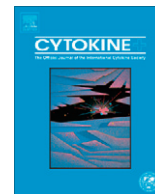


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Bronchial hyperresponsiveness in seasonal allergic rhinitis patients is associated with increased IL-18 during natural pollen exposure

Emel Kurt^{a,*}, Kurtuluş Aksu^a, Ali Dokumacioglu^b, Ozkan Alatas^b^a Departments of Allergy, Faculty of Medicine, Eskisehir Osmangazi University, Turkey^b Clinical Biochemistry, Faculty of Medicine, Eskisehir Osmangazi University, Turkey

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ABSTRACT

Background: The mechanism of bronchial hyperresponsiveness (BHR) is not certain in seasonal allergic rhinitis (SAR) patients.

Objective: We aimed to investigate the effects of natural pollen exposure on IL-18 and its relationship with BHR.

Methods: Thirty-two SAR patients with grass pollen sensitivity, 14 nonallergic rhinitis (NAR) patients and 17 normal-controls were included. Sixteen SAR patients had BHR during pollen season and off-season. Serum IL-18 levels were measured in SAR patients during pollen season between May–August and off-season between November–February. IL-18 levels were measured in NAR patients and normal controls once.

Results: During pollen season, SAR patients with BHR had significantly increased levels of IL-18 than those without BHR (279.2 ± 161.1 versus 145.3 ± 101.0 pg/ml, $p = 0.012$). Serum IL-18 levels were not different between SAR patients with and without BHR during off-season (233.8 ± 139.7 versus 183.2 ± 162.9 pg/ml, $p = 0.16$). Serum IL-18 levels in SAR patients during pollen season (212.3 ± 148.8 pg/ml) and off-season (208.5 ± 151.5 pg/ml) were not different than those NAR patients (224.8 ± 180.1 pg/ml, $p = 0.98$ and $p = 1.0$, respectively) and normal controls (174.8 ± 76.0 pg/ml, $p = 0.60$ and $p = 0.76$, respectively).

Conclusion: The results suggested us that BHR in SAR patients is associated with increased IL-18 during natural pollen exposure.

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1. Introduction

Although bronchial hyperresponsiveness (BHR) is a characteristic feature of asthma, patients with allergic rhinitis (AR) have also BHR even if they have no asthma symptoms. Asthma is the most common comorbid disease with AR [1]. Presence of BHR in AR is considered as a risk factor for asthma development [2,3]. Patients with seasonal allergic rhinitis (SAR) have increased BHR during pollen season that strengthens the thought that natural pollen exposure causes BHR. We previously showed that changes in the number of eosinophils and eosinophil apoptosis in induced sputum samples were associated with natural pollen exposure and might have a role in the seasonal changes of BHR in patients with SAR [4]. Although the seasonal increase of BHR during pollen season is well known in SAR patients, mechanism of BHR in these patients is not explained exactly. BHR is also observed in SAR patients during off-season when the patients are not exposed to allergens. The

mechanism of BHR in off-season is also not known exactly. We previously stated that IL-10 have a role in the continuation of BHR out of pollen season in SAR patients [5].

During the process of airway sensitivity, lymphocytes accumulate and send stimulus to eosinophils [6]. Cytokines play a crucial role in modulating these allergic and asthmatic inflammatory reactions. During the course of airway hyperresponsiveness a variety of Th2-type cytokines have been shown to play role [7]. Interleukin (IL)-18, previously named as IFN- γ inducing factor, has been shown to exert autoimmune regulatory activity on both Th1 and Th2 cytokines suggesting a complex role on Th1 and Th2 inflammatory responses [8,9]. IL-18 has effects on IFN- γ , proinflammatory cytokines, and other cytokines probably depending on the environment [10]. By these effects, IL-18 exerts a Th1-type response requiring co-stimulation with IL-12, IL-15 or IL-2, and a Th2 type response without IL-12 and IL-23 [11]. Murine models have indicated that IL-18 can increase allergic sensitization, serum IgE and Th2 type inflammation in the airways [8,12]. Moreover, when administered with allergen via nasal route, IL-18 activates Th1 cells to produce Th2-related cytokines and induces airway hyperresponsiveness with increase of eosinophils in their broncho-

* Corresponding author. Address: Allergy Department, Faculty of Medicine, 26040 Eskisehir, Turkey. Tel.: +90 2222392979; fax: +90 2222394714.

E-mail address: dremelkurt@yahoo.com (E. Kurt).

alveolar lavage fluid in mice [13]. We proposed that SAR patients with BHR might show different IL-18 profile during pollen season when they are exposed to allergen. In this study, we aimed to investigate the effect of natural pollen exposure on serum IL-18 and whether there was a relationship between IL-18 and BHR during pollen season and off-season in patients with SAR.

