

Dexamethasone Decreases IL-29 Expression in House Dust Mite-stimulated Human Bronchial Epithelial Cells

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Summary: The aim of this study was to explore the effect of IL-29 on the progression of airway allergic disease by detecting the level of IL-29 in airway allergic cell models stimulated by house dust mite (HDM) in the presence or absence of dexamethasone (DEX). The same batch of human bronchial epithelial cells in exponential growth phase was randomly divided into five groups: blank group (A), 300 ng/mL HDM group (B), 1000 ng/mL HDM group (C), 3000 ng/mL HDM group (D), and 300 ng/mL HDM+100 ng/mL DEX group (E). The IL-29 mRNA expression was detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR). The IL-29 protein expression in cell suspension was detected by ELISA. The results showed that after stimulation with HDM for 24 h, the expression of IL-29 was increased significantly, and after co-stimulation with HDM and DEX for 24 h, the expression of IL-29 in group E was significantly lower than that in the groups stimulated by HDM alone but higher than that in the group A. The differences between the different groups were significant ($F=132.957$, $P<0.01$). Additionally, the higher the concentration of HDM was, the more significant the increase in the IL-29 expression was. In conclusion, IL-29 may play a role in the progression of airway allergic disease including asthma.

Key words: IL-29; dexamethasone

Airway allergy is one of the most important pathogenesis of respiratory disease, including asthma. Furthermore, house dust mite (HDM) is a significant causative factor of airway allergy.

Recent extensive research has shown that anti-tumor effects, anti-viral activity, antiproliferative effect and modulation of immune cell functions are the main biological effects of IL-29. Some reports showed IL-29 expression in monocytes and dendritic cells (DCs) following stimulation with bacterial lipopolysaccharide (LPS), supporting production of this cytokine upon bacterial infections as well^[1,2].

Studies also demonstrated that IL-29 might modulate the Th1/Th2 response and cytokine secretion^[3]. Other studies demonstrated that IL-29 can be generated by peripheral blood mononuclear cells and monocyte-derived DCs, when stimulated by LPS or Toll-like receptor (TLR)^[2,4].

Bronchial epithelial cells were found to generate significant levels of IL-29 in response to HDM, and the molecular mechanisms underlying this response to allergy-inducing organisms have been recently proposed to lie in the triggering of TLR4^[5,6]. Significant differences were found in IL-29 mRNA levels in sputum between healthy and asthmatic adult patients, and the data indicated that in the absence of anti-inflammatory treatment, the induction of IL-29 is related to asthma symptoms and IL-29 could therefore play an immunoprotective role^[7]. In fact, researchers found the highest IL-29R levels in

organs of the gastrointestinal and respiratory systems, in the heart, and in several glands, whereas the brain had the lowest expression^[8].

Hence, we hypothesized that IL-29 may play a role in the progression of airway allergic diseases. However, its action mechanism is not yet known. In this study, we explored the effect of IL-29 on the progression of airway allergic diseases by measuring its expression in airway allergic cell models before and after treatment with dexamethasone (DEX).

1 MATERIALS AND METHODS

1.1 Cell Cultures

Primary human bronchial epithelial cells (HBECs) initially derived from a healthy adult volunteer were kept frozen in liquid nitrogen. These cells were rapidly thawed and cultured in RPMI-1640 medium (Gibco-Invitrogen, USA) supplemented with 10% fetal calf serum (Gibco, USA) at saturated humidity in a mixture of 5% CO₂ and air at 37°C. The medium was replaced daily. HBECs were plated in 6-well plates until 90% confluence, followed by the stimulation with HDM/DEX for 24 h.

1.2 Preparation of Der p Solution

Freeze-dried HDM allergen extract (ALK, Hørsholm, Denmark), containing several Der p subgroups, was reconstituted in the specified diluent according to the manufacturer's instructions. This resulted in a stock solution at a concentration of 9000 ng/mL Der p, which was further diluted with Medium 199 (Hyclone Europe Ltd., UK), to provide a working Der p solution at a con-

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centration of 300 ng/mL. This concentration was chosen since it was approximately one-tenth the concentration deemed to pose a risk of sensitization *in vivo*^[9]. For dose response studies solutions at concentrations of 1000 and 3000 ng/mL Der p were also prepared.

