

Glucocorticoids Modulate Th1 and Th2 Responses in Asthmatic Mouse Models by Inhibition of Notch1 Signaling

Chengping Hu Zhifang Li Juntao Feng Yongjun Tang Ling Qin Xinyue Hu
Yan Zhang Ruoxi He

Department of Respiratory Medicine, Key Cite of National Clinical Research Center for Respiratory Disease, Xiangya Hospital, Central South University, Changsha, China

Keywords

Asthma · Glucocorticoid · Notch signaling · γ -Secretase inhibitor

Abstract

Background: Notch1 has been linked to the pathogenesis of asthma due to its contribution on Th1/Th2 imbalance. γ -Secretase inhibitor (GSI) acts as an effective blocker of Notch1 signaling. Glucocorticoids (GCs) are the most effective anti-inflammatory drugs for asthma. The present study investigated the involvement of the Notch1 pathway in the anti-inflammatory effect of GCs and its association with Th1/Th2 balance. **Methods:** The asthma model was established in BALB/c mice by sensitization with ovalbumin (OVA). Dexamethasone (DEX; 1 mg/kg) and/or GSI (0.03 mg/kg) was orally or intranasally administered. **Results:** Compared to the OVA-sensitized mice, the administration of DEX and/or GSI significantly ameliorated the airway inflammation infiltration, goblet cell metaplasia, and airway hyper-responsiveness. The expression of IL-4 and IL-13, as well as the ratios of eosinophils and lymphocytes, were significantly decreased, whereas IFN- γ and IL-2 levels were significantly increased in bronchoalveolar lavage fluid after the administration of DEX

and GSI. The expressions of the Notch1/NICD1 pathway were decreased after DEX and/or GSI administration in lung tissues, especially in CD4⁺ T cells. Also, a reduction of GATA3 and elevation of T-bet levels were correlated with the up-regulation of Th1/Th2 ratios in lung tissues. **Conclusions:** Through the inhibition of Notch1 signaling, both GSI and GCs could regulate Th1/Th2 balance involved in allergic airway inflammation in OVA-induced asthma.

© 2018 S. Karger AG, Basel

Introduction

Asthma is a chronic inflammatory disease of the bronchial airways with T lymphocytes playing an indispensable role [1]. Th2 polarization and Th1/Th2 imbalance, characterized as an increase in Th2 cytokine levels (IL-4, IL-10, and IL-13) and decrease in Th1 cytokine levels (IFN- γ and IL-2), play a critical role in airway inflammation, hyper-reactivity, and the remodeling of asthma [2,

C.H. and Z.L. contributed equally to this study.
Edited by: D. Dombrowicz, Lille.

3]. Thus, the imbalance of Th1/Th2 is an essential target of asthma therapy [4]. Glucocorticoids (GCs) are the most effective anti-inflammatory drugs for asthma, which have been proven to reverse Th1/Th2 imbalance [5]. However, inhibitors of the Notch pathway [3, 6], PI3K signal pathway [6], or OX40-OX40L signaling [7] have also been reported to restore Th1/Th2 balance, but the mechanisms remain to be clearly delineated.

Notch signaling is an evolutionarily conserved pathway that regulates many cell-fate decisions during development. It consists of 4 different Notch transmembrane receptors (Notch1–4) and 5 types of transmembrane ligands (Delta1–3, Jagged1–2) in mammals [8]. The Notch pathway is initiated by receptor-ligand interactions followed by 2 successive proteolytic cleavages of Notch molecules, firstly by metalloproteases of the ADAM family, and subsequently by a γ -secretase complex [9]. Afterwards, the Notch-intracellular domain (NICD) is liberated, and translocated into the nucleus where it interacts with RBPj/CSL functioning as a potent transcriptional activator [10]. Notch1 plays a distinct role in T lineage development [11]. Previous studies have demonstrated that RBPj/CSL could increase expression of the GATA3 gene, which is a key transcription factor for Th2 differentiation [12], and oppositely repress T-bet, a Th1 differentiation gene [13]. The Notch1 protein level has been shown to increase in ovalbumin (OVA)-sensitized mice [3, 14]. Inhibition of Notch1 signaling by the γ -secretase inhibitor (GSI) could decrease Th2 cytokine production and increase Th1 cytokine levels, accompanied by alleviating asthma pathological changes [3]. Therefore, we hypothesized that Notch1 signaling may play a critical role in the pathogenesis of asthma through regulating the Th1/Th2 imbalance.

In this study, we investigated the changes of Th1/Th2 in responding to DEX and/or GSI treatment as well as Notch1 signaling in an asthma model of BALB/c mice.

Materials and Methods

Animal Model

All studies were approved by the Institutional Animal Care and Use Committee of Xiangya School of Medicine, Central South University. All animal procedures and experimental protocols complied with NIH guidelines. Ninety BALB/c mice (6–8 weeks old, females, mean weight 20 ± 2 g) were purchased from Hunan Slack King Laboratory Animal Co. and maintained under specific pathogen-free conditions at the animal center of Central South University. All animals were allowed to acclimatize for 5 days prior to treatments. The animals were assigned randomly into 6 groups ($n = 15/\text{group}$): the control group, OVA-sensitized group

(OVA group), OVA+DEX (dexamethasone) group, OVA+vehicle group, OVA+GSI group, and OVA+DEX+GSI group. The mice in the OVA group were sensitized by intraperitoneal administration of 50 μg of OVA (grade V, Sigma-Aldrich, St. Louis, MO, USA) emulsified with 2 mg of aluminum hydroxide (Sigma-Aldrich) in a total volume of 200 μL on days 0, 7, and 14. It was then followed by aerosol challenge with nebulized OVA (5%, 2 g of OVA in 40 mL of normal saline) for 1 h per day from day 21 to day 27 [15]. From day 21 to day 34, DEX (1 mg/kg body weight; Sigma-Aldrich) [16] was given orally and GSI (0.03 mg/kg body weight; L685485, Sigma-Aldrich) [3] was administered intranasally once a day, 30 min before aerosol challenge. For the OVA+DEX+GSI group, DEX and GSI were administered with a 30-min interval. In the OVA+vehicle group, saline was given orally and DMSO was given intranasally. The mice were sacrificed after the measurement of airway responsiveness.

