Bronchial Epithelial Cells Promote the Differentiation of Th2 Lymphocytes in Airway Microenvironment through Jagged/Notch-1 Signaling after RSV Infection

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Keywords

Airway hyperresponsiveness · Human bronchial epithelial cells · RSV · T helper cells

Abstract

Background: The aim of this study was to investigate the role of Notch-1 signaling through Notch-1 ligands on bronchial epithelial cells (BECs) in regulating the development of T helper 2 (Th2) lymphocytes after RSV infection. **Methods:** Firstly, we analyzed the expression of cytokines and Notch-1 ligands in BECs by using real-time PCR. Then, RSV-infected BECs were co-cultured with CD4⁺ T cells in a transwell chamber for 48 h, and differentiation of T cells in the lower chamber was determined using flow cytometry and real-time PCR. JAG1 siRNA was then used to determine the effects of Jagged/Notch-1 signaling on the differentiation of Th2. An RSV-infected mouse model was also used to analyze the secretion of Th differentiation-associated cytokines in serum and lung tissues using ELISA, the histopathological changes using HE staining, and the expression of JAG1 and JAG2 in BECs. **Results:** The results showed that RSV promoted the expression of Th2-type cytokines and Jagged-1 and inhibited the expression of Jagged-2 in normal BECs. RSV-infected BECs in-

duced Th2 differentiation. In addition, JAG1 downregulation inhibited the differentiation of Th2 and promoted differentiation of Th1. In the RSV-infected mouse model, the RSV titer, inflammation decreased with time. IL-4 and IL-17 increased on day 28 and 60, while IFN γ increased on day 7 and 28. Moreover, the expression of Jagged-1 increased and that of Jagged-2 decreased in BECs, which was consistent with IL-4 production in lung tissues. **Conclusion:** Our data showed that BECs had the potential to promote the differentiation of Th2 lymphocytes through Jagged-1/Notch-1 signaling.

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Introduction

Respiratory syncytial virus (RSV) dramatically increases the risk of airway hyper-responsiveness (AHR) after an acute infection in infants and young children [1, 2]. A number of studies have demonstrated a relationship between infants with severe RSV infection and

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the subsequent development of asthma later in childhood. However, the mechanisms by which RSV may be involved in the development of post-bronchiolitis asthma and allergy are poorly understood. The airway epithelium is the first line of defense against inhaled allergens, viruses, and environmental pollutants through inflammatory, immune, and regenerative responses. It also plays a significant role in initiating pulmonary diseases, particularly in the case of RSV, since this virus replicates and persists only in the respiratory epithelium [3, 4]. The long persistence of virus may undermine the physiological homeostasis of airway microenvironment and lead to adaptive remodeling of airway ultra-structure through interaction with host proteins [5]. Our previous study revealed that bronchial epithelial cells (BECs) can act as antigen-presenting cells. In addition, RSV infection in the epithelium promoted the differentiation of T helper 2 (Th2) and Th17 lymphocytes [6–8]. However, the mechanisms remain unclear.

According to previous results, we observed that Notch-1 in the lungs increased after RSV infection (unpublished data). The Notch signaling pathway is a highly evolutionarily conserved cell signaling pathway in most multicellular organisms that regulates proliferation, differentiation, cell fate, progenitor, and stem-cell self-renewal in both embryonic and adult tissues. Reports indicate that it is involved in AHR [9]. Several studies have demonstrated that BECs can express all the ligands of Notch-1, which is correlated with the differentiation of Th lymphocytes [10]. In mammals, there are 4 Notch receptors (Notch 1–4) and 5 Notch ligands (Delta1, Delta3, Delta4, Jagged1, and Jagged2), which can differentially affect the Notch-expressing cells [11]. Previous studies that focused on dendritic cells in the lungs revealed that Delta or Jagged ligands expressed on dendritic cells may influence the ability of naive CD4⁺ T cells to differentiate into Th1 or Th2 lineages [12]. However, it is likely that BECs also have an important role in Th cell differentiation or transdifferentiation, since all ligands of Notch-1 can be detected in the BECs.

It is therefore necessary to understand whether Notch-1, through different ligands on BECs, have distinct roles in regulating Th development. To address this question, we analyzed the expression of Notch-1 ligands in BECs and observed their effects in Th cell differentiation in this study. A mouse model was also established to analyze the correlation of JAG1 and Th differentiation.

