The Expression of IL-27, Th17 Cells and Treg 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 CD4+CD25+ regulatory T cells (Treg) as well as its associated cytokines in peripheral blood of patients with allergic rhinitis (AR).

Method: From March 2012 to May, the peripheral blood of 24 cases of AR patients (AR group) and 16 cases of healthy volunteers (control group) was collected, and the percentage of Th17 cells and Treg cells in the peripheral blood was detected by flow cytometry (FCM); the levels of IL-27, IL-17 and IL-10 in serum was detected by ELISA.

Result: The percentage of Th17 cells in AR group and the control group was 1.76% ± 0.60% and 0.59% ± 0.17%, respectively. It was higher in AR group than in control group, and the difference between two groups was statistically significant (P < 0.01); Treg cell percentage in AR group and control group was 1.65% ± 0.79% and 5.03% ± 1.92%, respectively. AR group was significantly lower than the control group, and the difference between two groups was statistically significant (P < 0.01). Serum IL-17 expression level (668.68 ± 62.59) pg/ml in AR group was higher than that of the control group (587.30 ± 28.00) pg/ml, and the difference was statistically significant (P < 0.01); the levels of IL-27 in AR group and the control group were (23.15 ± 10.12) pg/ml and (52.97 ± 10.08) pg/ml, and the difference was statistically significant (P < 0.01); IL-10 expression level (14.29 ± 6.16) pg/ml in AR group serum was lower than that in the control group (31.32 ± 21.20) pg/ml, and the difference between two groups was statistically significant (P < 0.01). In the peripheral blood of AR patients, there was a negative correlation between Th17 cell percentage and Treg cell percentage, IL-10 (r = −0.794, P < 0.01), and a negative correlation between IL-27 and Th17 cell percentage, IL-17 (r = −0.519 respectively, P < 0.01). IL-27 was positively correlated with Treg cell percentage and IL-10 (r = 0.622, P < 0.01). There was no correlation between IL-17 and Th17 cell percentage, Treg cell percentage, IL-10 (r = 0.225, P > 0.05).

Conclusion: In the peripheral blood of AR patients there was a reduction of IL-27 level and imbalance of Th17/Treg cell function. IL-27 on Th17/Treg cells adjustment may play an important role on the pathogenesis of the AR.

Keywords: Allergic Rhinitis; IL-27; Th17 Cells; Regulatory T Cells

1. Introduction

Allergic rhinitis (AR) is one of the most common disorders, which is characterized by sneezing, clear rhinorrhea, nasal itching and nasal congestion. Epidemiological studies have indicated that the prevalence of AR has progressively increased over the last three decades in developed and industrialized countries. Allergic rhinitis is a global health problem that affects patients of all ages and ethnic groups. Although not life-threatening, allergic rhinitis affects social life, sleep, and performance at school and work, and its economic impact is substantial [1].

Allergic rhinitis 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, EB3). 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-27 can also inhibit Th2 by decreasing GaTa3 expression. It inhibits the expression of RORyt through...
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 [3]. Studies have shown that the severity of allergic rhinitis is closely related to serum IL-17 level [4]. The regulatory T cell (Treg) which is involved in allergic diseases as another type of critical T cell subset can inhibit and regulate physiological and pathological immune responses, to achieve the maintenance of immune tolerance and immune balance. CD4+CD25+ Treg as one of the main types of regulatory T cells secretes cytokines TGF-β1 and IL-10, and forkhead box transcription factor p3 (Foxp3) is its important regulatory genes [5]. In this study, expressions of IL-17, Th17 and CD4+CD25+ Treg as well as its related cytokines IL-17, IL-10 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 as well as Treg in AR pathogenesis. It is reported as follows.

2. Material and Methods

2.1. Object of Study

Twenty-four cases of AR patients (AR group) admitted to our department from March to May in 2012, including 15 males and 9 females, with an average age of 29.7. All AR patients met the diagnostic criteria [1] 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. Sixteen cases of healthy volunteers (control group) from our hospital had no allergic rhinitis symptom, and were negative of inhaled allergens skin prick, including 7 males and 9 females with an average age of 30.8. All participants consented to accept the experiment.

2.2. 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 MultiSciences Company, and T-reg kit (Human Regulator T Cell Staining Kit) was purchased from Ebio-science Corporation. 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 eBioScience of United States. Fixative and amniotomy liquid were purchased from Invitrogen Corporation, USA. Anti-human IL-27/IL-17/IL-10 ELISA kits were purchased from eBioScience (United States).