Measurement of Airway Hyper-Responsiveness to Methacholine

Airway resistance (Raw) was evaluated by a Buxco's modular and invasive system (Buxco Electronics Inc., Troy, NY, USA) 24 h after the last application of DEX and/or GSI [17]. In short, mice were anesthetized by intraperitoneal injection of 10% chloral hydrate solution (0.6 mg/kg), tracheostomized, intubated with an appropriate cannula, and laid supine inside the body plethysmograph chamber connected to the ventilator. When a stable baseline airway pressure ($<5\%$ variation over 2.5 min) was achieved, mice were successively given aerosolized PBS or various concentrations of methacholine (0, 0.125, 0.25, 0.5, 1 mg/mL; Sigma-Aldrich) via a jet nebulizer into the head chamber. After delivery of each aerosol, forced oscillations were delivered every 15 s over a 5-min duration. Regular ventilation was resumed between impedance measurements. The peak response for each variable was determined.

Bronchoalveolar Lavage Fluid and Serum Analyses

After observing measurements of airway responsiveness, bronchoalveolar lavage fluid (BALF) and blood were collected and the lungs were removed. Blood samples were collected and centrifuged at 2,000 rpm for 10 min at room temperature (RT), and serum was stored at -80°C . BALF was obtained by 3 successive instillations of ice-cold sterile saline (0.5 mL). This procedure recovered approximately 90% of the infused fluid. Cell suspensions were kept on ice until centrifugation (1,000 rpm, 4°C , 10 min). Supernatants were collected, aliquoted, and stored at -80°C for further analyses. Total cells were counted on slides prepared with Diff-Quick staining (Scientific Products, Gibbstown, NJ, USA). The cell differentiation was based on their morphology, and a total of 300 white blood cells were counted. Cytokines (IL-2, IFN- γ , IL-4, and IL-13) in the BALF supernatant and serum were analyzed by enzyme-linked immunosorbent assay following the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

Real-Time PCR

The total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was synthesized from 1 μg of total RNA using a cDNA first-strand synthesis kit (Ferments, Vilnius, Lithuania). The *Notch1* gene was amplified using the forward primer 5'-GATGGCCTCAATGGGTACAAG-3' and reverse primer 5'-TCGTTGTTGTTGA-TGTCACAGT-3'. The *GATA3*

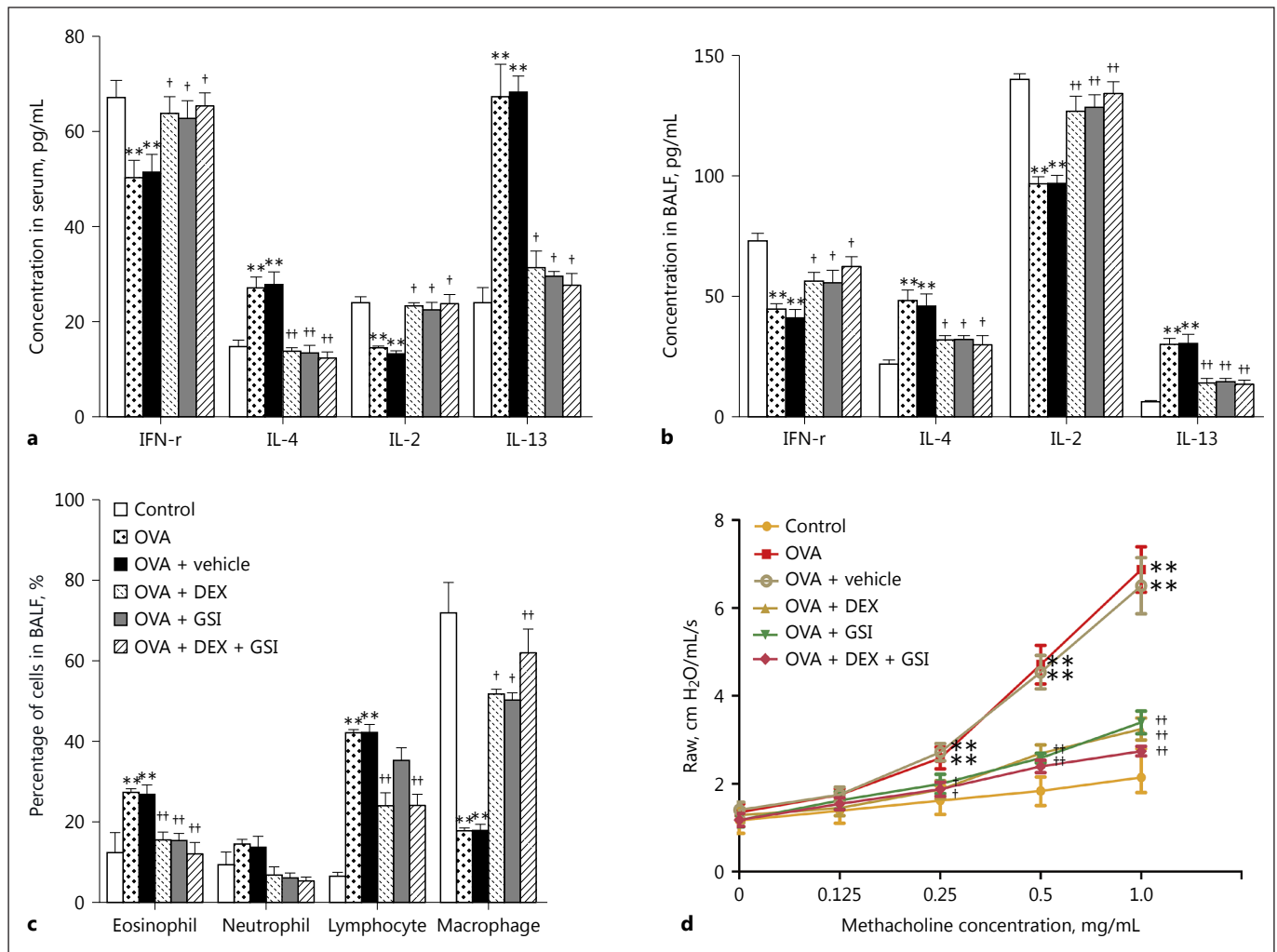


Fig. 1. Therapeutic effects of DEX and/or GSI on airway inflammation and Raw in OVA-sensitized mice. **a, b** The concentrations of Th2 and Th1 cytokines in serum and BALF were measured by enzyme-linked immunosorbent assay. **c** Inhibition of inflamma-