2. Methods

2.1. Patients

Three groups of subjects were selected: Thirty-two SAR patients, 14 nonallergic rhinitis (NAR) patients, and 17 normal-nonallergic controls. Only SAR patients with pollen allergy have been included in the study. The inclusion criteria for SAR patients were the following: having historical data defined by nasal itching, rhinorrhea, sneezing, and nasal obstruction for at least 2 years; positive physical examination finding with positive skin prick tests (SPTs) to grass pollen only; and no sensitization to perennial allergens.

NAR patients had perennial symptoms such as nasal itching, rhinorrhea, sneezing, and nasal obstruction for at least 2 years, but none of them had any allergic sensitivity determined by either historical data or SPTs.

Seventeen nonallergic–nonasthmatic subjects were taken as normal-controls. Patients were restricted to those living in Eskişehir to exclude possible effect of varying severity of pollen concentration on inflammatory parameters. All patients and controls did denied any history of chronic systemic disease. None of the SAR patients had previously been treated with immunotherapy. None of the subjects were smoker.

At the beginning of the study, the patients were asked for asthmatic symptoms and patients thought to be asthmatic by the medical story were excluded. None of the subjects did report any upper or lower respiratory tract infection and use of nasal or oral steroids during the previous 6 weeks or within the study period. During the study, none of the patients used nasal or oral corticosteroids that might affect BHR. Intranasal azelastin spray was allowed when necessary to suppress nasal symptoms during pollen season. The study was approved by the local ethics committee and informed consent was obtained from the subjects.

2.2. Evaluation of atopy

SPTs were performed by using a common panel including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, grass, tree and weed pollens, animal dander (cat, dog), animal feathers, and mold (including *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*) allergen extracts (ALK, Spain). Positive and negative controls were histamine (10 mg/ml) and phenolated glycerol saline, respectively. A mean wheal diameter of 3 mm or greater than that of obtained with control solution was considered as positive.

2.3. Bronchial provocation test (BPT)

Methacholine bronchial challenges were performed by a modification of the method described by Chai et al. [14]. A dosimeter (Zan 200; Zan, Oberthulpa, Germany), triggered by a valve set to remain open for 0.6 s, was used to deliver the aerosol. After inhalation of physiologic saline, subjects inhaled doubling concentration of methacholine from 0.06 mg/ml to 8 mg/ml diluted in buffered saline. Forced expiratory volume in one second (FEV₁) was measured with a spirometer (Alpha III; Vitalograph, Buckingham, England), and the largest value of triplicate FEV₁ after 60–90 s of each inhalation was adopted for analysis. The challenge was

stopped when the FEV₁ dropped by >20% from the post-physiologic saline or when the highest concentration of methacholine had been administered. The result was expressed as methacholine PC₂₀ (provocative concentration requires to produce a 20% fall in FEV₁). The PC₂₀ was obtained from the log concentration–percent fall in FEV₁ curve by linear interpolation of the last two points. Subjects were considered to have BHR if they showed a decline in FEV₁ of >20% at a concentration of methacholine of <8 mg/mL. At the end of the challenge, patients received 200 µg of salbutamol from a metered-dose inhaler to relieve airway obstruction.

2.4. Serum IL-18 detection

Blood samples were obtained from all patients and controls after an overnight fast by venipuncture. Samples were routinely centrifuged at 2500 g for 15 min in order to separate serum and stored at –80 °C until analysis. IL-18 levels in these serum samples were measured using a commercially available ELISA (Bender Med Systems, Vienna, Austria). The samples were processed according to the manufacturer's instructions. The lower limit of detection of this IL-18 assay was 9.2 pg/ml. The intra- and interassay coefficients of variation of this assay were 6.5% and 8.1%, respectively.

2.5. Study design

SAR patients: Peripheral blood samples to determine eosinophil and IgE levels and to detect serum IL-18 level have been obtained during the off-season between November and February and the pollen season between May and August. These seasonal intervals were selected according to the data regarding peak and off-grass pollen season in our city [15]. BPT with methacholine was also measured in both seasons. Only those SAR patients who had BHR and those who did not have BHR during both seasons were included in the study. Sixteen SAR patients had BHR and 16 did not have BHR during both seasons.

NAR patients and normal-controls: BPT and serum samples have been obtained once at any time regardless of the season.

