

**EXPRESSIONS OF IL-17, IL-21 AND IL-23 IN THE SERUM
OF ALLERGIC RHINITIS PATIENTS**

EKSPRESIJA IL-17, IL-21 I IL-23 U SERUMU PACIJENATA SA ALERGIJSKIM RINITISOM

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Summary: The present study aimed to investigate the expressions of interleukin-17 (IL-17), IL-21 and IL-23 in the serum of allergic rhinitis (AR) patients and to explore their relationship with special IgE (sFgE) in the serum. AR patients (n=24) and healthy subjects (n=12) were recruited and serum samples were collected. The serum level of IgE specific for inhalant allergens was determined using the automatic quantitative immunofluorescence analysis system, and the contents of IL-17, IL-21 and IL-23 in the serum were detected using ELISA. The level of serum IgE in the healthy individuals was categorized as grade 0 and that in the AR patients as grade 2–6. The mean contents of IL-17, IL-21 and IL-23 were 164.71 ± 39.37 pg/mL, 199 ± 97.86 pg/mL and 78.94 ± 26.33 pg/mL, respectively, in the AR patients, and 67.75 ± 18.24 pg/mL, 7.58 ± 5.49 pg/mL and 13.58 ± 3.93 pg/mL, respectively, in the healthy subjects. Statistical analysis showed the serum levels of IL-17 and IL-23 in the AR patients were markedly higher than those in the healthy subjects, however, no significant difference was noted in the content of IL-21. Furthermore, the IL-17 level was positively related to the levels of IL-23 and IgE and the IL-23 level was positively related to the IgE level among AR patients, but no relations were observed between the IL-21 level and levels of IL-17, IL-23 and IgE. Our study indicates IL-17 and IL-23 may play an important role in the pathogenesis of AR and maybe IL-21 is not involved in the occurrence of AR.

Keywords: interleukin-17, interleukin-21, interleukin-23, allergic rhinitis

Kratak sadržaj: Cilj ove studije bio je da se utvrdi ekspresija interleukina-17 (IL-17), IL-21 i IL-23 u serumu pacijenata sa alergijskim rinitisom (AR) kao i da se ispita njihov odnos sa specifičnim IgE u serumu. Oboje pacijenti sa AR (n=24) i zdravi ispitanici (n=12) od kojih su uzeti uzorci seruma. Nivo IgE specifičnog za inhalatorne alergene u serumu određen je pomoću sistema za automatsku kvantitativnu imunofluorescentnu analizu, dok su količine IL-17, IL-21 i IL-23 u serumu utvrđene pomoću ELISA. Nivo IgE u serumu zdravih osoba obeležen je kao multi stupanj a kod pacijenata sa AR stupnjeva od 2 do 6. Srednje vrednosti IL-17, IL-21 i IL-23 bile su $164,71 \pm 39,37$ pg/mL, $199 \pm 97,86$ pg/mL i $78,94 \pm 26,33$ pg/mL kod pacijenata sa AR, i $67,75 \pm 18,24$ pg/mL, $7,58 \pm 5,49$ pg/mL i $13,58 \pm 3,93$ pg/mL kod zdravih ispitanika. Statistička analiza pokazala je da su nivoi IL-17 i IL-23 u serumu pacijenata sa AR bili značajno viši nego kod zdravih ispitanika, dok za vrednosti IL-21 nije uočena značajna razlika. Štaviše, nivo IL-17 pokazao je pozitivnost u odnosu sa nivoima IL-23 i IgE, dok je nivo IL-23 imao pozitivan odnos sa nivoom IgE kod pacijenata sa AR, ali nije utvrđena povezanost između nivoa IL-21 i nivoa IL-17, IL-23 i IgE. Naša studija ukazuje na potencijalno važnu ulogu IL-17 i IL-23 u patogenezi AR, dok IL-21 možda ne učestvuje u nastanku AR.