tory cell infiltration by DEX and/or GSI. **d** Effects of DEX and/or GSI on Raw of OVA-sensitized mice. * $p < 0.05$, ** $p < 0.01$ versus the control group; † $p < 0.05$, †† $p < 0.01$ versus the OVA or OVA+vehicle group. $n = 5$.

gene was amplified using the forward primer 5'-CTCGGCCATTCGTACATGGAA-3' and reverse primer 5'-GGATACCTCTGCACCGTAGC-3'. The *Tbx21* gene was amplified using the forward primer 5'-GCCTACCAGAACGCAGAGAT-3' and reverse primer 5'-AGCAGTTGACAGTTGGGTCC-3'. The β -actin gene was amplified using the forward primer 5'-TGTTACCAACTGGGAAGACA-3' and reverse primer 5'-GGGGTGTTGAAGGTCTCAAA-3' as an internal control. Amplification was performed in duplicate by using a SYBR Green PCR kit (SYBR Premix Ex Taq, Takara, Japan) in a 7900HR Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). The real-time PCR programs were initiated by a 30-s denaturation at 95°C, followed by 40 cycles of 95°C for 5 s, 60°C for 30 s, and a final extension at 60°C for 15 s. Results are expressed as the ratios of the mean threshold cycle (Ct) values of experimental groups to those of the control group after normalization to β -actin.

Western Blot

Lung tissues were homogenized and 30 μ g of total protein was loaded on 8–12% SDS-PAGE gels. The proteins were electrotransferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were incubated with primary antibodies against Notch1 (1:1,000; Abcam, Cambridge, UK), NICD1 (1:500; Abcam), RBPj (1:1,000; Abcam), T-bet (1:1,000; Abcam), and GATA3 (1:1,000; Cell Signaling Technology, Boston, MA, USA) overnight at 4°C. It was then followed by incubation with horseradish peroxidase-conjugated secondary antibodies (Invitrogen, Carlsbad, CA, USA) for 1 h at RT. Reactions were visualized by enhanced chemiluminescence reagents (Pierce, Rockford, IL, USA) and the bands were scanned using Glyko BandsScan 5.0 software (Glyko, Novato, CA, USA). Results are expressed as the ratio of the mean band density of experimental groups to that of the control group after normalization to β -actin.

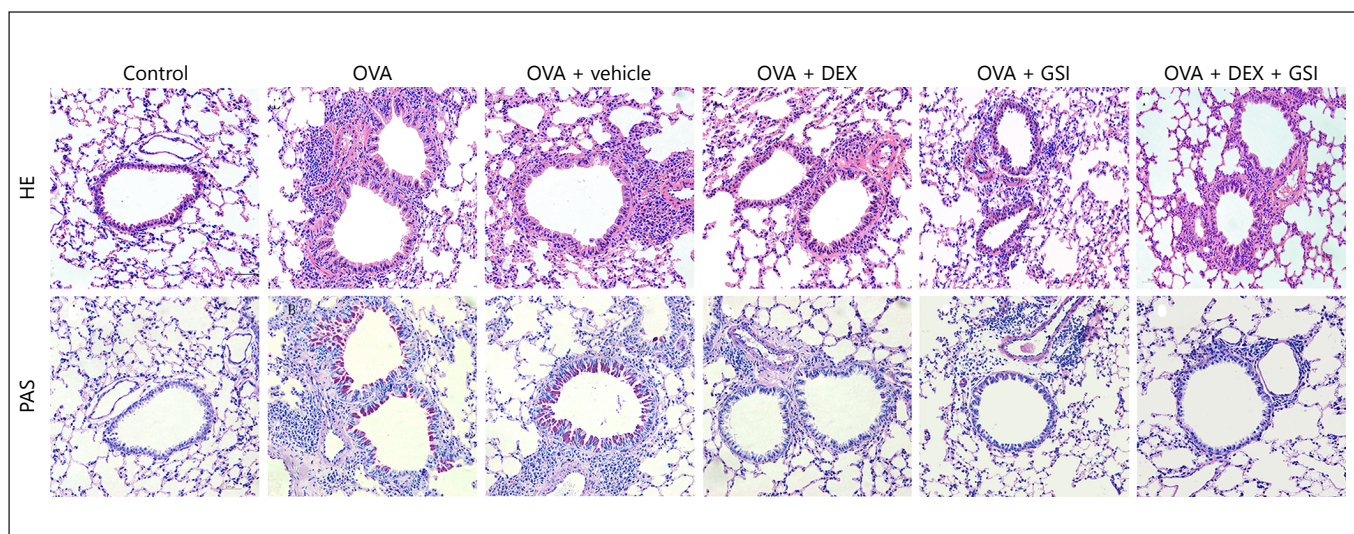


Fig. 2. DEX and/or GSI suppressed inflammatory cell infiltration and goblet cell metaplasia in lung tissue of OVA-sensitized mice. HE stain and periodic acid-Schiff staining. Original magnification. $\times 200$. Scale bar, 100 μm .