Materials and Methods

Culture and Infection of RSV

The RSV strain A2 was expanded in Hela cells supplemented with 5% fetal bovine serum (FBS). The infective process was allowed to proceed until syncytia were observed. The cells were subjected to three successive freeze-thaw cycles followed by re-suspension in fresh medium. The RSV was further purified by centrifugation at 2,500 g for 10 min at 4 °C and filtered through a 0.22-μm filter. The virus titer was determined by a plaque assay. 60–70% of the confluent BECs were infected with RSV at multiplicity of infection (MOI) of 0.1. The virus was allowed to incubate for 2 h at 37 °C in serum-free DMEM. Thereafter, the nonabsorbed virus was removed and washed twice. The cells were then cultured in fresh medium with 2% FBS and subcultured as needed. RSV infection was verified by immunofluorescence with a polyclonal rabbit anti-NS1 antibody (Santa Cruz Biotechnology, TX, USA) as a primary antibody and Cy3-conjugated secondary antibody (Santa Cruz Biotechnology, TX, USA).

Separation of CD4⁺ T Cells

Heparinized blood was obtained from the blood bank in Xiangya Hospital, Central South University. The blood samples were from healthy male adults of approximately similar age and who did not smoke. RSV antibody was negative in the samples. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Conray density gradient liquid (Haoyang Company, Tianjin, China). Subsequently, PBMCs were incubated in complete medium at 37 °C in 5% CO₂ for 2 h until the monocytes adhered to the bottom of culture flasks. The lymphocytes suspended in the medium were isolated using a CD4⁺ T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) by positive selection of CD45RA⁺CD4⁺ T cells and negative selection of CD45RO⁺CD4⁺ T cells. Cells were cultivated at 37 °C in 10% human AB plasma-containing RPMI 1640 medium, supplemented with penicillin-streptomycin. A total of 5 × 10⁵ CD45RA⁺CD4⁺ T cells were cultured in 96-well plates along with 1 × 10⁵ beads coated with 5 μg/mL of anti-CD3 and anti-CD28, according to the manufacturer's instructions.

Co-Culture of CD4⁺ T Cells with RSV-Infected BECs

Transwell chambers with 24-well inserts were used in the co-culture assay. RSV-infected BECs (10⁵ cells) with or without JAG1 RNAi were plated in the top chamber and incubated for 24 h. CD4⁺ T cells were placed in the lower chamber at a 1:1 ratio with BECs. After 48 h of incubation, lymphocytes were collected and assayed by flow cytometry.

Flow Cytometry Analysis

To detect the Th cell differentiation, the ratio of CD4⁺IFNγ⁺ cell (Th1), CD4⁺IL-4⁺ cell (Th2), CD4⁺IL-17⁺ cell (Th17), and CD25⁺Foxp3⁺ cell (Treg cells) were analyzed by flow cytometry analysis. Lymphocytes were then pretreated with 2 mL of monensin (BioLegend, CA, USA) for 6 h and then incubated for 30 min at 4 °C with FITC-anti-CD4, PE-conjugated anti-IFNγ, IL-4, IL-17, Alexa Fluor 647 anti-Foxp3, and PE-anti-CD25 mAbs (BD Biosciences, CA, USA). The cells were subsequently washed twice and resuspended in PBS containing 1% FBS and 0.1% NaN₃ (Sigma-Aldrich, Beijing, China) and immediately analyzed with FACS (Becton Dickinson, NY, USA). Isotype-matched antibodies were used as controls.