Ključne reči: interleukin-17, interleukin-21, interleukin-23, alergijski rinitis

Introduction

Allergic rhinitis (AR) is an IgE mediated non-infectious disease of the nasal mucosa following contact with allergens. Numerous studies have demonstrated the imbalance between Th1 cells and Th2 cells is closely related to AR. Recently, a study has shown that Th17 cells, a novel subset of CD4+ T cells, play an important role in the pathogenesis of

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allergic diseases (1), which renders a new mechanism underlying the occurrence of AR. Th17 cells can secrete interleukin-17 (IL-17) which further facilitates the production and secretion of lots of chemokines and matrix metalloproteinases, resulting in the recruitment of neutrophils and macrophages into the inflammatory sites and subsequent inflammation (2). IL-23 is a heterodimer protein and a member of the IL-12 family. IL-23 consists of two subunits, one called p40, which is shared with IL-12, and another called p19 (3). In conjunction with IL-6 and transforming growth factor- β 1 (TGF- β 1), IL-23 can stimulate the naive CD4+ T cells to differentiate into Th17 cells (4). IL-21 is a cytokine with a four-helix-bundle and the human IL-21 gene is mapped to 4q26-q27. IL-21 is secreted by the activated CD4+Th cells, especially the Th17 cells (5). Evidence reveals IL-21 can up-regulate the expression of IL-23 receptor and enhance the response of Th17 cells to IL-23, which is important for the stability and proliferation of IL-17 (6).

It is reported that there is an IL-23/IL-17 axis between acquired immunity and innate immunity (7). However, few studies report the role of IL-23/IL-17 axis in AR. The present study aimed to investigate the expressions of IL-17, IL-21 and IL-23 in the serum of AR patients and healthy subjects and to explore their relations with serum specific IgE (sIgE). Our study may provide evidence for the role of IL-23/IL-17 axis and IL-21 in the pathogenesis of AR.

Subjects and Methods

Subjects

A total of 24 patients with AR were recruited from the Otolaryngology Clinic between March 2010 and June 2010. The age of AR patients ranged from 5 to 53 years (mean: 27.33 ± 13.57 years) and there were 11 males and 13 females. No nasosinusitis, asthma or intolerance to aspirin were found among these AR patients. The diagnosis of AR was based on the ARIA guideline (Allergic Rhinitis and its Impact on Asthma; 2008 update) (8), and all patients did not receive treatment with steroids or antihistamines and immunotherapy within the past month. In addition, 12 healthy subjects were also enrolled and served as controls. These were 7 males and 5 females with a mean age of 26.08 ± 3.63 years (range: 22–34 years). The controls had no history of AR, nasosinusitis, asthma, intolerance to aspirin or other systemic diseases. All patients had not received treatments with steroids or antihistamines, or immunotherapy before, and physical examination showed normal. Furthermore, these patients were negative for sIgE. Informed consent was obtained before the study.

Sample collection

Three milliliters of whole blood were collected and centrifuged at 2000 r/min. The serum was divid-

ed into two parts: one was stored at -20°C for use and the other was immediately used for the detection of sIgE for common allergens.

Detection of serum sIgE

The level of sIgE for common inhalant allergens was determined using the automatic quantitative immunofluorescence analysis system (Pharmacia, Sweden) according to the manufacturer's instructions and reagents for detection were purchased from Sweden. The sIgE level was categorized into grade 0 to 6; 0: <0.35 kU/L; 1: $0.35\text{--}0.70$ kU/L; 2: $0.71\text{--}3.5$ kU/L; 3: $3.6\text{--}17.5$ kU/L; 4: $17.6\text{--}50$ kU/L; 5: $51\text{--}100$ kU/L; 6: >100 kU/L. Positive was defined as higher than grade 1, and the higher the level of sIgE, the higher the grade.

