The expression of IL-27 and Th17 cells in peripheral blood of patients with allergic rhinitis

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Abstract: Objective: To explore the expression of IL-27, Th17 cells and their related cytokines IL-17 in peripheral blood of patients with allergic rhinitis (allergic rhinitis, AR). Method: 18 Cases of allergic rhinitis patients (10 males, 8 females) whose allergen was dust mite were collected from April to June 2012 as the AR group, and 10 cases of healthy volunteers (4 males, 6 females) without allergic diseases were put into the control group. IL-27 and IL-17 levels in serum of peripheral blood of the two groups were detected by ELISA, and the percentage of Th17 cell was detected by flow cytometry. Result: IL-27 levels of AR group and control group were (21.69 ± 12.62) pg / ml and (53.10 ± 12.55) pg / ml respectively, and the difference was statistically significant (P <0.01); IL-17 levels of AR group and control group were (672.82±63.45) pg / ml and (576.62±22.81) pg / ml respectively, and the difference was statistically significant (P <0.01); Th17 cell percentage of AR group was 1.76 ± 0.60%, and in the control group it was 0.59 ± 0.17%. The difference between the two groups was statistically significant (P <0. 01). IL-27 was negatively correlated to Th17 cell and IL-17 (r was -0.361 and -0.435 respectively, P <0.05). Conclusion: The reduction of IL-27 level in the peripheral blood of patients with allergic rhinitis, the increase of Th17 cells percentage and IL-17 level, as well as the negative correlation of IL-27 with Th17 cell and IL-17 suggest that decline of IL-27 suppression to Th17 cell may play an important role in the pathogenesis of allergic rhinitis.

Keywords: Allergic rhinitis; Interleukin hormone-27; Th17 cell

Allergic rhinitis (allergic rhinitis, AR) is a chronic inflammation disease of nasal mucosa which involves IgE-mediated neurotransmitter release as well as a variety of immunocompetent cells and cytokines after exposure of atopic individuals to allergens. Cytokines play an important role in the immune regulation of allergic rhinitis. They participate in the immune cell proliferation, activation, differentiation, interaction and apoptosis. IL-27 is a new member of IL-12 cytokine family. It is a heterodimer composed of two subunits form of p28 and EB virus-induced gene 3 (E-pstein Barr Virus induced gene 3, EBI3). P28 is homologous to p35 subunits of IL-12, and EBI3 is homologous to IL-12 subunit of p40. IL-27 receptor is also a heterodimer of IL-27Ra (also known as WSX-1 or T-CCR) and gp130. They have the highest expression level in T cells and natural killer (NK) cell. In addition to this, they are also co-expressed in monocytes, mast cells, dendritic cells, NKT cells, endothelial cells and Langerhans cells. IL-27 can induce CD4+T cell proliferation, start the STAT1 pathway, induce the generation and differentiation of T-bet, and promote the differentiation of CD4+T cells to Th1; IL-27Can also inhibit Th2 by decreasing GaTA3 expression. It inhibits the expression of RORyt through STAT1-dependent pathway to prevent the differentiation of T cells to Th17 [1].
Th17 cell is a new kind of T cell subset. IL-17 is its main secretion cytokines, and it plays an important role in promoting inflammation and autoimmune diseases[2]. Studies have shown that the severity of allergic rhinitis is closely related to serum IL-17 level [3]. In this study, expressions of IL-27, IL-17 and Th17 in peripheral blood of AR patients were detected by ELISA and flow cytometry, in order to explore the role of IL-27 and Th17 cells in AR pathogenesis. It is reported as follow.

Materials and Methods

The research subject:

18 Cases of AR patients (AR group) admitted to our department from April to June in 2012, including 10 males and 8 females, with average age of 29.7. All AR patients met the diagnostic criteria [4] with allergen as dust mite and were not associated with sinusitis, asthma, aspirin intolerance and other diseases. They did not receive a local or systemic glucocorticoid treatment, and did not undergo anti-histamine and immunotherapy in the last one month. 10 Cases of healthy volunteers (control group) from our hospital had no allergic rhinitis symptom, and were negative of inhaled allergens skin prick, including 4 male and 6 female with average age of 30.8. All participants consent to accept the experiment.