1.3 Treatment with HDM and DEX

Commercially available natural Der p I was purchased from Indoor Biotechnologies (USA). A 30-min pre-activation of Der p I with 5 mmol/L cysteine HCl, pH 7.0, at 37°C was always performed before use in order to activate its thiol group^[10,11].

The cells in groups B, C and D were exposed to HDM at three different concentrations (300, 1000, and 3000 ng/mL) to examine the effect of HDM on IL-29 secretion. To examine the effect of DEX on IL-29 release induced by HDM, we exposed the cells of group E to HDM (300 ng/mL) in combination with 100 ng/mL DEX. After 24-h incubation, supernatants were clarified by centrifugation at 3000 g for 10 min at 4°C and were collected and frozen at -80°C for further analyses. The cells were frozen at -80°C for further RNA analyses.

The control samples were incubated for the same duration in the same medium, without the addition of DEX or HDM.

1.4 Real-time Polymerase Chain Reaction Analysis of IL-29 Expression in HBECs

Total cellular RNA was extracted using an RNA TRIZol kit (Sangon Biotech, China), and its concentration was spectrophotometrically measured. The RNA was reverse-transcribed to complementary DNA, using Moloney murine leukemia virus reverse transcriptase (M-MLV-RT) and oligo-dT (12–18) primers (Sangon Biotech, China).

Reverse transcription was primed with oligo (dT) in a volume of 20 µL. Real-time polymerase chain reaction (PCR) was performed using a fluorescent quantitation PCR instrument (Type ECO, USA).

The final volume (20 µL) of PCR reaction mixture contained 2× UltraSYBR mixture (*Taq*DNA Polymerase and dNTPs) 10 µL, 10 µmol/L forward primer 0.4 µL, 10 µmol/L reverse primer 0.4 µL, template DNA 0.8 µL, and RNase-free water 8.4 µL. After 10 min of DNA polymerase activation at 95°C, 40 cycles of amplification were performed. Each PCR cycle consisted of denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and the final melting curve program consisting of 95°C for 15 s, 61°C for 55 s, 95°C for 15 s. Amplification specificity of PCR products was confirmed by melting curve analysis.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression was used as the endogenous control for normalization. Primer sequences were as follows: GAPDH (forward, 5'-GTCAGTGGTGGACCTGACCT-3'; reverse, 5'-TGCTGTA GCCAAAT TCGTTG-3'); IL-29 (forward: 5'-TATCCAGCCTCAGCCACA-3', and reverse: 5'-TCAGACACAGGTTCCCATCG-3').

Relative quantification of mRNA was performed using a comparative cycles threshold (CT) method.

1.5 IL-29 Protein Detection

Culture supernatants were collected for ELISA. IL-29 protein levels were determined using human IL-29 ELISA kits according to the manufacturer's protocols. The absorbance (*A*) values of IL-29 were assayed at a wavelength of 450 nm.

1.6 Statistical Analysis

Multiple comparisons were performed by one-way ANOVA with LSD's correction. *P*<0.05 was considered as significant.

2 RESULTS

There were no significant changes in HBECs after stimulation with HDM or DEX under a light microscope.

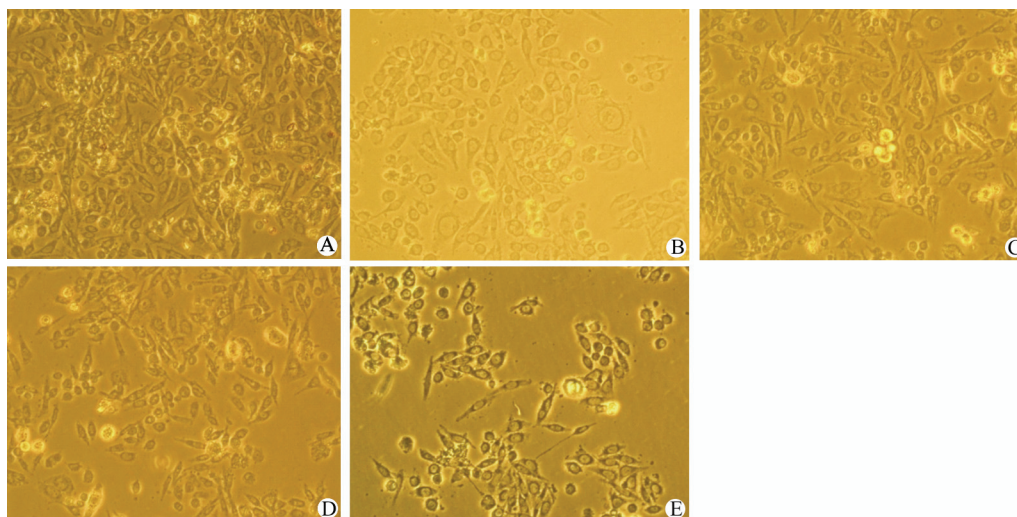


Fig. 1 Appearance of HBECs before and after stimulation with HDM or DEX (inverted phase contrast microscope, ×400)

No significant difference was observed in the appearance of HBECs.