Real-Time PCR

The five Notch-1 ligands including Jagged-1, Jagged-2, DLL1, DLL3, and DLL4 on BECs and transcription factors of Th differentiation including T-bet (Th1), GATA-3 (Th2), ROR λ (Th17), and Foxp3 (Treg cells) in Th cells were assayed by real-time PCR. Following the manufacturer's guidelines, RNA was isolated from BECs in the top chamber or CD4⁺ T cells in the lower chamber using TRIZOL[®] Reagent (Invitrogen, CA, USA). Afterwards, the RNA was incubated at 37 °C for 30 min in DNase I solution to remove the contaminating DNA. The RNA was subsequently treated using RNeasy[®] MinElute[™] Cleanup Kit (Qiagen, MA, USA). RNA yield and quality were assayed by detecting the ratio of A260 to A280 values and denaturing agarose gel electrophoresis. After the first strand cDNA synthesis using SuperScript III Reverse Transcriptase, real-time PCR was performed by SYBR Green Real-Time PCR Kit (Takara, Beijing, China) at 95 °C for 10 min, then at 95 °C for 15 s, and at 60 °C for 1 min for 35 amplification cycles. The Δ Ct (average Ct – average Ct of Homo-18S rRNA) and $\Delta\Delta$ Ct [$\Delta\Delta$ Ct = Δ Ct (experimental) – Δ Ct (control)] were calculated. The relative expression was calculated from experiment to control as $2^{-\Delta\Delta$ Ct} [13]. The following primers were used in the assays: Jagged-1: 5-GCTACTACTGCGACTGTCTTCCCG-3 and 5-AGATACAGCGATAACCATTAACCAAA-3 (121 bp); Jagged-2: 5-CGCCCCTAACAACCGTCTC-3 and CAATAACTGTGCGCCGCTCAC (330 bp); DLL1: 5-GGACTCGGGCTGTCAACTTC-3 and AGTCTTGCCATCTCACTTCCATTT (118 bp); DLL3: 5-CAGGTGCGGATGGAGGAGAD-3 and GGGCTTGAGGAGGTCACGAT (150 bp); DLL4: 5-GACAGCCCCGAAAGACAGATAGG-3 and 5-GTGGGTCAGAACTGGTTATTGGA-3 (229 bp); T-bet: 5-ACTGCTCGAACTCAGCCTCAT-3 and 5-ACCCAGTTCATTGCCGTGAC-3 (316 bp); GATA-3, 5-GCTTTGAACAAATGATTCGCTA-3 and 5-AGGATGCCAAGAAGTTTAAGGAATAT-3 (203 bp); ROR λ : 5-TCTTCTCTCAAGTATTGGCAGGTTT-3 and 5-TCTTCCCTACTGTTCGTTCCACCA-3 (119 bp); Foxp3, 5-GTTGAGTGAGGGACAGGATTG-3 and 5-AAGGAAAGGAGGATGGACGAAC-3 (381 bp).

RNAi

JAG1 siRNA and control siRNA were purchased from ThermoFisher (Shanghai, China). Cells were seeded into 6-well plates at 10⁶ cells per well. 90% of the confluent monolayer cultures were incubated with a final concentration of 10 nM, siRNA, or negative control using Lipofectamine 2000 for 24 h. Subsequently, the medium was replaced by a fresh medium and co-cultured with CD4⁺ T cells for 48 h.

Mouse Models

One-week-old male C57 mice weighing 8–10 g from the same mother were obtained from the experimental animal center, Central South University, and maintained in a pathogen-free environment. The mice were randomly divided into control group ($n = 20$) and RSV group ($n = 20$) by random number table method. All the procedures were reviewed and approved by the Institutional Animal Care and Use Committee. The virus titer in the inoculum was determined by plaque assay on the same day as the inoculation of mice. Mice were made to inhale 5% halothane before 50 μ L of PBS containing 10⁶ pfu of RSV was intranasally inoculated. Control mice received placebo inoculums. The mice were studied on day 7, 28, and 60 with 6 mice from each group after being anesthetized with a lethal dose of chloral hydrate. RSV infection was assayed by real-time PCR and immunohistochemistry.

Immunohistochemistry

Mice were perfused with PBS after sacrifice, followed by perfusion fixation with 4% paraformaldehyde diluted in 0.1 M PBS for 4 h. The left lung was then removed, rinsed with 0.9% sodium chloride solution, and fixed in 10% formaldehyde solution for 24 h ahead of paraffin sectioning. Following routine deparaffin, hydration, tissue antigen retrieval, endogenous peroxidase inactivation, and nonspecific staining blockage, rabbit anti-JAG1 or JAG2 polyclonal antibody (Santa Cruz Biotechnology, TX, USA) was added and incubated overnight at 4 °C. After thorough rinsing, the slides were incubated with biotinylated universal secondary antibody and then horseradish peroxidase-labelled streptomycin-avidin in turn. The slides were colored with DAB staining solution for 15 min and then observed under a high-power microscope. The integral optical density values of JAG1 and JAG2 protein in BECs were measured by CMIAS-8 color pathological image analysis system.

