiate airway inflammation. Of note, this upregulation is dependent on STAT3 and IL-6 (88). In the present study, both the STAT1 and STAT3 pathways were downregulated in the OVA group, whereas they were upregulated in the OVA + IL-27 group (Fig. 5), which suggested that IL-27 upregulated the phosphorylation of the STAT1 and STAT3 proteins and restored the STAT1 and STAT3 signaling pathways in the asthmatic mouse model. When administrated preventatively, IL-27 subverted naïve $CD4^+$ T-cells into Th2 cells and led to an improvement in the pathological changes of asthma. Thus,

the ameliorated Th2 inflammatory environment may help to attenuate allergic asthma by restoring the STAT1 and STAT3 pathways.

In conclusion, the present study demonstrated that IL-27 did not improve airway inflammation, AHR and airway remodeling when administrated in a therapeutic manner in a mouse model of OVA-induced asthma. The preventive administration of IL-27 decreased the production of Th2 cytokines in BALF and increased the proportion of Tr1 cells in lung tissue. The ameliorated Th2 inflammatory environment is instrumental in attenuating allergic asthma by STAT1 and STAT3 pathway-dependent mechanisms. Collectively, these findings may provide insight into the multifaceted role of IL-27 in allergic asthma. Therapeutic intervention with IL-27 may be relevant to the improvement of the inflammatory environment associated with dysregulated type-2 responses in allergic asthma. With further studies, IL-27 is expected to be used as an immunotherapeutic treatment target for asthma.

Acknowledgements

The authors would like to thank Dr Xianchen Liu (Center for Public Health Initiatives, University of Pennsylvania, Philadelphia, PA, USA) for providing a critical appraisal of the manuscript.

Funding

The present study was supported by the Collaborative Innovation Center for Intelligent Molecules with Multi-effects and Nanomedicine (grant no. 2019-01), Shandong province, China.

Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Authors' contributions

JL, XJ and DL contributed to the conception and design of the present study. JL, XJ, LW, YJ, XL, ZG, YJ, CH, HP and FS performed the experiments and analyzed the data. JL, XJ, HP and DL wrote and revised the manuscript. DL reviewed the article. JL and FS confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

All protocols were approved by the Ethics committee for Laboratory Animal care and Use in Shandong Qianfoshan Hospital, Shandong University (approval no. 2019-S-306). All procedures using mice were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Papi A, Brightling C, Pedersen SE and Reddel HK: Asthma. Lancet 391: 783-800, 2018.
2. Adcock IM, Caramori G and Chung KF: New targets for drug development in asthma. Lancet 372: 1073-1087, 2008.
3. Barnes CB and Ulrik CS: Asthma and adherence to inhaled corticosteroids: Current status and future perspectives. Respir Care 60: 455-468, 2015.
4. Conner JB and Buck PO: Improving asthma management: The case for mandatory inclusion of dose counters on all rescue bronchodilators. J Asthma 50: 658-663, 2013.