Main instruments and reagents:

Flow cytometry is U.S. BD FACSCalibur model, using Cellquest software (BD Company) to obtain the cell data and experimental data analysis. A U.S.Bio-Tek ELX-800 microplate reader was used. PMA (phorbol ester), Ionomycin Calcium (ionomycin) and BFA were purchase from Multi-Sciences company, and APC-labeled anti-human CD8 mAb was purchased from BD Company, US. PerCP-Cy5.5-labeled anti-human CD3 mAb, PE-labeled anti-human IL-17 mAb and its matching isotype control were purchased from eBio- science of United States. Fixative and amniotomy liquid were purchased from Invitrogen Corporation, USA. Anti-human IL-27 and IL-17ELISA kits were purchased from eBioScience.

Specimen collection

4ml of patients’ peripheral venous blood was collected in early morning, and heparin was used as anticoagulant. 2ml of blood was centrifuged at 3000r/min for 15 minutes to obtain serum. It was stored at −20°C for the detection of serum IL-27 and IL-17 concentration. 2ml of blood was subject to flow cytometry for detection of Th17 cells within 3 hours.

ELISA detection of serum IL-27 and IL-17 concentration

Detection was performed accordance with the instructions of the ELISA kit. The sensitivity of IL-27 was 9.5pg/ml and IL-17 sensitivity was 0.5pg/ml.

Flow cytometry detection of the percentage of Th17 cells

Peripheral blood 250μl was added PMA50μg/L, Golgi blocker monensin 2.0μmol/L, and ion neomycin 750μmol/L and mixed well. It was cultured in a CO2 incubator (50 mL/L) at 37 °C for 4 h and the cell suspension was transferred to a 1.5 ml EP tube. The suspension was centrifuged for 6 min at 2500r/min, and the supernatant was discarded. It was washed twice with PBS for flow cytometry analysis. 10μl of PECy5-anti-CD3 and 10μl of FITC-anti-CD8 were added, and the mixture was incubated at room temperature away from light for 30 min. 300μl fixative liquid was added after twice PBS wash. It was incubated at 4°C away from light for 15min, and the supernatant was discarded after centrifugation. Amniotomy liquid was added and the mixture was centrifuged at 3000r/min to discard the supernatant. It was washed with PBS twice and divided into two. Each one was added 20μl PE-anti-IL-17 and 10μl of isotype control PE-IgG1, respectively. They were incubated at room temperature away from light for 30min. Twice PBS wash was followed by resuspension of cells with 0.3 ml PBS. They were subject to machine testing and CellQuest software was used for data analysis.

Statistical analysis

SPSS16.0 software was used for statistical analysis. The normal distribution of measurement data was described with (' c ± s) and independent samples t-test was applied for the comparison between groups. The relationship between variables used Pearson linear correlation analysis. If P <0.05, the difference was statistically significant.

Results

Comparison of IL-27 and IL-17 levels in peripheral blood of AR group and control group

IL-27 level in the AR group was lower than that of the control group (Table 1), and the difference between the two groups was statistically significant (P <0.01). IL-17 level in AR group was higher than that in the control group (Table 1), and the difference was statistically significant (P <0.01).

Comparison of Th17 cell percentage in AR group and control group

The flow cytometry results showed that Th17 cell percentage of the AR group was higher than that of the control group (Figure 1). Th17 cell percentage in AR group was 1.76 ± 0.60% and in the control group was 0.59 ± 0.17%. The difference between the two groups was statistically significant.

Correlation analysis of IL-27 level with Th17 cell percentage

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and IL-17 level in peripheral blood

The IL-27 level in peripheral blood was negatively related to Th17 cell percentage (P <0.05) as well as IL-17 level (Table 2).