Western Blot Analysis

Lysates of BECs or lung tissues (75 μ g) were quantified by A280 (Eppendorf, Hamburg, Germany) and separated on 10% SDS-PAGE and then transferred to polyvinylidene fluoride membrane (Millipore, MA, USA). Membranes were blocked with 5% skim milk and incubated with rabbit anti-human Notch-1 and CSL (Cell Signaling Technology Inc., MA, USA) at 4 °C for the entire night. After being washed, membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:4,000; ProteinTech Group Inc., IL, USA) for 1 h at room temperature. Antibody-antigen complexes were then detected using an ECL chemiluminescent detection system (Gene Co., Ltd., Hong Kong, China). GAPDH was used as a loading control. A densitometry analysis was performed using Alpha-Ease software version 2200.

Cytokine Measurement

The eyeball blood was extracted from mice, left to stand for 20 min, and then centrifuged at 400 g/min for 15 min to obtain serum. The total protein from lung tissues was extracted according to the instructions. Mouse IFN γ , IL-4, and IL-17 concentrations in serum and lung tissues were determined using ELISA kits from R&D Systems (USA).

Statistical Analysis

Statistical analysis was done using SPSS software 16. All values were expressed as mean $\pm$ SE. Means were compared by the use of one-way analysis of variance (ANOVA) and Bonferroni post-hoc analysis between two groups. A p value <0.05 was considered significant.

Results

RSV Promoted Th2-Type Cytokines Secretion from BECs

RSV infection was confirmed by observing cytopathic effects and indirect immunofluorescence at 24 and 48 h after infection (MOI = 0.1). Red fluorescence was noted within the cells (Fig. 1a). Previous reports [14–17] showed thymic stromal lymphopoietin (TSLP), granulocyte-

Fig. 1. Effects of RSV on the expression of cytokines and Notch-1 ligands in BECs. **a** RSV infection was assayed by indirect immunofluorescence. **b** Effects of epithelium-derived cytokines on the differentiation of Th. **c** The expression levels of cytokines from

BECs were determined by real-time PCR. **d** The expression levels of Notch-1 ligands were determined by real-time PCR. Results are mean values $\pm$ SD of 6 independent experiments. * $p < 0.05$ and ** $p < 0.01$ vs. zero. Zero represents normal control.

macrophage colony stimulating factor (CSF2), IL-25, and IL-33 produced by epithelial cells were crucial for Th2 differentiation. Epithelium-derived CSF2, IL-6, and TGF β promote Th17 differentiation mainly through activation dendritic cells. TGF β promotes Treg differentiation, but IL-6 inhibits Treg differentiation. IL-12 promotes Th1 differentiation (Fig. 1b). Therefore, the above cytokine expressions from RSV-infected BECs were assayed by real-time PCR. The results showed that RSV promoted the expression of CSF2 and TSLP (Fig. 1c).

The expression of the five ligands of Notch-1 in BECs was determined. Results of real-time PCR showed that RSV promoted the expression of JAG1 at 24 and 48 h but inhibited the expression of Jagged 2 at 48 h (Fig. 1d).

RSV Promoted Th2 Differentiation through JAG1/Notch-1 Signaling

BECs, with or without RSV infection, were cultured and subsequently co-cultured with CD4 $^{+}$ T cells. The results of flow cytometry analysis showed that RSV promoted the differentiation of Th2 cells (Fig. 2a). Furthermore, the mRNA expression of GATA-3 also increased significantly after RSV infection (Fig. 2b).

To understand the effects of JAG1 on the differentiation of Th cells, the BECs were cultured for 48 h after pre-treatment with JAG1 RNAi or control RNAi. The inhibitory effects of the RNAi to JAG1 were verified by real-time PCR (Fig. 3a). The BECs were then infected with RSV for 24 h and co-cultured with CD4 $^{+}$ T cells for an

Fig. 2. Differentiation of Th cells after co-culture with BECs was assayed by flow cytometry (a) and real-time PCR (b). RSV-infected BECs promoted the differentiation of Th2 cells. The results were

from 6 independent experiments. * $p < 0.05$ and ** $p < 0.01$ vs. zero. Zero represents normal control; T represents Th cells without co-culture with BECs.

additional 48 h. Using real-time PCR, the study revealed that JAG1 RNAi inhibited the expression of GATA3 mRNA (Th2 cells) and promoted the expression of T-bet mRNA (Th1 cells) (Fig. 3b).