Flow Cytometry of Th1/Th2 Cells in Blood and Lungs

The peripheral blood and left lobe lung were used to prepare a single-cell suspension for flow cytometry. In brief, mononuclear cells were isolated from peripheral blood with Ficoll solution (Sigma-Aldrich) and from lungs by Percoll gradient centrifugation (GE Healthcare, Marlborough, MA, USA). To isolate lymphocytes, lungs were mildly cut with a razor blade and digested for 3 h at 37°C with collagenase IV (Sigma-Aldrich), and a single-cell suspension was prepared by forcing the digestion through a $70\text{-}\mu\text{m}$ nylon tissue strainer (BD Falcon, Bedford, MA, USA). The resultant suspension was washed twice and the cells were counted. Single-cell suspensions were then incubated with BV421 anti-mouse CD4 antibody (BD Biosciences, San Jose, CA, USA) at 4°C for 30 min. The cells were then fixed and permeabilized according to the manufacturer's instructions, and then stained with FITC anti-mouse IFN- γ (BD Biosciences) for Th1 detection and APC anti-mouse IL-4 (BD Biosciences) for Th2 detection. FACS analysis was performed using FACSCalibur and Cell Quest software (BD Biosciences). The fluorescence strength was represented by the mean value (mean fluorescence intensity) and the data were analyzed using FlowJo software version 10.0.7.

Immunofluorescence

Lung tissues were cut into sections ($10\text{ }\mu\text{m}$) with a cryostat (Leica, Wetzlar, Germany) at -20°C . Slides were rinsed in PBS 3 times at RT, and then incubated with blocking buffer (10% goat serum, 0.4% Triton-X100) for 30 min. The slides were incubated with primary antibodies CD3e (1:200, BD Biosciences), CD4 (1:200, BD Biosciences), and NICD1 (1:500, Abcam) overnight at 4°C . After washing the slides with PBS 3 times, they were incubated with secondary antibodies (Lianke, Hangzhou, China) at RT in the dark for 1 h. The images were acquired by confocal microscope (Nikon, Tokyo, Japan) at $\times 20$ magnification.

Statistical Analysis

Data were expressed as the mean $\pm$ SD (standard deviation) and analyzed using 1-way analysis of variance followed by the Duncan multiple range test using SPSS version 19.0 software. $p < 0.05$ was considered statistically significant.

Results

DEX and/or GSI Inhibit Th2 Cytokine Productions, but Increase Th1 Cytokine Levels in BALF and Peripheral Serum

As shown in Figure 1, OVA challenge significantly increased IL-4 and IL-13, but significantly decreased IL-2 and IFN- γ concentrations in BALF and serum ($p < 0.05$). In OVA-sensitized mice, DEX and/or GSI significantly decreased IL-4 and IL-13 levels in BALF and serum ($p < 0.05$). In contrast, IL-2 and IFN- γ levels were significantly increased in the OVA+DEX, OVA+GSI, and OVA+DEX+GSI groups compared to OVA-sensitized mice ($p < 0.05$).

Inhibitory Effects of DEX and/or GSI on Airway Inflammation, Airway Remodeling, and Airway Hyper-Responsiveness

The proportion of eosinophils and lymphocytes was significantly increased in the BALF of the OVA group (Fig. 1c). The elevated Raw to methacholine was observed. The Raw value in the OVA group was signifi-

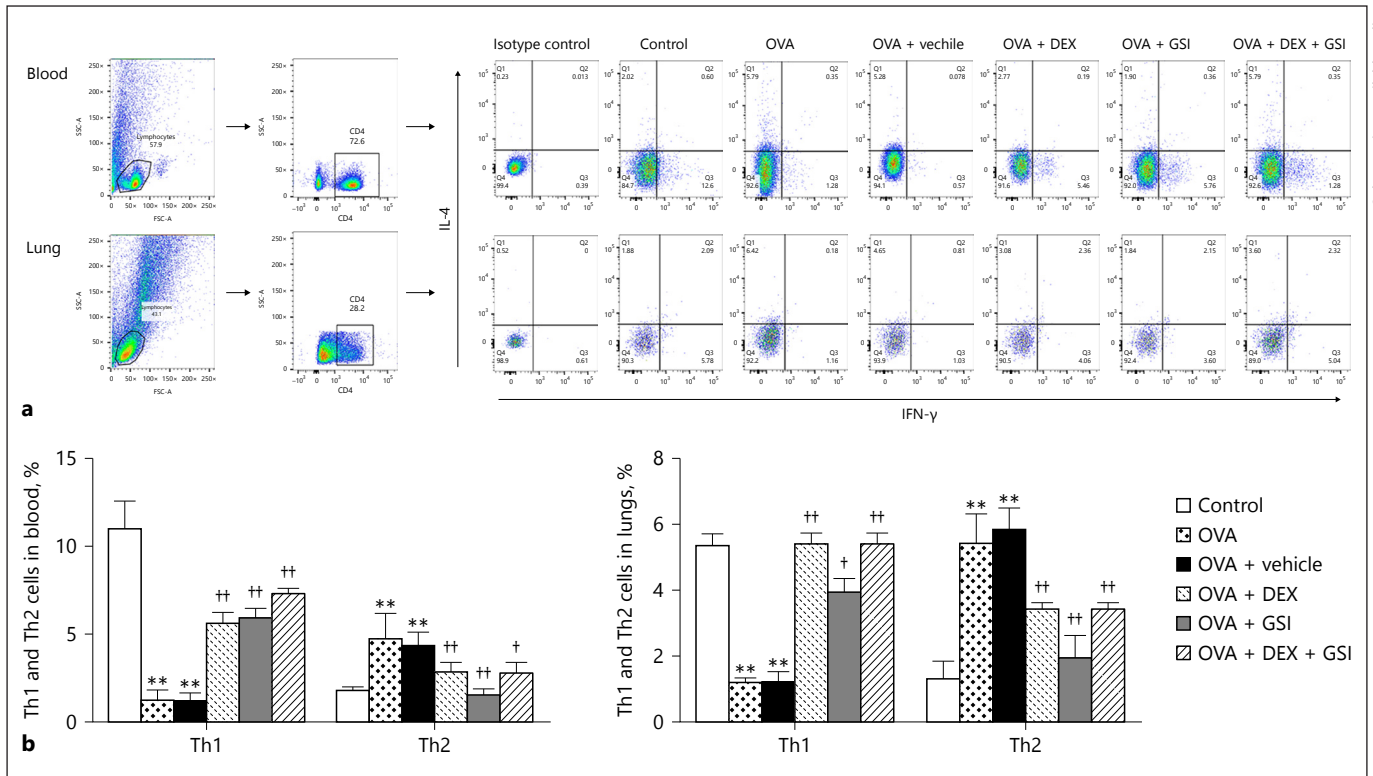


Fig. 3. DEX and/or GSI skew to Th1 from Th2 immunity in OVA-sensitized mice. **a** CD4+ T-cell subsets in the blood or lungs of different groups were tested by flow cytometric analysis. **b** Th1 and Th2 ratios. * $p < 0.05$, ** $p < 0.01$ versus the control group; + $p < 0.05$, ++ $p < 0.01$ versus the OVA or OVA+vehicle group. $n = 3$.