2.3. Specimen Collection

4 ml of patients’ peripheral venous blood was collected in early morning, and heparin was used as anticoagulant. 2 ml of blood was centrifuged at 300 × g for 15 minutes to obtain serum. It was stored at –20°C for the detection of serum IL-27, IL-17 and IL-10 concentrations. 2 ml of blood was subject to flow cytometry for detection of Th17 and Treg cells within 3 hours.

2.4. Flow Cytometry Detection of the Percentage of Th17 Cells

Peripheral blood (250 μl) was added PMA 50 μg/L, Golgi blocker monensin (2.0 μmol/L), and ion neomycin (750 μmol/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 300 × g, and the supernatant was discarded. It was washed twice with PBS for flow cytometry analysis. 10 μl of PECy5-anti-CD3 and 10 μl of FITC-anti-CD8 were added, and the mixture was incubated at room temperature away from light for 30 min. 300 μl fixative liquid was added after twice PBS wash. It was incubated at 4°C in dark for 15 min, and the supernatant was discarded after centrifugation. Amniotomy liquid was added and the mixture was centrifuged at 300 × g to discard the supernatant. It was washed with PBS twice and divided into two. Each one was added 20 μl PE-anti-IL-17 and 10 μl of isotype control PE-IgG1, respectively. They were incubated at room temperature away from light for 30 min. Twice PBS wash was followed by resuspension of cells with 0.3 ml PBS. They were subject to flow cytometry testing and Cell Quest software was used for data analysis.

2.5. Flow Cytometry Detection of the Percentage of Treg Cells

Mark sample tubes, control tubes, and each tube was added the following antibodies: CD4/CD25/FoxP3 (sample tube), FoxP3 isotype control mAb CD4/CD25/Mouse IgG (control tube). 100 μl anticoagulated whole blood was added to each tube and incubated at room temperature in dark for 20 min. 1ml of hemolytic agent was added and incubated in dark at room temperature for 10 min. It was centrifuged at 300 × g for 5 min, and the supernatant was removed followed by addition of 1ml PBS to resuspend cells. It was centrifuged under 300 × g for 5 min to remove the supernatant, and 0.5 ml of Foxp3 fixative was added to each tube and mixed well. After the reaction in the dark at room temperature for 20 min, the mixture was washed with 1 ml PBS followed by 300 × g centrifugation for 5 min. After the supernatant was re-
moved, each tube was added 0.5 ml Foxp3 Amniotomy mixture to wash once. Centrifugation at 300 × g for 5 min and removal of the supernatant was followed by addition of 0.5 ml Foxp3 rupture liquid to resuspend cells. The reaction was allowed in the dark at room temperature for 15 min followed by centrifugation (300 × g) for 5 min and the removal of supernatant. The sample tube was added 10 μl PE Foxp3 antibody, and the control tube was added the same type of PE monoclonal antibody. They were incubated in the dark at room temperature for 30 min followed by PBS (1 ml) wash once. Centrifugation (300 × g) for 5 min and removal of the supernatant was followed by resuspension of cells with 0.4 ml PBS. They were subject to flow cytometry testing and Cell Quest software was used for data analysis.

2.6. ELISA Detection of Serum IL-27, IL-17 and IL-10 Concentrations

Detection was performed in accordance with the instructions of the ELISA kit. The steps were followed: 1) Determine the number of microwell strips required; 2) Wash microwell strips twice with Wash Buffer; 3) Standard dilution on the microwell plate: Add 100 μl Assay Buffer (1×), in duplicate, to all standard wells. Pipette 100 μl prepared standard into the first wells and create standard dilutions by transferring 100 μl from well to well. Discard 100 μl from the last wells. Alternatively external standard dilution in tubes. Pipette 100 μl of these standard dilutions in the microwell strips; 4) Add 100 μl Assay Buffer (1×), in duplicate, to the blank wells; 5) Add 50 μl Assay Buffer (1×) to sample wells; 6) Add 50 μl sample in duplicate, to designated sample wells; 7) Prepare Biotin-Conjugate; 8) Add 50 μl Biotin-Conjugate to all wells; 9) Cover microwell strips and incubate 2 hours at room temperature (18°C to 25°C); 10) Prepare Streptavidin-HRP; 11) Empty and wash microwell strips 3 times with Wash Buffer; 12) Add 100 μl diluted Streptavidin-HRP to all wells.; 13) Cover microwell strips and incubate 1 hour at room temperature (18°C to 25°C); 14) Empty and wash microwell strips 3 times with Wash Buffer; 15) Add 100 μl of TMB Substrate Solution to all wells; 16) Incubate the microwell strips for about 10 minutes at room temperature (18°C to 25°C); 17) Add 100 μl Stop Solution to all wells; 18) Blank microwell reader and measure colour intensity at 450 nm. The sensitivity of IL-27 was 9.5 pg/ml; IL-17 sensitivity was 0.5 pg/ml and IL-10 sensitivity was 1 pg/ml.