5. Campo P, Rodriguez F, Sanchez-Garcia S, Barranco P, Quirce S, Pérez-Francés C, Gómez-Torrijos E, Cárdenas R, Olaguibel JM, Delgado J, *et al*: Phenotypes and endotypes of uncontrolled severe asthma: New treatments. J Investig Allergol Clin Immunol 23: 76-88; quiz 71 p follow 88, 2013.
6. Martin MJ, Beasley R and Harrison TW: Towards a personalised treatment approach for asthma attacks. Thorax 75: 1119-1129, 2020.
7. Pflanz S, Timans JC, Cheung J, Rosales R, Kanzler H, Gilbert J, Hibbert L, Churakova T, Travis M, Vaisberg E, *et al*: IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells. Immunity 16: 779-790, 2002.
8. Owaki T, Asakawa M, Morishima N, Hata K, Fukai F, Matsui M, Mizuguchi J and Yoshimoto T: A role for IL-27 in early regulation of Th1 differentiation. J Immunol 175: 2191-2200, 2005.
9. Hunter CA: New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. Nat Rev Immunol 5: 521-531, 2005.
10. Meka RR, Venkatesha SH, Dudics S, Acharya B and Moudgil KD: IL-27-induced modulation of autoimmunity and its therapeutic potential. Autoimmun Rev 14: 1131-1141, 2015.
11. Owaki T, Asakawa M, Fukai F, Mizuguchi J and Yoshimoto T: IL-27 induces Th1 differentiation via p38 MAPK/T-bet- and intercellular adhesion molecule-1/LFA-1/ERK1/2-dependent pathways. J Immunol 177: 7579-7587, 2006.
12. Wang RX, Yu CR, Mahdi RM and Egwuagu CE: Novel IL27p28/IL12p40 cytokine suppressed experimental autoimmune uveitis by inhibiting autoreactive Th1/Th17 cells and promoting expansion of regulatory T cells. J Biol Chem 287: 36012-36021, 2012.
13. Artis D, Villarino A, Silverman M, He W, Thornton EM, Mu S, Summer S, Covey TM, Huang E, Yoshida H, *et al*: The IL-27 receptor (WSX-1) is an inhibitor of innate and adaptive elements of type 2 immunity. J Immunol 173: 5626-5634, 2004.
14. Moro K, Kabata H, Tanabe M, Koga S, Takeno N, Mochizuki M, Fukunaga K, Asano K, Betsuyaku T and Koyasu S: Interferon and IL-27 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses. Nat Immunol 17: 76-86, 2016.
15. Qin L, Li Z, Fan Y, Fang X, Zhang C, Yue J, Xu Y, Wenzel SE and Xie M: Exploration of plasma interleukin-27 levels in asthma patients and the correlation with lung function. Respir Med 175: 106208, 2020.
16. Liu Z, Niu C, Ying L, Zhang Q, Long M and Fu Z: Exploration of the serum interleukin-17 and interleukin-27 expression levels in children with bronchial asthma and their correlation with indicators of lung function. Genet Test Mol Biomarkers 24: 10-16, 2020.
17. Jirmo AC, Daluege K, Happle C, Albrecht M, Dittrich AM, Busse M, Habener A, Skuljec J and Hansen G: IL-27 is essential for suppression of experimental allergic asthma by the TLR7/8 Agonist R848 (Resiquimod). J Immunol 197: 4219-4227, 2016.
18. Lu D, Lu J, Ji X, Ji Y, Zhang Z, Peng H, Sun F and Zhang C: IL27 suppresses airway inflammation, hyperresponsiveness and remodeling via the STAT1 and STAT3 pathways in mice with allergic asthma. Int J Mol Med 46: 641-652, 2020.
19. Roncarolo MG, Gregori S, Bacchetta R and Battaglia M: Tr1 cells and the counter-regulation of immunity: Natural mechanisms and therapeutic applications. Curr Top Microbiol Immunol 380: 39-68, 2014.