cantly increased compared to the control group when the concentration of methacholine reached 0.25 mg/mL (Fig. 1d). The infiltration of inflammatory cells was significantly increased in both the peribronchiolar and perivascular areas in OVA-sensitized mice (Fig. 2). Goblet cell metaplasia, elevated mucin production, and smooth muscle hypertrophy were also observed in the OVA group (Fig. 2). The application of DEX and/or GSI remarkably alleviated these pathological alterations of airway inflammation and airway remodeling in OVA-sensitized mice (Fig. 1, 2). Administration of DEX and GSI markedly attenuated the ratio of eosinophils and lymphocytes in BALF and Raw in OVA-sensitized mice.

Effects of DEX or/and GSI on the Percentages of Th1 and Th2 Cells in the Peripheral Blood and Lungs

As shown in Figure 3, the percentages of CD4+IFN- γ + cells (Th1 cells) in the peripheral blood and lung tissue of mice were markedly lower in the OVA group than those in the control group ($p < 0.01$), whereas the proportions

of Th1 cells in the OVA+DEX, OVA+GSI, and OVA+DEX+GSI groups were significantly higher than those in the OVA group ($p < 0.05$). The percentages of CD4+IL-4+ cells (Th2 cells) in the OVA group were notably higher than those in the control group ($p < 0.01$). The proportions of Th2 cells were significantly lower in the OVA+DEX, OVA+GSI, and OVA+DEX+GSI groups than in the OVA group in the peripheral blood and lung tissue ($p < 0.05$).

DEX and/or GSI Inhibit the Activation of Notch1 Signaling, Downregulate GATA3 Expressions, and Upregulate T-bet Levels in Lung Tissues

To investigate the involvement of Notch signaling in anti-inflammation, we assessed the protein levels of Jagged1 (Notch1 ligand) and the protein and mRNA expressions of the Notch1 pathway. The protein expressions of Jagged1, Notch1, NICD1, RBPj, and the mRNA expression of the *Notch1* gene were significantly elevated in the OVA group compared to the control group ($p < 0.05$; Fig. 4). The augmentation effects of OVA sensitization on