2.7. Statistical Analysis

SPSS16.0 software was used for statistical analysis. The normal distribution of measurement data was described with (X̄ ± s) and independent samples t-test was applied for comparisons between groups. The relationship among IL-27 level, Th17 cell percentage, Treg cell percentage, IL-17 and IL-10 level used Pearson linear correlation analysis. If P < 0.05, the difference was statistically significant.

3. Results

3.1. Comparison of Th17 Cell Percentage and Treg Cell Percentage in AR Group and Control Group

The results of flow cytometry showed that Th17 cell percentage in the AR group was higher than that of the control group (Table 1), and the difference between the two groups was statistically significant (t = 4.59, P < 0.01). Treg cell percentage of AR group patients was significantly lower than that of the control group (Table 1), and the difference was statistically significant (t = 3.36, P < 0.01).

3.2. Comparison of IL-27, IL-17 and IL-10 Levels in Serum of AR Group and Control Group

The expression levels of serum IL-27, IL-17 and IL-10 in AR group and the control group are shown in Table 2. The IL-27 expression level in the AR group was lower than that of the control group, and the difference between the two groups was statistically significant (t = 9.12, P < 0.01); the IL-17 expression level in the AR group was higher than that of the control group, and the difference between the two groups was statistically significant (t = 3.81, P < 0.01); the IL-10 expression level in the AR group was lower than that of the control group, and the difference between the two groups was statistically significant (t = 3.50, P < 0.01).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Th17 (pg/ml)</th>
<th>Treg (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR group (n = 24)</td>
<td>1.76 ± 0.601</td>
<td>1.65 ± 0.791</td>
</tr>
<tr>
<td>Control group (n = 16)</td>
<td>0.59 ± 0.17</td>
<td>5.03 ± 1.92</td>
</tr>
</tbody>
</table>

1Compared with control group, P < 0.01; 2Compared with control group, P < 0.01.

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-27 (pg/ml)</th>
<th>IL-17 (pg/ml)</th>
<th>IL-10 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR group (n = 24)</td>
<td>23.15 ± 10.12</td>
<td>668.68 ± 62.59</td>
<td>14.29 ± 6.16</td>
</tr>
<tr>
<td>Control group (n = 16)</td>
<td>52.97 ± 10.08</td>
<td>587.30 ± 28.00</td>
<td>31.32 ± 21.20</td>
</tr>
</tbody>
</table>

1Compared with control group, P < 0.01; 2Compared with control group, P < 0.01.
3.3. Correlation Analysis of IL-27 Level, Th17 Cell Percentage, Treg Cell Percentage, IL-17 and IL-10 Level in Peripheral Blood of AR Group

Pearson correlation analysis showed that in the peripheral blood of AR patients Th17 cell percentage and Treg cell percentage, IL-10 was negatively correlated ($r = -0.794, -0.483, P < 0.01$); IL-27 and Th17 cell percentage, IL-17 was negatively correlated ($r = -0.758, -0.519, P < 0.01$). There was a positive correlation between IL-27 and Treg cell percentage, IL-10 ($r = 0.722, 0.646, P < 0.01$), and a positive correlation between the percentage of Treg cells and IL-10 ($r = 0.622, P < 0.01$). There was no correlation between IL-17 and Th17 cells percentage, Treg cell percentage, IL-10 ($r = 0.225, -0.183, -0.176, P > 0.05$).

4. Discussion

IL-27 is primarily secreted by dendritic cells, and its receptor is composed by two subunit WSX-1 and gp130. It is expressed in a variety of immune cells and non-immune cell surfaces, with T cell and natural killer (NK) cell expression levels the highest [6]. After binding to its receptor, IL-27 regulates the proliferation and differentiation of naive T cells through activation of downstream STAT1/STAT3 [1]. Studies have shown that IL-27 has played a crucial role in many of inflammation such as colitis [7], and autoimmune diseases such as multiple sclerosis [8]. The airway responsiveness of mice missing IL-27 receptor increases; eosinophils increases in airway, and serum IgE levels, and Th2 cytokine also increase [9]. In mouse asthma model, IL-27 secreted by NK cells inhibits Th2 response and allergic inflammation [10]. In this study, serum IL-27 level of AR patients was lower than that of the control group, suggesting that IL-27 may be involved in the pathogenesis of AR.