A: blank group; B: 300 ng/mL HDM group; C: 1000 ng/mL HDM group; D: 3000 ng/mL HDM group; E: 300 ng/mL HDM+100 ng/mL DEX group

2.1 Comparison of Expression Levels of IL-29 mRNA

Melting temperature of each melting curve was

uniform, and the shape of each peak was sharp and specific (fig. 2).

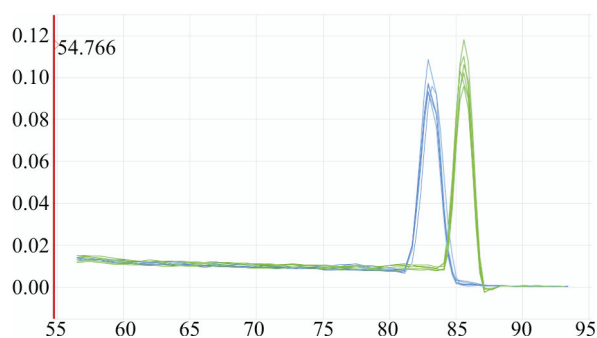


Fig. 2 Melting curve of IL-29 and GAPDH expression

Blue curve: the melting curve of GAPDH expression; green curve: the melting curve of IL-29 expression

After stimulation with HDM for 24 h, the expression of IL-29 mRNA in HBECs in groups B, C and D was significantly higher than that in group A, and after co-stimulation with HDM and DEX for 24 h, the expression of IL-29 mRNA in HBECs of group E was significantly lower than that in the groups stimulated by HDM alone but higher than that in the blank group. The differences between the different groups were significant ($F=132.957$, $P<0.01$). Additionally, the higher the concentration of HDM, the more significant the increase in IL-29 mRNA expression (fig. 3).

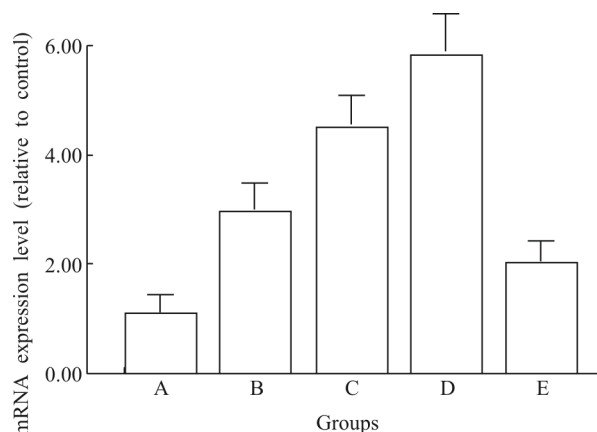


Fig. 3 Comparison of the expression levels of IL-29 mRNA

A: blank group; B: 300 ng/mL HDM group; C: 1000 ng/mL HDM group; D: 3000 ng/mL HDM group; E: 300 ng/mL HDM+100 ng/mL DEX group

2.2 Comparison of Protein Levels of IL-29 in Cell Suspensions

After stimulation with HDM for 24 h, the IL-29 protein level in cell suspensions of groups B, C and D was significantly higher than that in group A, and after co-stimulation with HDM and DEX for 24 h, the protein level of IL-29 in cell suspensions of group E was significantly lower than that in the groups stimulated by HDM alone but higher than that in the blank group. The differences between the different groups were significant ($F=191.983$, $P<0.01$). Additionally, the higher the concentration of HDM, the more significant the increase in

the protein level of IL-29 (table 1).