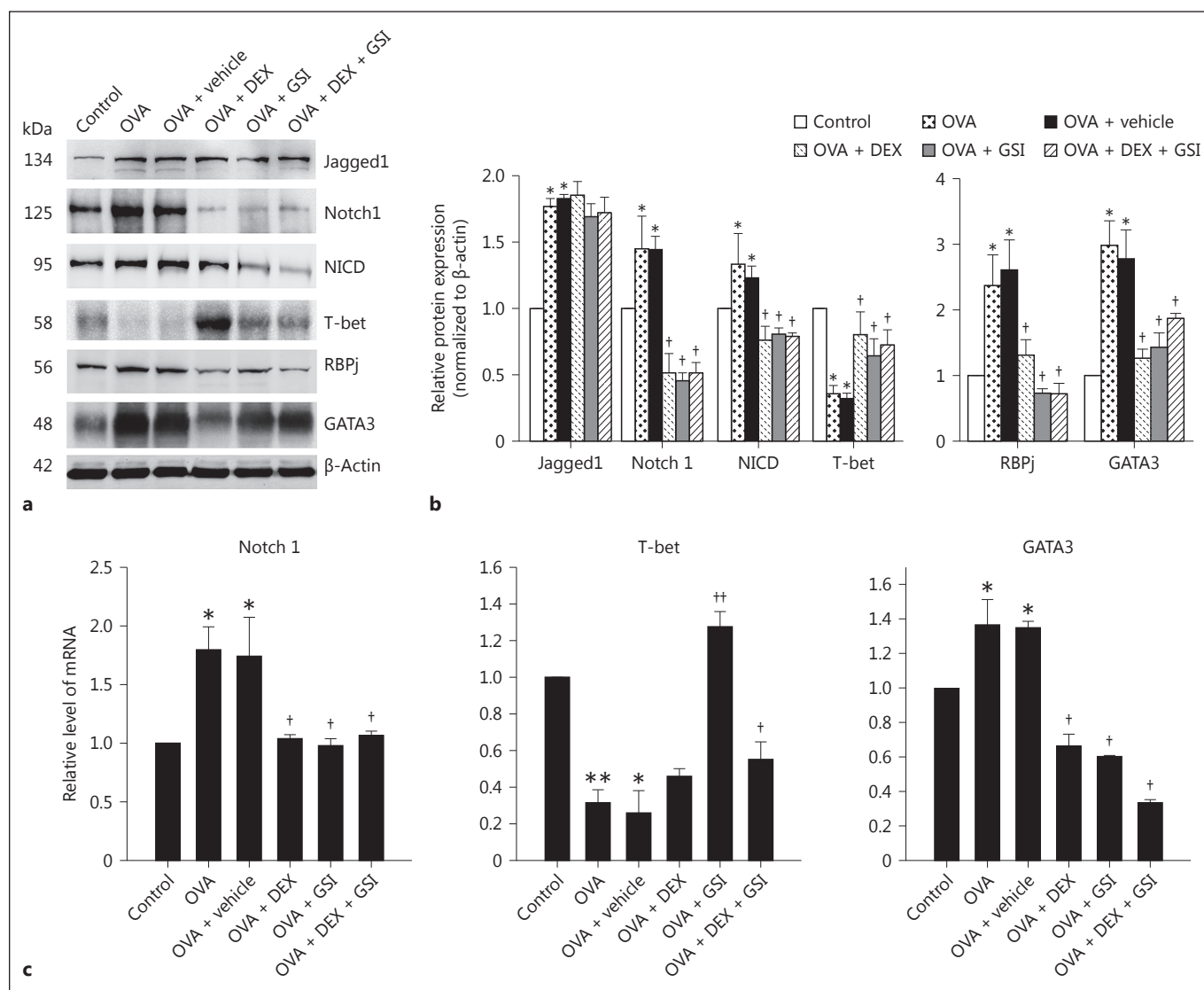


Fig. 4. DEX and/or GSI suppressed Notch1 signaling components in the lungs of OVA-sensitized mice. Jagged1, Notch1, NICD1, T-bet, RBPj, and GATA3 protein levels were measured by Western blot (a) while mRNA levels were measured by real-time PCR (c) in mice lungs. β -Actin was the internal control. The density ratios

of bands in Western blot (b) were calculated and represented as the relative expression level. Each value represents the mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$ versus the control group; † $p < 0.05$, †† $p < 0.01$ versus the OVA or OVA+vehicle group. $n = 5$.

Notch1, NICD1, and RBPj protein expressions and mRNA were significantly repressed by DEX and/or GSI treatment ($p < 0.05$).

To detect the potential mechanisms of DEX on Th1 and Th2 ratios, *T-bet* and *GATA3* mRNA and protein expressions in lung tissues were detected. *GATA3* mRNA and protein expressions were significantly upregulated, but *T-bet* mRNA and protein expressions were downregulated in the OVA group compared to the control group

($p < 0.05$; Fig. 4). *GATA3* protein and mRNA levels were significantly decreased, whereas *T-bet* protein levels were significantly increased in the OVA+DEX, OVA+GSI, and OVA+DEX+GSI groups compared to the OVA group ($p < 0.05$). In the OVA+DEX group, the incremental change in *T-bet* mRNA expression was marginal, but the *T-bet* mRNA levels were markedly elevated in OVA+GSI and OVA+DEX+GSI groups compared to the OVA group ($p < 0.05$).

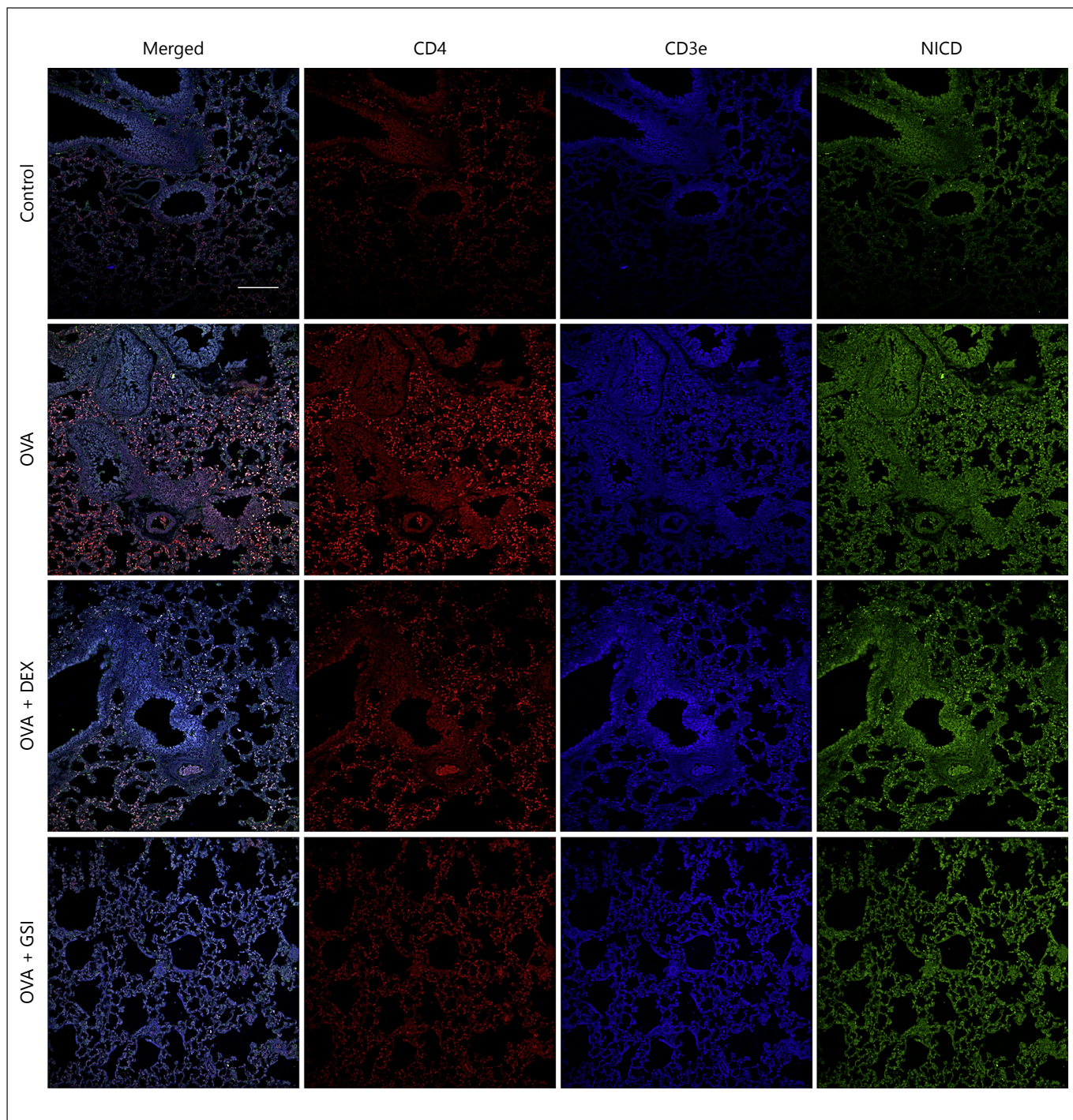


Fig. 5. Activation of Notch1 signaling in lung CD4+CD3e+ T lymphocytes of OVA-sensitized mice. Immunofluorescent staining of mice lung tissues shows that NICD1 is colocalized with CD4+CD3e+ T lymphocytes. The level of NICD1 was increased in the OVA group and decreased in the OVA+DEX and OVA+GSI groups. Scale bar, 100 μ m.

