



The role of regulatory T cells in allergic rhinitis and their correlation with IL-10, IL-17 and neopterin levels in serum and nasal lavage fluid

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Abstract

Purpose Allergic rhinitis (AR), is an IgE-mediated inflammation of the nose. Regulatory T cells (Tregs) and inflammatory cytokines have been shown to play a critical role in allergic airway inflammation. The aim of the study was to compare the levels of blood T lymphocyte subsets and IL-10, IL-17 and neopterin concentrations in serum and nasal lavage of patients with AR compared to healthy subjects.

Methods The study included 38 subjects with moderate-severe AR and 36 sex- and age-matched controls. Peripheral blood CD3+, CD3+CD4+ and CD4+CD25+Foxp3 percentages were evaluated using flow cytometry. Levels of IL-10, IL-17 and neopterin were measured both in serum and nasal lavage fluid with ELISA and HPLC, respectively.

Results No difference was found in the percentages of T lymphocyte subsets between the two groups ($p > 0.05$). Serum IL-10 levels were similar ($p > 0.05$), whereas nasal IL-10 was lower in AR subjects compared to control group (2.22 ± 0.91 and 3.12 ± 1.45 pg/ml, respectively) ($p < 0.05$). Mean serum and nasal IL-17 were higher in AR (107.7 ± 79.61 and 527.36 ± 738.7 pg/ml) than the control group (76.29 ± 28.94 and 328.9 ± 430.8 pg/ml) ($p < 0.05$ and $p > 0.05$). There were no significant differences in serum and nasal neopterin levels ($p > 0.05$).

Conclusions Although there were no differences in the distribution of lymphocyte subsets between the AR and control groups, the finding of higher levels of serum and nasal IL-17 and lower levels of nasal IL-10 support the cytokine imbalance in the pathogenesis of AR.

Keywords Allergic rhinitis · Regulatory T cells · IL-10 · IL-17 · Neopterin

Introduction

Allergic rhinitis (AR), the most common form of non-infectious rhinitis, is characterised by sneezing, nasal congestion, nasal itching and rhinorrhea [1]. It is an immunoglobulin E (IgE)-mediated inflammation of the membranes lining the

nose. Antigen-presenting cells located in the nasal mucosa present allergens to naive T lymphocytes and they initiate a complex series of events, leading to the differentiation of effector T lymphocytes and the production of T helper 2 (Th2) cytokines and antigen-specific IgE [1].

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CD4+CD25+ regulatory T cells (Tregs) represent about 5–10% of human CD4+ T cells, and are commonly identified by the constitutive expression of interleukin-2 (IL-2) receptor α (IL-2Ra; CD25), as well as the transcription factor *scurfin*, encoded by the forkhead family transcription factor 3 (Foxp3) gene [2–5]. CD4+CD25+ Tregs are essential for healthy immune responses to environmental proteins and the generation of immune tolerance. They suppress the production of IgE and induce IgG4 isotype allergen-specific antibodies that are particularly mediated by interleukin-10 (IL-10) and transforming growth factor (TGF)- β .

The cytokine IL-17 is a proinflammatory Th1 cytokine that was shown to play a role in allergic and autoimmune diseases [6–9]. It activates the expression of a variety of proinflammatory cytokines, chemokines, and cell adhesion molecules by macrophages, dendritic cells, T cells, synovial cells, and endothelial cells.

IL-10 which is mainly produced by CD4+CD25+ Tregs, inhibits the expression of many proinflammatory cytokines and decreases the lifespan of eosinophils, supports the allergen tolerance of T cells, mediates allergen tolerance after allergen-specific immunotherapy and, conversely to these inhibitory effects, prolongs differentiation, proliferation and the lifespan of B cells and increases the production of IgG4 [10–18].

Neopterin is produced by human monocyte-derived macrophages and dendritic cells upon stimulation with interferons and due to its stability in biological fluids, it has become a useful marker in cell-mediated Th1-type inflammation, such as infectious and inflammatory diseases [19–21].

The aim of this study was to evaluate the role of Tregs in the pathogenesis of AR and their correlation with inflammatory and anti-inflammatory cytokines. Therefore, we compared the levels of blood T lymphocyte subsets, the ratio of CD4+CD25+ Tregs and IL-10, IL-17 and neopterin concentrations in serum and nasal lavage of patients with AR with the healthy subjects.