20. Song Y, Wang N, Chen L and Fang L: Tr1 cells as a key regulator for maintaining immune homeostasis in transplantation. Front Immunol 12: 671579, 2021.
21. Battaglia M, Gregori S, Bacchetta R and Roncarolo MG: Tr1 cells: From discovery to their clinical application. Semin Immunol 18: 120-127, 2006.
22. Yoshimoto T, Yoshimoto T, Yasuda K, Mizuguchi J and Nakanishi K: IL-27 suppresses Th2 cell development and Th2 cytokines production from polarized Th2 cells: A novel therapeutic way for Th2-mediated allergic inflammation. J Immunol 179: 4415-4423, 2007.
23. Su X, Pan J, Bai F, Yuan H, Dong N, Li D, Wang X and Chen Z: IL-27 attenuates airway inflammation in a mouse asthma model via the STAT1 and GADD45γ/p38 MAPK pathways. J Transl Med 14: 283, 2016.
24. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals: Guide for the Care and Use of Laboratory Animals. 8th edition. National Academies Press (US), Washington, DC, 2011.
25. Reddy AT, Lakshmi SP and Reddy RC: Murine model of allergen induced asthma. J Vis Exp 63: e3771, 2012.
26. Liu X, Li S, Jin J, Zhu T, Xu K, Liu C, Zeng Y, Mao R, Wang X and Chen Z: Preventative tracheal administration of interleukin-27 attenuates allergic asthma by improving the lung Th1 microenvironment. J Cell Physiol 234: 6642-6653, 2019.
27. Overmyer KA, Thonusin C, Qi NR, Burant CF and Evans CR: Impact of anesthesia and euthanasia on metabolomics of mammalian tissues: Studies in a C57BL/6J mouse model. PLoS One 10: e0117232, 2015.
28. Kirstein F, Nieuwenhuizen NE, Jayakumar J, Horsnell WGC and Brombacher F: Role of IL-4 receptor α-positive CD4(+) T cells in chronic airway hyperresponsiveness. J Allergy Clin Immunol 137: 1852-1862 e9, 2016.
29. O'Byrne PM and Inman MD: Airway hyperresponsiveness. Chest 123 (3 Suppl): 411S-416S, 2003.
30. Hoymann HG: Lung function measurements in rodents in safety pharmacology studies. Front Pharmacol 3: 156, 2012.
31. Martin TR, Gerard NP, Galli SJ and Drazen JM: Pulmonary responses to bronchoconstrictor agonists in the mouse. J Appl Physiol (1985) 64: 2318-2323, 1988.
32. Cataldo DD, Tournoy KG, Vermaelen K, Munaut C, Foidart JM, Louis R, Noël A and Pauwels RA: Matrix metalloproteinase-9 deficiency impairs cellular infiltration and bronchial hyperresponsiveness during allergen-induced airway inflammation. Am J Pathol 161: 491-498, 2002.
33. Polte T, Behrendt AK and Hansen G: Direct evidence for a critical role of CD30 in the development of allergic asthma. J Allergy Clin Immunol 118: 942-948, 2006.
34. Lambrecht BN, Hammad H and Fahy JV: The cytokines of asthma. Immunity 50: 975-991, 2019.
35. Hammad H and Lambrecht BN: The basic immunology of asthma. Cell 184: 2521-2522, 2021.
36. Albrecht M, Chen HC, Preston-Hurlburt P, Ranney P, Hoymann HG, Maxeiner J, Staudt V, Taube C, Bottomly HK and Dittrich AM: T(H)17 cells mediate pulmonary collateral priming. J Allergy Clin Immunol 128: 168-177.e8, 2011.
37. Kalidhindi RSR, Ambhore NS and Sathish V: Cellular and biochemical analysis of bronchoalveolar lavage fluid from murine lungs. Methods Mol Biol 2223: 201-215, 2021.
38. Gregorczyk I and Maslanka T: Blockade of RANKL/RANK and NF-kB signalling pathways as novel therapeutic strategies for allergic asthma: A comparative study in a mouse model of allergic airway inflammation. Eur J Pharmacol 879: 173129, 2020.
39. Chauhan PS, Subhashini, Dash D and Singh R: Intranasal curcumin attenuates airway remodeling in murine model of chronic asthma. Int Immunopharmacol 21: 63-75, 2014.
40. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
41. Loke WS, Freeman A, Garthwaite L, Prazakova S, Park M, Hsu K, Thomas PS and Herbert C: T-bet and interleukin-27: Possible TH1 immunomodulators of sarcoidosis. Inflammopharmacology 23: 283-290, 2015.
42. Kelly-Welch AE, Melo ME, Smith E, Ford AQ, Haudenschild C, Noben-Trauth N and Keegan AD: Complex role of the IL-4 receptor alpha in a murine model of airway inflammation: Expression of the IL-4 receptor alpha on nonlymphoid cells of bone marrow origin contributes to severity of inflammation. J Immunol 172: 4545-4555, 2004.