Activation of Notch1 Signaling in CD4+CD3e+ T Lymphocytes of Lung Tissues

Immunofluorescent staining of mice lung tissues showed that NICD1 staining (activation marker of Notch1 signaling) is colocalized with CD4+CD3e+ T lymphocytes (Fig. 5). NICD1 expression was increased in CD4+CD3e+ T lymphocytes in the OVA group compared to the control group. DEX or GSI treatment could reduce the level of NICD1.

Discussion

Asthma affects more than 300 million people worldwide and is one of the most common chronic diseases with well-defined pathological features, including airway inflammation, mucin hypersecretion, airway remodeling, and airway hyper-responsiveness [18]. The mechanisms of asthma are not fully elucidated, but it is commonly thought to be associated with Th1/Th2 imbalance. A mouse model of asthma was successfully established using OVA sensitization and challenge to mimic all the above mentioned pathological changes in asthma patients. This study observed a Th1/Th2 imbalance in OVA-sensitized mice, a decrease in Th1 concomitant with an increase in Th2.

We found an elevated Notch1 protein level in OVA mice. Antigen presentation cells (such as dendritic cells) could provide instructive signals for Th cells differentiation by expressing different Notch ligands upon stimulations [8, 19, 20]. However, the involvement of Notch in T-cell differentiation remains to be elucidated. This study showed that Jagged1 protein expressions were significantly increased in the lung of OVA-sensitized mice. NICD1 is the activation marker of Notch1 signaling. In this study, OVA stimulated Notch1 signaling in the CD4+ lymphocytes. Our results reinforce previous findings [3] that the increased RBPj expression paralleled to the increase in GATA3 and decrease in T-bet expression. RBPj and Notch1 are shown to bind to the GATA3 promoter [21] or the chromatin regions of Tbx21 (*T-bet* gene) [22], which implicate a physiological role of these molecules in the regulation of Th1 and Th2 balance. Therefore, we propose that allergens like OVA challenge could increase the expressions of Jagged1 on dendritic cells and Notch1 on CD4+ lymphocytes, which subsequently induce more Jagged1-Notch1 combinations and activate the Notch1 pathway to induce Th2 differentiation.

GSI is a specific inhibitor of the γ -secretase complex. In agreement with previous studies [3, 23], the blockade

of Notch1 signaling by GSI inhibited Th2 and stimulated Th1 cytokine production. Besides, GSI treatment might reduce allergic pulmonary inflammation by modulating Th1 and Th2 responses. Thus, Th2 cell differentiation and Th1/Th2 imbalance caused by Notch1 (NICD1)-RBPj signaling may play a role in asthma pathogenesis.

GCs are the most effective therapy for the treatment of asthma, while the associated molecular and cellular mechanisms are still unclear. Previous studies suggested that GCs could inhibit the recruitment of inflammatory cells, suppress the multiple inflammatory genes that are activated in asthma, and reverse the goblet-cell hyperplasia [24]. This hormone also exerts the potent anti-inflammatory effect partly through modulating the behavior and function of immune cells. In Th cells, GCs may restore Th1/Th2 balance by altering the secretion of various cytokines, which induce Th cell differentiation [5]. In our study, we confirmed that DEX decreased airway responsiveness and inflammation [25], and we also found that DEX could inhibit Th2 cytokine production and increase Th1 cytokine production in OVA-sensitized mice. A novel finding in our study is that DEX enhanced T-bet expression but inhibited GATA3 expression through the Notch1 (NICD1)-RBPj pathway, which in turn induced changes in the Th1 and Th2 phenotype. The effects of DEX are analogous to GSI in OVA mice. These findings raise the possibility that GCs might exert anti-inflammation and immunoregulatory effects, partly through the blockade of Notch1 signaling. However, the direct link between GCs and the Notch pathway needs further investigation.

This study found no positive collaborative effects between DEX and GSI. The reason for this is unclear, although similar observations were also reported [26]. Previous studies on GC resistance in T-cell acute lymphoblastic leukemia demonstrated that the combination therapy with GSI plus GC can improve the anti-leukemic effects of GSI and reduce their gut toxicity in vivo [27]. It implies that GSI may be a potent treatment to GC-resistant asthma. However, a complete understanding of the relationship between GCs and GSI still needs to be elucidated.

In conclusion, our study provided evidence for the first time that GCs as well as GSI might reverse the Th1/Th2 imbalance and reduce allergic pulmonary inflammation though inhibiting Notch1 signaling.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81400022, 81270080) and the Natural Science Foundation of Hunan Province (No. 2015JJ3170).

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

Z.L. and R.H. performed the experiments and prepared the figures; J.F. and Y.T. analyzed the data; X.H. and Y.Z. interpreted the results of the experiments; Z.L., C.H., and R.H. drafted the manuscript; R.H. was responsible for the conception and design of the research; C.H., R.H., and L.Q. edited and revised the manuscript, and R.H. approved the final version of the report.