Discussion

IL-27 is produced by antigen presenting cells (APC). It was detected that the highest levels of human p28 and EBI3 mRNA are in lipopolysaccharide activated monocytes, monocyte derived dendritic cells and activated macrophages [5]. The receptors of IL-27 are IL-27Ra (also known as WSX-1 or T-CCR) and gp130 which belong to immunoglobulin superfamily. They are coordinately expressed in a variety of cells, and T cells and natural killer (NK) cells have the highest expressing level [6].

Studies have shown that WSX-1 (-/-) mice airway reactivity increased: goblet cell hyperplasia, increased infiltration of pulmonary eosinophils, elevated serum IgE levels, increased Th2 cytokine [7]. The airway reactivity of EBI3/- mice elevated, with increased eosinophils in airway, serum IgE levels and Th2 cytokine (IL-4, IL-5, IL-13) [8]. The above research suggests that in allergic asthma, IL-27 plays an important role in reducing airway hyperresponsiveness and lung inflammation by inhibiting the generation of Th2 cytokines.

In vitro, the initial CD4+T cells treated with IL-27 inhibit Th17 cell development, so IL-27 is a potent inhibitor of Th17 cell development [9]. The key nuclear transcription factor of Th17 cells is RORγt, and it is critical in promoting Th17 cell differentiation. The excessive expression of RORγt will promote the differentiation of Th17 cells, and T cells with RORγt expression defects cannot complete the differentiation of Th17 cells [10]. IL-27 inhibits the expression of Th17-specific transcription factor RORγt through STAT1-dependent pathway, thus preventing the differentiation of initial CD4 + T cells to Th17 [11]. In this study, IL-27 level in the serum of patients with allergic rhinitis was lower than the control group; Th17 percentage in peripheral blood was higher than the control group; IL-27 and Th17 cell percentage was negatively correlated. These results suggest that the inhibition function decline in AR peripheral blood may be due to IL-27 level decreasing, leading to increased Th17 cell differentiation.

IL-17 is a specific cytokines of Th17 cell. It can promote the maturation of neutrophils, with a strong role in the recruitment of neutrophils. It also promotes a variety of cell maturation and differentiation, leading various cytokines to generate synergies to enlarge the inflammatory response [12,13,14]. IL-17

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<th>Table 1 – Comparison of IL-27 and IL-17 levels in serum of AR group and control group (±s) pg/ml</th>
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<tr>
<td>AR group (n=18)</td>
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<tr>
<td>21.69±12.62*</td>
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<td>control group (n=10)</td>
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1) compared with control group, P <0.01; 2) compared with control group, P < 0.01

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<th>Table 2 The correlation analysis of IL-27 level with Th17 cell percentage and IL-17 level in peripheral blood</th>
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<td>Correlation coefficient</td>
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<td>IL-27 and Th17 cell</td>
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Figure 1. Th17 cells flow cytometry diagram (a: AR group b: control group)
expression levels were significantly increased in the sputum, bronchoalveolar lavage fluid and serum of patients with asthma, and the increased level was related to the degree of inflammation of airway hyperresponsiveness[15]. IL-17 expression level in serum of AR patients was also significantly higher than that of the normal control group[16]. Ciprandi[17] reported that for allergic rhinitis patients with pollen as the allergen, the serum IL-17 level is related to the severity of clinical symptoms in pollen seasons. This study found that IL-17 level increased in peripheral blood of AR patients while IL-27 level deceased; IL-27 and IL-17 were negatively related (P <0.05). These suggest the reduced inhibition function of IL-27 in peripheral blood of AR not only promoted the differentiation of Th17 cells, but also promoted the secretion of IL-17.

The results of this study showed that IL-27 level in peripheral blood of AR patients reduced, the Th17 cell percentage and IL-17 level increased. IL-27 level was negatively correlated with Th17 cell percentage and IL-17 levels. These results suggest that IL-27 may play an inhibiting role on Th17cell differentiation and function, so IL-27 could be a target to provide new ideas for the treatment of AR.

REFERENCES