43. Tang X, Nian H, Li X, Yang Y, Wang X, Xu L, Shi H, Yang X and Liu R: Effects of the combined extracts of Herba Epimedii and Fructus Ligustrilucidi on airway remodeling in the asthmatic rats with the treatment of budesonide. BMC Complement Altern Med 17: 380, 2017.
44. Komai M, Tanaka H, Masuda T, Nagao K, Ishizaki M, Sawada M and Nagai H: Role of Th2 responses in the development of allergen-induced airway remodelling in a murine model of allergic asthma. Br J Pharmacol 138: 912-920, 2003.
45. Kohan M, Breuer R and Berkman N: Osteopontin induces airway remodeling and lung fibroblast activation in a murine model of asthma. Am J Respir Cell Mol Biol 41: 290-296, 2009.
46. Eifan AO, Orban NT, Jacobson MR and Durham SR: Severe persistent allergic rhinitis. Inflammation but No histologic features of structural upper airway remodeling. Am J Respir Crit Care Med 192: 1431-1439, 2015.
47. Apetoh L, Quintana FJ, Pot C, Joller N, Xiao S, Kumar D, Burns EJ, Sherr DH, Weiner HL and Kuchroo VK: The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. Nat Immunol 11: 854-861, 2010.
48. Aron JL and Akbari O: Regulatory T cells and type 2 innate lymphoid cell-dependent asthma. Allergy 72: 1148-1155, 2017.
49. Afkarian M, Sedy JR, Yang J, Jacobson NG, Cereb N, Yang SY, Murphy TL and Murphy KM: T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells. Nat Immunol 3: 549-557, 2002.
50. Lighvani AA, Frucht DM, Jankovic D, Yamane H, Aliberti J, Hissong BD, Nguyen BV, Gadina M, Sher A, Paul WE and O'Shea JJ: T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. Proc Natl Acad Sci USA 98: 15137-15142, 2001.
51. Doganci A, Eigenbrod T, Krug N, De Sanctis GT, Hausding M, Erpenbeck VJ, Haddad el-B, Lehr HA, Schmitt E, Bopp T, *et al*: The IL-6R alpha chain controls lung CD4+CD25+ Treg development and function during allergic airway inflammation in vivo. J Clin Invest 115: 313-325, 2005.
52. Finotto S, Eigenbrod T, Karwot R, Boross I, Doganci A, Ito H, Nishimoto N, Yoshizaki K, Kishimoto T, Rose-John S, *et al*: Local blockade of IL-6R signaling induces lung CD4+ T cell apoptosis in a murine model of asthma via regulatory T cells. Int Immunol 19: 685-693, 2007.
53. Chen Z, Wang S, Erekosima N, Li Y, Hong J, Qi X, Merkel P, Nagabhushanam V, Choo E, Katial R, *et al*: IL-4 confers resistance to IL-27-mediated suppression on CD4+ T cells by impairing signal transducer and activator of transcription 1 signaling. J Allergy Clin Immunol 132: 912-921.e1-5, 2013.
54. Boonpiyathad T, Sozener ZC, Satitsuksanoa P and Akdis CA: Immunologic mechanisms in asthma. Semin Immunol 46: 101333, 2019.
55. Ray A and Kolls JK: Neutrophilic Inflammation in asthma and association with disease severity. Trends Immunol 38: 942-954, 2017.
56. El-behi M, Ciric B, Yu S, Zhang GX, Fitzgerald DC and Rostami A: Differential effect of IL-27 on developing versus committed Th17 cells. J Immunol 183: 4957-4967, 2009.
57. McHedlidze T, Kindermann M, Neves AT, Voehringer D, Neurath MF and Wirtz S: IL-27 suppresses type 2 immune responses in vivo via direct effects on group 2 innate lymphoid cells. Mucosal Immunol 9: 1384-1394, 2016.
58. Ho J, Bailey M, Zaunders J, Mrad N, Sacks R, Sewell W and Harvey RJ: Group 2 innate lymphoid cells (ILC2s) are increased in chronic rhinosinusitis with nasal polyps or eosinophilia. Clin Exp Allergy 45: 394-403, 2015.
59. Kabata H, Moro K, Koyasu S, Fukunaga K, Asano K and Betsuyaku T: Mechanisms to Suppress ILC2-induced airway inflammation. Ann Am Thorac Soc 13 (Suppl 1): S95, 2016.
60. Kato A: Group 2 innate lymphoid cells in airway diseases. Chest 156: 141-149, 2019.
61. Kabata H, Moro K and Koyasu S: The group 2 innate lymphoid cell (ILC2) regulatory network and its underlying mechanisms. Immunol Rev 286: 37-52, 2018.
62. Matsuda M, Doi K, Tsutsumi T, Fujii S, Kishima M, Nishimura K, Kuroda I, Tanahashi Y, Yuasa R, Kinjo T, *et al*: Regulation of allergic airway inflammation by adoptive transfer of $CD4^+$ T cells preferentially producing IL-10. Eur J Pharmacol 812: 38-47, 2017.
63. Pot C, Apetoh L and Kuchroo VK: Type 1 regulatory T cells (Tr1) in autoimmunity. Semin Immunol 23: 202-208, 2011.