References

- Long H: Esculetin attenuates Th2 and Th17 responses in an ovalbumin-induced asthmatic mouse model. *Inflammation* 2016;39:735–743.
- Zhou M, Cui ZL, Guo XJ, Ren LP, Yang M, Fan ZW, Han RC, Xu WG: Blockade of Notch signaling by γ -secretase inhibitor in lung T Cells of asthmatic mice affects T cell differentiation and pulmonary inflammation. *Inflammation* 2015;38:1281–1288.
- Kang JH, Kim BS, Uhm TG, Lee SH, Lee GR, Park CS, Chung IY: γ -Secretase inhibitor reduces allergic pulmonary inflammation by modulating Th1 and Th2 responses. *Am J Resp Crit Care* 2009;179:875–882.
- Effros RM, Nagaraj H: Asthma: new developments concerning immune mechanisms, diagnosis and treatment. *Curr Opin Pulm Med* 2007;13:37–43.
- Emmanuel O, Andrew CBC: Effects of glucocorticoids in the immune system; in Wang JC, Harris C (eds): *Glucocorticoid Signaling: From Molecule to Mice to Man*. New York, Springer, 2015, vol 872, pp 217–233.
- Zhang W, Nie Y, Chong L, Cai X, Zhang H, Lin B, Liang Y, Li C: PI3K and Notch signal pathways coordinately regulate the activation and proliferation of T lymphocytes in asthma. *Life Sci* 2013;92:890–895.
- Wu Q, Tang Y, Hu X, Wang Q, Lei W, Zhou L, Huang J: Regulation of Th1/Th2 balance through OX40/OX40L signaling by glycyrrhizic acid in a murine model of asthma. *Respirology* 2016;21:102–111.
- Amsen D, Antov A, Flavell RA: The different faces of Notch in T-helper-cell differentiation. *Nat Rev Immunol* 2009;9:116–124.
- Wakabayashi N, Chartoumpekis DV, Kensler TW: Crosstalk between Nrf2 and Notch signaling. *Free Radic Biol Med* 2015;88:158–167.
- Ayaz F, Osborne BA: Non-canonical Notch signaling in cancer and immunity. *Front Oncol* 2014;4:345.
- Maillard I, Fang T, Pear WS: Regulation of lymphoid development, differentiation, and function by the Notch pathway. *Annu Rev Immunol* 2005;23:945–974.
- Amsen D, Antov A, Jankovic D, Sher A, Radtke F, Souabni A, Busslinger M, McCright B, Gridley T, Flavell RA: Direct regulation of *Gata3* expression determines the T helper differentiation potential of Notch. *Immunity* 2007;27:89–99.
- Pei J, Tang Z, Zang G, Yu Y: Blockage of Notch1 signaling modulates the T-helper (Th)1/Th2 cell balance in chronic hepatitis B patients. *Hepatology* 2010;40:799–805.
- Guo XJ, Zhou M, Ren LP, Yang M, Huang SG, Xu WG: Small interfering RNA-mediated knockdown of Notch1 in lung. *Chin Med J* 2009;122:2647–2651.
- Wang N, Yan D, Liu Y, Liu Y, Gu X, Sun J, Long F, Jiang S: A HuR/TGF- β 1 feedback circuit regulates airway remodeling in airway smooth muscle cells. *Respir Res* 2016;17:117.
- Liu R, Bai J, Xu G, Xuan L, Zhang T, Meng A, Hou Q: Multi-allergen challenge stimulates steroid-resistant airway inflammation via NF- κ B-mediated IL-8 expression. *Inflammation* 2013;36:845–854.
- Wang J, Wu J, Kong L, Nurahmat M, Chen M, Luo Q, Li B, Wu X, Dong J: BuShenYiQi Formula strengthens Th1 response and suppresses Th2-Th17 responses in RSV-induced asthma exacerbated mice. *J Ethnopharmacol* 2014;154:131–147.
- Holgate ST, Wenzel S, Postma DS, Weiss ST, Renz H, Sly PD: Asthma. *Nat Rev Dis Primers* 2015;1:15025.
- Amsen D, Blander JM, Lee GR, Tanigaki K, Honjo T, Flavell RA: Instruction of distinct CD4 T helper cell fates by different Notch ligands on antigen-presenting cells. *Cell* 2004;117:515–526.
- Okamoto M, Matsuda H, Joetham A, Lucas JJ, Domenico J, Yasutomo K, Takeda K, Gelfand EW: Jagged1 on dendritic cells and Notch on CD4+ T cells initiate lung allergic responsiveness by inducing IL-4 production. *J Immunol* 2009;183:2995–3003.
- Amsen D, Antov A, Jankovic D, Sher A, Radtke F, Souabni A, Busslinger M, McCright B, Gridley T, Flavell RA: Direct regulation of *Gata3* expression determines the T helper differentiation potential of Notch. *Immunity* 2007;27:89–99.
- Minter LM, Turley DM, Das P, Shin HM, Joshi I, Lawlor RG, Cho OH, Palaga T, Gottipati S, Telfer JC, Kostura L, Fauq AH, Simpson K, Such KA, Miele L, Golde TE, Miller SD, Osborne BA: Inhibitors of gamma-secretase block in vivo and in vitro T helper type 1 polarization by preventing Notch upregulation of Tbx21. *Nat Immunol* 2005;6:680–688.
- Gu W, Xu W, Ding T, Guo X: Fringe controls Naïve CD4+T cells differentiation through modulating Notch signaling in asthmatic rat models. *PLoS One* 2012;7:e47288.
- Barnes PJ, Adcock IM: How do corticosteroids work in asthma? *Ann Intern Med* 2003;139:359–370.
- Ram A, Mabalirajan U, Jaiswal A, Rehman R, Singh VP, Ghosh B: Parabromophenacyl bromide inhibits subepithelial fibrosis by reducing TGF- β 1 in a chronic mouse model of allergic asthma. *Int Arch Allergy Immunol* 2015;167:110–118.
- Roderick JE, Gonzalez-Perez G, Kuksin CA, Dongre A, Roberts ER, Srinivasan J, Andrzejewski C Jr, Fauq AH, Golde TE, Miele L, Minter LM: Therapeutic targeting of NOTCH signaling ameliorates immune-mediated bone marrow failure of aplastic anemia. *J Exp Med* 2013;210:1311–1329.
- Real PJ, Tosello V, Palomero T, Castillo M, Hernandez E, de Stanchina E, Sulis ML, Barnes K, Sawai C, Homminga I, Meijerink J, Aifantis I, Basso G, Cordon-Cardo C, Ai W, Ferrando A: γ -Secretase inhibitors reverse glucocorticoid resistance in T cell acute lymphoblastic leukemia. *Nat Med* 2009;15:50–58.