Detection of contents of IL-17, IL-21 and IL-23 in the serum

The detection of the contents of IL-17, IL-21 and IL-23 was carried out using the double antibody avidin biotin peroxidase complex enzyme-linked immunosorbent assay (ABC-ELISA). The microplates were coated with anti-human IL-17 antibody (Bio-source, USA), anti-human IL-21 antibody (R&D, USA) or anti-human IL-23 antibody (Bender, Austria) and the IL-17, IL-21 and IL-23, separately, in the standards and the samples bound to these antibodies followed by addition of biotinylated anti-human IL-17, IL-21 and IL-23, separately. Then, the St reptavidin was added and bound to the immune complexes. Subsequently, the working solution was added and, when the mixture became blue, the reaction was discontinued by addition of sulfuric acid. The optical density (OD) was detected at 450 nm with a microplate reader (MK3, Finland). The contents of IL-17, IL-21 and IL-23 were positively proportional to the OD, and standard curve was delineated followed by calculation of the contents of IL-17, IL-21 and IL-23. Blank controls were included in each detection.

Statistical analysis

Data were expressed as mean \pm standard deviation (\pm s) and statistical analysis was performed with the SPSS version 15.0 statistic software package. Comparisons were done with t test and correlation was analyzed with Pearson correlation analysis. A value of $P < 0.05$ was considered statistically significant.

Results

Contents of IL-17, IL-21 and IL-23

The contents of IL-17, IL-21 and IL-23 in the AR patients and healthy subjects are displayed in *Table 1*.

The levels of IL-17 and IL-23 in the AR patients were significantly higher than those in the healthy subjects ($P < 0.05$). Moreover, the serum IL-17 level was positively related to that of IL-23 ($r = 0.533$, $P = 0.001$), but the IL-21 level was not relevant with the levels of IL-17 and IL-23 ($r = -0.200$, $P = 0.242$ and $r = -0.162$, $P = 0.344$, respectively).

Correlations between serum sIgE and levels of IL-17, IL-21 and IL-23

The sIgE level in the 12 healthy controls was categorized as grade 0 (< 0.35 kU/L). Among AR patients, 9 had grade 2 of sIgE level (0.71–3.5 kU/L), 4 had grade 3 (3.6–17.5 kU/L), 8 had grade 4 (17.6–50 kU/L), 1 had grade 5 (51–100 kU/L) and 2 had grade 6 (> 100 kU/L).

Discussion

Human IL-17 is a homodimer consisting of 155 amino acids and IL-17 gene is mapped to 2q31. Its molecular weight is 3 kD and the N terminal serves as a signal peptide composed of 19–23 residuals. IL-17 is produced by not only Th17 cells, but other types of cells including thymocytes, epithelial cells and endothelial cells. The IL-17 receptor gene is mapped to chromosome 22 and IL-17 receptor is widely expressed to different extents in the intestinal epithelial cells, osteoblasts, fibroblasts, myocytes, T lymphocytes and cells in the spleen, kidney, lung and liver (9). IL-17 can enhance the development and maturation of neutrophils, recruit neutrophils, promote the maturation and differentiation of multiple cells and reconcile numerous cytokines leading to an inflammation cascade (10–12). Studies show IL-17 is involved in the pathogenesis of allergic airway diseases. IL-17 in the sputum, bronchoalveolar lavage fluid and serum has been found to markedly increase in asthma patients, and the extent of increase of the IL-17 level is related to the airway hyperresponsiveness and the degree of inflammation (13). Klemens et al. (14) reported that IL-17 was detectable in the excretions of viral rhinitis patients and AR patients, but the IL-17 level in the viral rhinitis was dramatically higher than that in the AR patients. In addition, Ciprandi et al. (15) found, in the pollen season, the serum level of IL-17 was closely relevant with the severity of clinical manifestations in patients with

pollen induced AR. In the present study, our result revealed the serum level of IL-17 was markedly higher in AR patients than in healthy controls ($P < 0.05$), which suggests IL-17 may play a crucial role in the pathogenesis of AR.