64. McGee HS and Agrawal DK: TH2 cells in the pathogenesis of airway remodeling: Regulatory T cells a plausible panacea for asthma. Immunol Res 35: 219-232, 2006.
65. Smith N and Broadley KJ: Optimisation of the sensitisation conditions for an ovalbumin challenge model of asthma. Int Immunopharmacol 7: 183-190, 2007.
66. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, Thunberg S, Deniz G, Valenta R, Fiebig H, *et al*: Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. J Exp Med 199: 1567-1575, 2004.
67. Wirtz S and Neurath MF: Animal models of intestinal inflammation: New insights into the molecular pathogenesis and immunotherapy of inflammatory bowel disease. Int J Colorectal Dis 15: 144-160, 2000.
68. Akdis CA and Akdis M: Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. World Allergy Organ J 8: 17, 2015.
69. Zhao ST and Wang CZ: Regulatory T cells and asthma. J Zhejiang Univ Sci B 19: 663-673, 2018.
70. O'Farrell AM, Liu Y, Moore KW and Mui AL: IL-10 inhibits macrophage activation and proliferation by distinct signaling mechanisms: Evidence for Stat3-dependent and -independent pathways. EMBO J 17: 1006-1018, 1998.
71. Stumhofer JS, Silver JS, Laurence A, Porrett PM, Harris TH, Turka LA, Ernst M, Saris CJ, O'Shea JJ and Hunter CA: Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat Immunol 8: 1363-1371, 2007.
72. Li MO, Wan YY, Sanjabi S, Robertson AK and Flavell RA: Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 24: 99-146, 2006.
73. Rowshanravan B, Halliday N and Sansom DM: CTLA-4: A moving target in immunotherapy. Blood 131: 58-67, 2018.
74. Mandapathil M, Szczepanski MJ, Szajnik M, Ren J, Jackson EK, Johnson JT, Gorelik E, Lang S and Whiteside TL: Adenosine and prostaglandin E2 cooperate in the suppression of immune responses mediated by adaptive regulatory T cells. J Biol Chem 285: 27571-27580, 2010.
75. Magnani CF, Alberigo G, Bacchetta R, Serafini G, Andreani M, Roncarolo MG and Gregori S: Killing of myeloid APCs via HLA class I, CD2 and CD226 defines a novel mechanism of suppression by human Tr1 cells. Eur J Immunol 41: 1652-1662, 2011.
76. Pot C, Apetoh L, Awasthi A and Kuchroo VK: Molecular pathways in the induction of interleukin-27-driven regulatory type 1 cells. J Interferon Cytokine Res 30: 381-388, 2010.
77. Muallem G, Wagage S, Sun Y, DeLong JH, Valenzuela A, Christian DA, Harms Pritchard G, Fang Q, Buza EL, Jain D, *et al*: IL-27 limits type 2 immunopathology following parainfluenza virus infection. PLoS Pathog 13: e1006173, 2017.
78. Akdis M: T-cell tolerance to inhaled allergens: Mechanisms and therapeutic approaches. Expert Opin Biol Ther 8: 769-777, 2008.
79. Pot C, Jin H, Awasthi A, Liu SM, Lai CY, Madan R, Sharpe AH, Karp CL, Miaw SC, Ho IC and Kuchroo VK: Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. J Immunol 183: 797-801, 2009.
80. Bauquet AT, Jin H, Paterson AM, Mitsdoerffer M, Ho IC, Sharpe AH and Kuchroo VK: The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. Nat Immunol 10: 167-175, 2009.
81. Nurieva RI, Duong J, Kishikawa H, Dianzani U, Rojo JM, Ho Ic, Flavell RA and Dong C: Transcriptional regulation of th2 differentiation by inducible costimulator. Immunity 18: 801-811, 2003.
82. Diegelmann J, Olszak T, Goke B, Blumberg RS and Brand S: A novel role for interleukin-27 (IL-27) as mediator of intestinal epithelial barrier protection mediated via differential signal transducer and activator of transcription (STAT) protein signaling and induction of antibacterial and anti-inflammatory proteins. J Biol Chem 287: 286-298, 2012.
83. Kamiya S, Owaki T, Morishima N, Fukai F, Mizuguchi J and Yoshimoto T: An indispensable role for STAT1 in IL-27-induced T-bet expression but not proliferation of naive CD4+ T cells. J Immunol 173: 3871-3877, 2004.
84. Karwacz K, Miraldi ER, Pokrovskii M, Madi A, Yosef N, Wortman I, Chen X, Watters A, Carriero N, Awasthi A, *et al*: Critical role of IRF1 and BATF in forming chromatin landscape during type 1 regulatory cell differentiation. Nat Immunol 18: 412-421, 2017.
85. Pereira ABM, de Oliveira JR, Teixeira MM, da Silva PR, Rodrigues Junior V and Rogerio AP: IL-27 regulates IL-4-induced chemokine production in human bronchial epithelial cells. Immunobiology 226: 152029, 2021.
86. Wang H, Meng R, Li Z, Yang B, Liu Y, Huang F, Zhang J, Chen H and Wu C: IL-27 induces the differentiation of Tr1-like cells from human naive CD4+ T cells via the phosphorylation of STAT1 and STAT3. Immunol Lett 136: 21-28, 2011.
87. Pot C, Apetoh L, Awasthi A and Kuchroo VK: Induction of regulatory Tr1 cells and inhibition of T(H)17 cells by IL-27. Semin Immunol 23: 438-445, 2011.
88. Harb H, Stephen-Victor E, Crestani E, Benamar M, Massoud A, Cui Y, Charbonnier LM, Arbag S, Baris S, Cunnigham A, *et al*: A regulatory T cell Notch4-GDF15 axis licenses tissue inflammation in asthma. Nat Immunol 21: 1359-1370, 2020.