Nasal symptoms, rhinorrhea, nasal blockage, and sneezing were recorded by rhinitis patients over a period of 4 weeks. SAR patients recorded symptom scores during the pollen season. The scores graded from 0 to 3 according to the severity considering the whole day (0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, and 3 = severe symptoms). Mean nasal symptom scores were calculated as the sum of scores divided by the number of days recorded for each patient. SAR patients were allowed to take topical nasal azelastine and ophthalmic olopatadine as required during pollen season. Drugs were stopped 24 h before the study during pollen season.

2.6. Statistical analysis

The results were presented as mean ± standard deviation (SD). Multiple comparisons between groups were made using Kruskal–Wallis analysis. Two-sided comparisons were made using the Mann–Whitney U test, chi-square (χ^2) tests, and parametric and nonparametric types of *t*-test for unpaired data when appropriate. The pollen season and off-pollen season values of SAR patients were compared using Wilcoxon's paired *t*-test. P value less than 0.05 was considered as statistically significant. SPSS for Windows (version 13.0; SPSS Inc. Chicago, IL) was used for the calculations.

3. Results

Mean ages were not different between groups ($p = 0.11$) (Table 1). Duration of disease and nasal symptom scores were not different

Table 1

Characteristics of the subjects in each study group.

	Seasonal allergic rhinitis	Nonallergic rhinitis	Normal controls
N	32	14	17
Gender (female/male)	22/10	8/6	10/7
Age (year)	31.0 ± 9.3	34.9 ± 10.3	37.2 ± 10.5
Duration of disease (year)	5.7 ± 3.6	5.1 ± 3.3	–
Nasal symptom score, mean	1.39 ± 0.49	1.21 ± 0.47	–

between those SAR and NAR patients ($p = 0.67$ and $p = 0.16$ respectively) (Table 1).

Blood eosinophil numbers and serum total IgE levels were higher in SAR patients during pollen season when compared with off-season ($p < 0.001$ and $p = 0.017$ respectively) (Table 2). Blood eosinophil numbers were not different in NAR patients than SAR patients during both seasons ($p = 0.23$ and $p = 0.42$, respectively) (Table 2). NAR patients had lower total serum IgE levels when compared with SAR patients during pollen season ($p = 0.001$) and off-season ($p = 0.01$) (Table 2). Serum IL-18 levels were not different between groups ($p = 0.98$) (Table 2).

Mean age of the patients ($p = 0.34$) and mean duration of the disease ($p = 0.96$) were not different between those patients with BHR and without BHR (Table 3). There was no statistical difference in respect to nasal symptom scores between BHR positive and negative SAR patients ($p = 0.61$) during pollen season (Table 3). Blood eosinophil numbers ($p = 0.38$ and $p = 0.42$, respectively) and serum total IgE levels ($p = 0.32$ and $p = 0.17$, respectively) were not different between those patients with and without BHR during both seasons (Table 3). During pollen season, SAR patients with BHR had significantly increased levels of IL-18 than those patients without BHR ($p = 0.012$). Power analysis of this outcome showed 78% power when $\alpha = 0.05$. Serum IL-18 levels were not different between SAR patients with and without BHR during off-season ($p = 0.16$).

When we considered SAR patients during pollen season, serum IL-18 levels were not correlated with severity of rhinitis obtained by nasal symptom scores ($r = 0.19$, $p = 0.31$), blood eosinophil numbers ($r = 0.22$, $p = 0.22$), and total IgE levels ($r = -0.09$, $p = 0.64$). In addition, there was positive correlation between nasal symptom scores and the number of blood eosinophils ($r = 0.45$, $p = 0.011$). There was no correlation between serum IL-18 levels and nasal symptom score ($r = 0.34$, $p = 0.24$), blood eosinophil numbers ($r = 0.48$, $p = 0.08$), and total IgE levels ($r = 0.38$, $p = 0.18$) in NAR patients.

4. Discussion

Even though BHR is a characteristic feature of asthma, it can be seen in SAR patients without asthma symptoms as in the present study [4,5]. The relationship between BHR and airway inflammation in the asthmatic patients is well known. But, the pathogenesis of BHR in AR patients is not clear. The proposed mechanisms for BHR in AR patients are the contraction of bronchial smooth muscle by mediators releasing from inflammatory cells reaching to the bronchi directly or by blood circulation and eosinophilic inflammation of the airways [4,16,17]. In the present study, BHR in SAR pa-

tients was not associated with blood eosinophil numbers, total IgE levels, and rhinitis severity during pollen season. Showing BHR also continues during off-season confirmed that BHR occurs as a result of systemic effects of allergic inflammation rather than rhinitis or its severity. This opinion was also supported by the result that there was no relation between BHR and the severity of rhinitis which was determined by nasal symptom scores during pollen season. However, SAR patients with BHR had increased levels of IL-18 during pollen season indicating that allergen exposure causes BHR through IL-18 related mechanisms.