Activation of Notch-1 in Th cells was detected and found to decrease significantly after co-culture with JAG1 RNAi-treated BECs when compared with that of the control group ($p < 0.05$, Fig. 3c). However, the DNA-binding protein CSL of Notch-1 also decreased significantly after co-culture with JAG1 RNAi-treated BECs ($p < 0.05$, Fig. 3d).

RSV Infection Significantly Induced Airway Pathological Changes

The expression of RSV in lung tissue was very strong on the 7th day of RSV infection, although the expression gradually decreased with time. On the 60th day after infection, the expression of RSV in lung tissue was the lowest

(Fig. 4a). The pathological changes in the lung included significant epithelial denudation and inflammatory infiltration which were noted to decrease with time in the RSV group (Fig. 4b). The influence of RSV on the cytokines in lung tissues was found to be greater than in serum. It was discovered that the three cytokines in the RSV group were not significantly different from those in the control group in serum (Fig. 4c). However, in the lung tissues, IL-4 increased significantly on the 28th and 60th day after infection, IL-17 was significantly higher on the 28th and 60th day after infection, while IFN γ was significantly higher on the 7th and 28th day after infection ($p < 0.05$) (Fig. 4d).

Expression of JAG1 and JAG2 in Lung Tissues

The expression levels of JAG1 and JAG2 in BECs were examined using immunohistochemical staining 7, 28, and 60 days after infection. Although JAG1 expression de-

Fig. 3. The effects of JAG1 RNAi on the differentiation of Th cells and the activation of Notch-1. **a** BECs were incubated with a final concentration of 10 nM, siRNA, or control RNAi using Lipofectamine 2000 for 24 h. The inhibitory effects of RNAi on JAG1 were verified by real-time PCR. **b** Subsequently, the JAG1 RNAi-treated BECs were co-cultured with CD4⁺ T cells for 48 h. The ef-

fects of JAG1 RNAi on the differentiation of Th cells were assayed by real-time PCR. The expression of Notch-1 (**c**) and CSL (**d**) in CD4⁺ T cells was assayed by Western blot. The results were from 6 independent experiments. * $p < 0.05$ and ** $p < 0.01$ vs. control RNAi.

creased with time in control groups, when compared with the control group, the expression of JAG1 increased significantly in BECs at 28 and 60 days after infection (Fig. 5a). On the contrary, the expression of JAG2 in BECs was significantly decreased on day 60 after infection (Fig. 5b).

Discussion

The respiratory tract was covered with a continuous sheet of epithelial cells which is critical for preserving airway patency and defending against inhaled pathogens and allergens. The first line of defense was initiated when BECs were stimulated by pathogens and allergens through the induction of various inflammatory cytokines and che-

mokines. Chronic and repetitive exposure of BECs to stimuli led to airway remodeling and AHR. The development of AHR is one of the defining features of asthma. It is associated with dysfunction of BECs. A large number of clinical and basic studies have verified that RSV, which replicates and persists only in the epithelium, is an important risk factor for subsequent AHR.

Previous reports have shown that epithelium-derived CSF2, TSLP, IL-25, and IL-33 are crucial for Th2 differentiation [14]. Epithelium-derived CSF2, IL-6, and TGFβ promote Th17 differentiation mainly through activation of dendritic cells [15]. TGFβ promotes Treg differentiation, but IL-6 inhibits Treg differentiation [16]. IL-12 promotes Th1 differentiation [17]. The lung epithelium of asthmatic patients contains a higher number of TSLP⁺ cells. More-

Fig. 5. The expression levels of JAG1 (a) and JAG2 (b) in BECs were assayed by immunohistochemistry. The integral optical density (IOD) values of JAG1 and JAG2 protein in BECs were mea-

sured by CMIAS-8 color pathological image analysis system. The results were from 6 mice. * $p < 0.05$ and ** $p < 0.01$ vs. control.