Previously, the imbalance of Th1/Th2 has been considered to be important for allergic rhinitis. This concept has been modified since two T lymphocyte subsets, Treg cells and Th17 cells were discovered recently [11]. Th17 cells are characterized by the mainly production of cytokines IL-17. IL-17 may be involved in allergic disorders since this cytokine has been demonstrated to reduce neutrophil infiltration in an experimental asthma model [12], and on the other hand increases eosinophil infiltration. Furthermore, IL-17 induces recruitment and is a survival factor for airway macrophages [13]. Tregs release IL-10. IL-10 inhibits proinflammatory cytokine production and both Th1 and Th2 cell activation. It also impairs the activation of mast cells and eosinophils and promotes the synthesis of IgG4 [14].

The results of this study showed that Th17 percentage in peripheral blood of AR patients was higher compared with the control group ($P < 0.01$); IL-17 level was higher than that of the control group ($P < 0.01$); CD4$^+$/CD25$^+$ Treg percentage was lower ($P < 0.01$) and IL-10 was lower than the control group ($P < 0.01$). Ciprandi et al. [15] also confirmed after mononuclear cell in peripheral blood of AR patients was cultured in vitro, proportion of Th17 cells in AR patients increased detected by flow cytometry with stimulation of pollen allergen, and the severity of allergic rhinitis was closely related to the serum IL-17 level in blood [4]. Xu et al. [16] found that the Foxp3$^+$ lymphocyte count and the expression level of Foxp3m RNA in nasal mucosa and peripheral blood mononuclear cells of AR patients were significantly lower than those in the normal control group. The above studies suggest that Th17 cells and Treg cells may play an important role in the pathogenesis of AR. Th17 and Treg cells both come from naive T cells. Th17 cells mediate inflammatory responses, so they are “pro-inflammatory cells”; while Treg cells mediate immune tolerance, so they belong to “suppression inflammatory cells”. Their function and differentiation process work against each other and a balance is maintained between the two, which is beneficial for the stability of the immune state. The results of this study confirm Th17 cells and Treg cell percentage in peripheral blood of AR patients was negatively correlated ($P < 0.01$), indicating that Th17 and Treg cell immune imbalance may play a key role in AR incidence.

IL-27 is a cytokine which can effectively inhibit Th17 cell development. It inhibits the expression of Th17-specific transcription factor RORγt through STAT1-dependent pathway, thereby preventing initial differentiation of CD4$^+$ T cells to Th17 [17]. In this study, serum IL-27 level of patients with allergic rhinitis was lower than that of the control group; Th17 cell percentage was higher in the peripheral blood; IL-27 and Th17 cell percentage, IL-17 level was negatively correlated; suggesting that the inhibition function may decline due to the decrease of IL-27 level in peripheral blood of AR patients, resulting in increased differentiation of Th17 cells and promotion of IL-17 secretion.

Awasthi et al. [18] found that IL-27 is the main factor in inducing the Trl cells production, and promotes Trl cell differentiation together with TGF-β, thus further contributing to the generation of IL-10. It also has been reported that IL-27 receptor is highly expressed in CD4$^+$ CD25$^+$ Treg cell surface [19]. In experimental autoimmune uveitis (EAU), IL-27 can inhibit Th1 and Th17 inflammatory response by promoting Trl and CD4$^+$ CD25$^+$ Treg proliferation [20]. In this study IL-27 level reduced; CD4$^+$ CD25$^+$ Treg percentage lowered ($P < 0.01$); IL-10 was lower than that of the control group ($P < 0.01$), and IL-27 was positively correlated to CD4$^+$ CD25$^+$ Treg percentage, expression level of IL-10, suggesting IL-27 in peripheral blood of AR patients may promote CD4$^+$...
CD4+CD25+ Treg cell differentiation.

In summary, the present study shows that there is an immune imbalance between Th17 cells and CD4+CD25+ Treg cell, a negative correlation between IL-27 and Th17 cell percentage, a positive relation between IL-27 and CD4+CD25+ Treg cell percentage. These suggest that IL-27 may have a regulatory role on Th17 cell and CD4+CD25+ Treg cell differentiation and function. Therefore, IL-27 may be a new target for the treatment of AR.

REFERENCES