IL-23 is mainly derived from activated macrophages and dendritic cells, and has multiple functions. IL-23 can promote not only the growth and survival of Th17 cells, but the production of interferon (IFN- γ) and IL-12 from the T cells and antigen presenting cells which may regulate the costimulatory function of dendritic cells. In addition, IL-23 has anti-tumor and anti-metastasis activities and is closely related to autoimmune diseases and inflammatory diseases (16). Evidence shows IL-23 deficient mice have severe impairment of humoral immunity and the ability to activate the secretion of IL-17 by T cells was also compromised in the IL-23 deficient antigen presenting cells. Furthermore, the IL-23 deficient mice have similar phenotype to IL-17 deficient mice. These findings suggest IL-23 can induce the production of IL-17 by the CD4⁺ T cells of mouse. Thus, IL-23 may play a key role in the T cell dependent immune response and there may be an IL-23/IL-17 axis between acquired immunity and innate immunity (7). Our results showed the serum levels of IL-23 and IL-17 in AR patients were markedly higher than those in healthy subjects and moreover the IL-23 level was related to the IL-17 level in AR patients, which suggests the IL-23/IL-17 axis may be an important participant in the pathogenesis of AR and there is a close relationship between IL-23 and IL-17.

AR is an IgE mediated non-infectious disease of the nasal mucosa following contact with allergens. Once they enter the body, allergens can activate the plasma cells to produce sIgE which then binds to the IgE Fc receptor on the basophils and mast cells. When one re-contacts with the same allergen, sIgE then interact with each other which may lead to degranulation of the basophils and mast cells resulting in the release of histamine, leukotrienes and neutrophil chemotactic factor. These mediators act on the nasal mucosa and result in the features of allergy characterized by telangiectasias, increase of vascular permeability and elevation of gland secretion (17). sIgE can be used for the diagnosis of hypersensitivity to a specific allergen. Our results showed serum level of sIgE was positively relevant to the levels of IL-17 and IL-23. We speculate that the IL-17/IL-23

Table I Levels of IL-17, IL-21 and IL-23 in the serum of AR patients and healthy controls ($\bar{x} \pm SD$, pg/mL).

Group	IL-17 (pg/mL)	IL-21 (pg/mL)	IL-23 (pg/mL)
AR patients (n=24)	164.71 \pm 39.37	199 \pm 97.86	78.94 \pm 26.33
Healthy controls (n=12)	67.75 \pm 18.24	7.58 \pm 5.49	13.58 \pm 3.93
t	2.24	1.958	2.46
P	0.033	0.062	0.022

Table II Correlations between the serum sIgE level and the levels of IL-17, IL-21 and IL-23 in AR patients.

	Correlation coefficient	P
sIgE and IL-17	0.687	0.000
sIgE and IL-21	-0.244	0.251
sIgE and IL-23	0.513	0.010

axis may promote the production of IgE and the chemotaxis of neutrophils resulting in AR, and IL-17/IL-23 may facilitate the hypersensitive response.

IL-21 has been shown to regulate the cellular immunity and humoral immunity which is mainly characterized by the differentiation of naïve CD4+ T cells into Th17 cells, differentiation of B cells into plasma cells and regulation of immunoglobulin production. Evidence showed the serum IgG level was markedly decreased in IL-21R deficient mice, but that of IgE significantly increased. Immunization of IL-21R^{-/-} mice with T cell dependent antigens may dramatically reduce specific IgG1 and increase the specific IgE (18). In an OVA induced AR model, IL-21 can obviously improve the symptoms of allergy when given at the early stage of antigenic stimulation. Furthermore, the IL-21 treated mice with AR had

significantly reduced serum levels of sIgE and decreased levels of Th2 cytokines (IL-4, IL-5 and IL-13) in the nasal tissues. In addition, the eosinophil selective chemokine-1 and 2 were reduced in IL-4 induced fibroblasts, which inhibited the migration of eosinophils into the nasal tissues (19). Our result indicated there was no significant difference in the serum IL-21 level between AR patients and healthy controls ($P > 0.05$), and IL-21 was not associated with serum sIgE, IL-21, IL-17 and IL-23. Our results were not consistent with those above, which suggests the IL-21 may not be crucial in the pathogenesis of human AR and there is no relationship between IL-21 and IL-17/IL-23. However, the present study had a small sample size and the IL-21 was detected at the protein level. Future studies with a larger sample size are required to investigate the role of IL-21 in AR at the mRNA level.

Our results indicate IL-23 and IL-17 may be pivotal cytokines involved in the pathogenesis of AR and the IL-23/IL-17 axis may become a novel target in the treatment of AR.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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