Methods

Patients

The study included AR and healthy subjects aged between 18 and 45 years. After a detailed otolaryngological examination, the subjects were evaluated by an immunology and allergy specialist for family history and for associated systemic allergies. The inclusion criteria for AR group were symptoms of rhinitis that should be related to SPT results, positive nasal endoscopic examination and at least one positive SPT and/or serum specific IgE. Subjects were classified as moderate to severe AR according to the ARIA report. They had remarkable symptoms that affect sleep pattern,

school performance and quality of life. Exclusion criteria were as follows: (a) use of intranasal or systemic steroids and/or antihistamine use within the last month; (b) having active rhinosinusitis; and (c) previous history nasal or sinus surgery. The control group included healthy subjects with no history of AR, no allergic symptoms and a normal endoscopic examination. Blood tests included peripheral blood cell counts, immunoglobulin (Ig) and specific IgE levels. All subjects underwent a skin prick test (SPT) with commercial extracts.

Collection of nasal lavage fluid

Nasal lavage was collected while the patient was seated and extended his neck approximately 45° forwards. During this procedure, the patient was not allowed to breathe or swallow. Nasal lavage fluid was collected by giving 5 cc isotonic saline solution to each nasal cavity. The supplied liquid was collected in a clean kidney cuvette.

Cytokine assay

The levels of cytokines (IL-10 and IL-17) and neopterin in serum and nasal lavage fluids were measured with enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC), respectively.

Flow cytometric analysis

Peripheral blood (PB) samples were collected into collection tubes containing sodium heparin. The PBMCs were prepared by Ficoll–Hypaque density centrifugation. Then, the mononuclear cells were carefully collected and washed with PBS and cells were stained with Human Treg Kit (Biolegend, San Diego). Monoclonal antibodies included CD3 peridinin chlorophyll protein (PerCp), CD4 allophycocyanin (APC), CD25 phycoerythrin (PE) and FOXP3 fluorescein isothiocyanate (FITC). Flow cytometry analysis was performed on an FACS Aria III flow cytometer (BD Biosciences, San Jose, USA). The data were further analysed in dot plots and histogram plots using the FACS Diva software package (Becton–Dickinson, CA, USA). Treg cells were defined as CD3+CD4+CD25+FOXP3+ (Fig. 1).

Statistical analysis

All the analyses were performed using a statistical software package (SPSS for Windows, version 16.0, SPSS Inc., USA). Numerical data were expressed as the mean $\pm$ standard deviation (SD). In the analysis of the data, χ^2 Chi square test, *T* test, NPar test and Mann–Whitney tests were used. Regulatory T cell levels and correlation of IL-10, IL-17 and

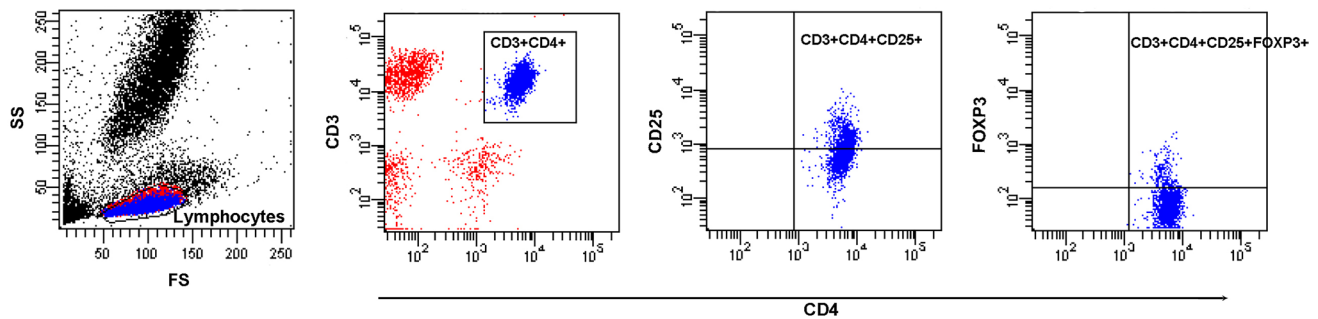


Fig. 1 Flow cytometry dot-plot and histogram surface marker analysis

neopterin levels were evaluated by Pearson and Spearman correlations. *p* values less than 0.05 indicate significance.

Results

The study included 38 subjects (20 women, 18 men) and 36 sex- and age-matched controls (19 women, 17 men). The mean age of the AR group was 25.55 ± 5.25 years (range 18–39 years) and 26.64 ± 5.9 years in the control group (range 18–40 years), with no statistically significant difference ($p = 0.4$). Complete demographic features of the

patients, including medication, smoking status, comorbidities and ARIA classifications are given in Table 1.