Table 1 Comparison of the protein levels of IL-29 in cell suspensions ($\bar{x} \pm s$, $n=12$)

Groups	Protein level of IL-29 in cell suspension (pg/mL)
A	50.76 $\pm$ 6.55
B	149.73 $\pm$ 16.44*
C	236.53 $\pm$ 19.98* Δ
D	281.14 $\pm$ 38.55* Δ *
E	105.14 $\pm$ 23.94* Δ

* $P<0.01$ vs. group A, $\Delta P<0.01$ vs. group B, * $P<0.01$ vs. group C

3 DISCUSSION

Airway allergy is one of the pathogeneses of asthma. HDM is an important factor affecting airway allergy. Extensive research in the recent years has revealed that anti-tumor effects^[12, 13], anti-viral activity^[14-17], antiproliferative effect^[18-20] and modulation of immune cell functions are the main biological effects of IL-29. Primary human B-cells show a very limited responsiveness, whereas, interestingly, some B-cell-derived cancer cells are responsive and can be inhibited in their growth by IL-29^[12, 13]. The few side-effects of IL-29 include chills, fever, nausea and arthralgia. Therefore, IL-29 is a potential new drug with good therapeutic effect and few side-effects^[21-24]. In fact, researchers found the highest IL-29 receptor levels in organs of the gastrointestinal and respiratory systems and the main targets of IL-29 were the epithelial cells and liver cells^[8].

Some studies demonstrated that IL-29 can be generated by peripheral blood mononuclear cells and monocyte-derived DCs upon stimulation with LPS/viruses, and it may play a role in the defense against bacterial/viral infections^[4, 25].

Bossios *et al*^[5] found that bronchial epithelial cells generated high levels of IL-29 in response to HDM. Significant differences were found in IL-29 protein and mRNA levels between healthy and asthmatic adult patients^[7]. Studies also showed deficient induction of IL-29 by rhinovirus in asthmatic primary bronchial epithelial cells and alveolar macrophages, which was highly correlated with severity of rhinovirus-induced asthma exacerbation and virus load in experimentally infected human volunteers^[25].

So we suspect that IL-29 may play a role in the progression of asthma. Hence, we explored the effect of IL-29 on the progression of asthma by measuring its expression in airway allergic cell models stimulated by HDM in the presence or absence of DEX.

Our study showed that after stimulation with HDM for 24 h, the expression levels of IL-29 mRNA and protein in cell suspensions in HBECs in groups B, C and D were significantly higher than those in group A, and after co-stimulation with HDM and DEX for 24 h, the two measures in HBECs of group E were significantly lower than those in the groups stimulated by HDM alone but higher than those in group A. The differences between the different groups were significant. Additionally, the higher the concentration of HDM, the more significant the increase in IL-29 expression.

In conclusion, the expression of IL-29 increased

significantly in HBECs after stimulation with HDM alone, and DEX decreases IL-29 expression in HDM-stimulated HBECs. Previous studies have shown that asthmatic adults have increased IL-29 mRNA expression in sputum as compared to healthy subjects^[7], which are consistent with our findings.

Our findings also indicate that IL-29 may play a role in the progression of airway allergic diseases induced by inhaled allergens including asthma. But the mechanism is still unknown. We suspect the mechanism may lie in the following aspects: First, IL-29 could modulate the Th1/Th2 response and cytokine secretion. Some studies showed that IL-29 induced the production of IL-10, IL-6, IL-8, CXCL9, CXCL10 and CXCL11 in peripheral blood mononuclear immune cells. However, it was only effective at extremely high concentrations^[26, 27]. Second, IL-29 may reduce Th2 prototypic markers in human T-cells, including the production of IL-13 and IL-4 and the expression of IL-4R and GATA3^[28, 29], which indicates an immunoprotective role of IL-29. Last, human monocyte-derived DCs treated with IL-29 acquire a tolerogenic phenotype and induce expansion of CD25⁺ T regulatory cells *in vitro*^[30].

Furthermore, this study also demonstrated that IL-29, if given to immature monocyte-derived DCs, provoked an incompletely mature phenotype of these cells characterized by the upregulation of MHC class I molecules in the absence of CD86 and CD83 upregulation and by the ability to promote the proliferation of regulatory T cells^[30].

Our findings indicate that IL-29 may play a role in the progression of airway allergic diseases, furthermore, it might be a protective mechanism against allergic sensitization towards inhaled allergens, which is consistent with above findings, and provides novel insights for further research on the diagnosis and treatment of asthma.

Conflict of Interest Statement

The authors declare that there are no competing interests and financial relationship with the commercial identities mentioned in this paper.

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