Fig. 4. RSV infection significantly induced airway pathological changes. Mice weighing 8–10 g from the same mother were randomly divided into control and RSV group and made to inhale 5% halothane before 50 μ L of PBS containing 10^6 pfu of RSV was intranasally inoculated. Control mice received placebo inoculums. The mice were studied on day 7, 28, and 60 with 6 mice from each group. **a** RSV infection on day 7, 28, and 60 was assayed by real-time PCR and immunohistochemistry. **b** The pathological changes in the lung were examined by HE staining. **c** The production of cytokines in serum was assayed by ELISA. **d** The production of cytokines in lung tissues was assayed by ELISA. IL-4 increased significantly on the 28th and 60th day; IL-17 was significantly higher on the 28th and 60th day, while IFN γ was significantly higher on the 7th and 28th day after infection in the lung tissues. * $p < 0.05$ vs. control.

over, the bronchoalveolar lavage samples of these patients have higher concentrations of TSLP protein compared to those of healthy individuals [18]. Higher concentration of TSLP mRNA expression in the lung epithelium is crucial for development of allergic diseases such as asthma [19]. A polymorphism has been identified in the promoter of IL33R which predisposes to a greater risk of developing asthma [20]. IL-25 in epithelial cells is a derived cytokine in Th2-high corticosteroid-responsive asthma [21]. In the current study, we found that CSF2 and TSLP increased significantly. The other cytokines showed no changes 48 h after RSV infection, indicating RSV-infected BECs have the potential to drive the differentiation of Th2 cells.

The epithelium also plays a critical role in the development and function of the T cells. Five membrane-bound ligands of Notch-1 are commonly expressed on BECs and are closely associated with the development of Th cells [22]. The present study reveals that BECs can express all five ligands of Notch-1. In addition, RSV promoted the expression of JAG1 and inhibited the expression of JAG2 in BECs 48 h after RSV infection. Further, JAG1 silencing inhibited the differentiation of Th2 cells and promoted the differentiation of Th1 cells. Consistent with the previous reports, JAG1 promoted Th2 differentiation on dendritic cells [23]. The Th1/Th2 balance plays an important role in the AHR. Th2 inhibits the differentiation of Th1. When Th2 is suppressed, Th1 begins to express itself in large quantities. So, we posit that the increased differentiation of Th1 cells may be a feedback mechanism due to decreased differentiation of Th2 cells. Previous reports also show that JAG2 inhibits the differentiation of B cells [24], indicating that decreased JAG2 promoted the differentiation of B cells and subsequent antibody production.

A mouse model was also used to analyze the correlation of JAG1 and Th differentiation. During acute infection, infants may have extensive and prolonged epithelial damage and denudation. However, on the mouse model after RSV infection, we found epithelial denudation and inflammatory infiltration after 7 days and no significant morphological changes on day 28 and 60. There were no significant changes in cytokines in serum. However, in the lung tissues, on day 7 and 28, the main differentiation was Th1 and, on day 28 and 60, Th2 and Th17. Further, we verified that increased JAG1 and decreased JAG2 were found in BECs. Studies on animal models and humans have shown that simple RSV infection induces Th1 response [25], which may be related to the increase of Th1 on 7 and 28 days after RSV infection. Compared with the control group, JAG1 increased significantly on 28 and 60 days after RSV infection, which was consistent with the expression of IL-4 in the lung and may be related to the imbalance of Th1/Th2 and the induction of Th2 differentiation in the later stage. The increased expression of IL-17 on 28 and 60 days after RSV infection may be related to Th17 differentiation, which may be related to the activation of dendritic cells in the lungs. These indicate that the JAG1 on BECs is a strong factor that drives Th2 differentiation. In addition, we found RSV maintained JAG1 expression, while in control animals it was highly diminished after 60 days, indicating that JAG1 expression was closely related to the age of mice.

There are two potential limitations to the current study. First, in vivo experiments are more complex because various antigen-presenting cells can express

Notch-1 ligands, especially the dendritic cells. Therefore, the effects of Notch-1 ligands on differentiation of Th lymphocytes should be further studied through additional knock-out model for Notch-1 ligands. Second, many scholars believe that the BECs are not the main antigen-presenting cells and that naïve T cells commonly do not reach the BECs. However, BECs are some of the important effectors and are often in contact with various lymphocytes, indicating that BECs may be important in regulation of Th differentiation or transdifferentiation. Hence, comprehensive studies should be conducted to elucidate the role played by epithelial cells and other antigen-presenting cells in T lymphocyte differentiation.

In summary, activation of JAG1 in BECs promoted the differentiation of Th2 cells, which may be associated with the pathogenesis of RSV causing AHR.

Statement of Ethics

The study was approved by the Institutional Ethics Committee of Central South University.

Disclosure Statement

The authors declare no competing interests.

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