Flow cytometry results

The percentages of CD3 + cells, CD4 + cells and Tregs cells were not different between AR (61.86 ± 7.29 , 34 ± 5.87 and 2.32 ± 0.94 , respectively) and control groups (59.27 ± 8.71 , 33.6 ± 6.38 and 2.07 ± 0.77 , respectively) ($p > 0.05$) (Table 2).

Table 1 Demographic details of the AR group & controls (AR: Allergic rhinitis, AC: allergic conjunctivitis, CRS: Chronic rhinosinusitis, SPT: skin prick test, HDM: house dust mite)

	AR group	Controls
Gender (<i>n</i> , %)		
Female	20 (52.6%)	19 (52.7%)
Male	18 (47.4%)	17 (47.3%)
Age (years) (mean $\pm$ std)	25.55 ± 5.25	26.64 ± 5.89
ARIA classification (<i>n</i> , %)		
Moderate	14 (36.8%)	0
Severe	24 (63.2%)	0
Smoking (<i>n</i>)	0	0
Comorbidities (<i>n</i> , %)		
AC	16 (42.1%)	0
CRS	4 (10.5%)	0
Asthma	2 (5.3%)	0
None	16 (42.1%)	36 (100%)
Medication*	0	0
IgE (U/ml) Median (min–max)	235 (60–560)	24 (2.80–150)
SPT (<i>n</i> , %)		
Tree mixture	9 (23.6%)	0
Grass pollen	12 (31.6%)	0
HDM	10 (26.4%)	0
Other	7 (18.4%)	0
Negative	0	36 (100%)
Eosinophil# (K/uL) Median (min–max)	0.32 (0.15–0.97)	0.06 (0.01–0.13)

*Not using any medication within last month

Table 2 Flow cytometry and ELISA results

	Control	Allergic Rhinitis	<i>p</i>
CD3+CD4+CD25+FOXP3+	2.07 ± 0.77	2.32 ± 0.94	0.23
CD3+CD4 ⁺	33.6 ± 6.38	34 ± 5.87	0.87
CD3+	59.27 ± 8.71	61.86 ± 7.29	0.17
Nasal Neopterin	0.44 ± 0.13	0.42 ± 0.16	0.21
Serum Neopterin	5.85 ± 1.58	6.53 ± 2.16	0.24
Nasal IL-17	328.9 ± 430.8	527.36 ± 738.7	0.14
Serum IL-17	76.29 ± 28.94	107.7 ± 79.61	0.002
Nasal IL-10	3.12 ± 1.45	2.22 ± 0.91	0.002
Serum IL-10	32.02 ± 32.06	41.51 ± 40.65	0.27

ELISA results

Mean serum IL-10 levels were similar between the two groups, whereas nasal IL-10 was lower in AR subjects compared to healthy subjects (2.22 ± 0.91 and 3.12 ± 1.45 pg/ml, respectively) ($p < 0.05$). Mean serum and nasal IL-17 were higher in the AR (107.7 ± 79.61 and 527.36 ± 738.7 pg/ml) than the control group (76.29 ± 28.94 and 328.9 ± 430.8 pg/ml; $p = 0.002$ and $p = 0.14$). However, there were no significant differences in serum and nasal neopterin levels between the AR and control groups ($p > 0.05$, both).

Discussion

In the present study, serum IL-17 level was higher and nasal IL-10 level was lower in patients with AR compared to the healthy control group. Meanwhile, no significant difference was found between the AR and control groups in terms of nasal IL-17 and serum IL-10 levels. In addition, there were no significant differences in the rates of total T cells, CD3+ T cells, CD4+ T cells and Tregs between AR group and the control group. There was no significant difference in neopterin levels both in serum and in nasal lavage fluid.

Allergic diseases such as asthma and AR are characterized by a Th2-skewed immune environment. Th2-type cytokines are upregulated in allergic inflammation, IL-17 is a cytokine released by the Th17 subgroup of CD4+ T cells and play a pathological role in allergic diseases [6–9]. Serum IL-17 level was found to be increased in patients with allergic asthma compared to the non-asthmatic control group [6]. IL-17 level in sputum was found to be correlated with airway hyperresponsiveness [7]. Li et al. showed that Th17 response decreased after specific immunotherapy in patients with house dust mite-induced AR [8]. In another study, the rates of T cells that produce IL-17 and serum IL-17 levels were found to be higher in the allergic group [9].

IL-10 is an inhibitor cytokine that plays an important role in the suppression of allergic reactions [11]. IL-10 decreases IL-4-induced IgE switching [14]. Rapid switch

and expansion of IL-10-producing Tregs and the use of suppressive factors represent essential mechanisms in immune tolerance to a high dose of allergens [15]. In the study of Takanashi et al. IL-10 was shown to inhibit survival of, and cytokine production from, eosinophils induced by lipopolysaccharide [16]. In the study of Hussein et al. the IL-10RA gene SNP S138G was shown to contribute to susceptibility to atopic diseases [17]. Grunig et al. found airway hyperactivity, eosinophilia and an exaggerated Th2-type response in mice with IL-10 deficiency [18]. In the study of Baumann et al. [22] the nasal lavages of 15 symptomatic allergic and 14 nonallergic subjects were collected during the pollination season and nasal secretion was collected from six allergic subjects after nasal challenge test. After challenge, allergic subjects had a significant immediate response of nasal symptoms and a significant increase of IL-4, IL-10, IL-17 and eotaxin-3. During natural pollen exposure, eotaxin-3 and MCP-4 levels were significantly elevated and IL-10 and, unexpectedly, IL-4 were significantly lower in allergic subjects compared with nonallergic subjects. In the study by Genç et al., the IL-10 level was decreased in the peripheral blood mononuclear cell culture in the AR group compared to the control group, while no significant difference was found in the nasal fluid [23]. In our study, no significant difference was found in serum IL-10 levels between the control and patient groups, while nasal lavage IL-10 levels were significantly lower in the patient group. A low nasal IL-10 level was consistent with the studies published in the literature, and it indicated that the cells producing IL-10 should be separately evaluated in the pathogenesis. In our study, the serum IL-17 level was significantly higher in the AR group than the control group, while no significant difference was found in nasal lavage fluid, while nasal IL-10 was lower and serum IL-10 was not significant in patients with AR, suggesting that the factors inducing allergic inflammation were systemic and its inhibition occurred locally.

Neopterin, which is a pteridine derivative, increases in many diseases as a specific marker of monocyte/macrophage activation [20]. Increased neopterin levels are found in body fluids as a result of stimulation by IL-2 and IFN- γ ,

which are the main cytokines of cellular immunity. It has been shown that neopterin is elevated in infections caused by viruses, parasites and intracellular bacteria, malignancies, autoimmune diseases, allograft rejection and infections that may develop after transplantation and acute coronary syndrome [21]. Neopterin secretion begins 3 days before T cell proliferation, and it can be detected in serum 2–4 weeks before specific antibody formation [21]. In the study of Unuvar et al. neopterin, tryptophan, and kynurenine levels were found to be higher in patients with asthma and AR than in controls [24]. In this study, we could not find any differences in serum and nasal lavage fluid neopterin levels between AR and control groups.

Treg cells play a basic role in response to allergens by stimulating Th2 cells and the maintenance of immune tolerance by preventing cytokine secretion [25]. Treg cells have a crucial role, where peripheral tolerance is important, such as in allergic diseases, including asthma, AR, autoimmune diseases and transplant rejection [26, 27]. Lee et al. [28] and Xu et al. [3] found a decrease in Treg in allergic patients. Lee et al. found that decreased numbers of T(reg) cells in children with allergic airway disease might represent a defect of the T(reg) population while Xu et al. reported that their results indicate that CD4(+) CD25(+) regulatory T cells as well as Foxp3 may play a crucial role in immunological imbalance of AR and increasing Foxp3 and CD4(+) CD25(+) T cells have the potential to be new therapeutic targets for the treatment of AR.

Zhang et al. [29] found a significant decrease in Treg cells in the acute exacerbation period compared to the stable disease stage in asthmatic patients. Akdiş et al. found a decrease in the number of allergen-specific Treg cells in allergic persons [30]. In the study of Stelmazczyk-Emmel et al., Treg concentration was lower in allergic patients compared to the healthy group, and showed a significant correlation with serum IgE concentration [31].

In the study of Genc et al. the percentage of FoxP3(+) Tregs were found to be lower in AR patients compared with healthy subjects [23]. In PBMC culture supernatants, IL-10 levels were significantly lower, whereas IL-4, IL-5, and TNF- α levels in nasal lavage fluids were higher in AR patients compared with healthy subjects ($p < 0.05$).

However, there are some authors reporting no significant difference in Tregs between allergic and nonallergic populations [32–35].

In conclusion, although there were no differences in the distribution of lymphocyte subsets between the AR and control groups, the finding of higher levels of serum and nasal IL-17 and lower levels of nasal IL-10 support the inflammatory and antiinflammatory cytokine imbalance in the pathogenesis of AR. Systemic and local inflammatory cytokine increase with a relative decrease of anti-inflammatory IL-10

might play a determinant role in the development of AR symptoms.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee of Selcuk University (2015/65) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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