IL-18 have pleiotrophic role in Th1 and Th2 responses depending on cytokine milieu. IL-18 is produced by monocytes/macrophages, and IL-18 receptor is expressed on variety of cells including Th1 cells, naive T cells, B cells, dendritic cells, macrophages, neutrophils, NK cells, endothelial, and smooth muscle cells [11]. IL-18 appears to have a variety of immunological effects on both Th1 and Th2 cytokines, suggesting a complex role on Th1 and Th2 inflammatory responses [8,9]. IL-18 has effects on IFN- γ , proinflammatory cytokines, and other cytokines probably depending on the environment [10]. By these effects, IL-18 exerts a Th1-type response requiring co-stimulation with IL-12, IL-15 or IL-2, and a Th2 type response without IL-12 and IL-23 [11]. Besides, IL-18 is able to generate Th2 responses under certain conditions and promote allergic inflammation through the stimulation of IL-4, IL-13, and histamine release [18]. Several studies have demonstrated that IL-18 activity is increased in Th2 type diseases such as asthma, allergic rhinitis, and atopic dermatitis [19–21].

IL-18 may show Th2-type inflammatory response in the airways when given together with allergen. When administered with ragweed allergen, IL-18 has been shown to increase intrapulmonary eosinophilia and airway mucus and induces IgE response in a mouse model [9]. Verhaeghe et al. [22] demonstrated that IL-18 expression in nasal secretion was increased after natural allergen exposure in SAR patients suggesting enhanced expression of IL-18 modulated by allergen exposure. Our study also confirmed that IL-18 may contribute to BHR in SAR patients with natural allergen exposure during pollen season. This is also supported with our data that IL-18 was not associated with BHR during off-season when the patients are not exposed pollen allergen. The mechanism of BHR in these patients during off-season when there is no allergen exposure is not certain yet. We previously suggested that continuation of BHR in SAR patients during off-season in which there is no allergen exposure is probably related to IL-10 dependant regulatory mechanisms on airway inflammation [5].

Several cytokines mediate allergic inflammation and bronchial hyperresponsiveness [23]. Previous studies have shown that

Table 2

Blood eosinophils, total IgE levels, bronchial hyperresponsiveness, and serum IL-18 levels in each group.

	Seasonal allergic rhinitis (N = 32)	Nonallergic rhinitis (N = 14)	Normal controls (N = 17)
	Off-season		
Blood eosinophils (mm ⁻³)	193.8 ± 123.7	265 ± 212.5	–
Total IgE (IU/ml)	177.3 ± 250.7	66.2 ± 76.3	–
BHR, yes/no	16/16	2/12	0/17
Serum IL-18 (pg/ml)	208.5 ± 151.5	224.8 ± 180.1	174.8 ± 76.0

Table 3
Blood eosinophils, serum total IgE, and IL-18 levels in seasonal allergic rhinitis (SAR) patients with and without bronchial hyperresponsiveness during off-season and pollen season.

	Off-season Bronchial hyperresponsiveness		Pollen season Bronchial hyperresponsiveness	
	Yes (n = 16)	No (n = 16)	Yes (n = 16)	No (n = 16)
Age (year)	32.6 ± 8.4	29.4 ± 10.1		
Duration of the disease (year)	5.7 ± 3.6	5.6 ± 3.6		
Nasal symptom score	1.43 ± 0.48	1.34 ± 0.53	–	–
Blood eosinophils (mm ⁻³)	224.4 ± 151	163.1 ± 82.6	379.4 ± 253.9	293.8 ± 198.2
Total IgE (IU/ml)	107.7 ± 82.9	240.9 ± 339	126.7 ± 89.7	277.4 ± 374.1
Serum IL-18 (pg/ml)	233.8 ± 139.7	183.2 ± 162.9	279.2 ± 161.1*	145.3 ± 101

* p = 0.012 (SAR patients with BHR had higher serum IL-18 level than those without BHR during pollen season).

IL-18 might regulate inflammation in the airways by modulating cytokine responses. Kodama et al. [24] have shown that administration of IL-18 causes increased levels of IL-5 in the bronchoalveolar lavage fluid in OVA-sensitized mice. IL-4 and IL-13 have important role in allergic inflammation. Yamagata et al. [13] found diminished levels of OVA-specific IgE in IL-18 deficient mice together with low levels of IL-4 and IL-13 in bronchoalveolar lavage than those in wild-type mice, suggesting that IL-18 might affect airway inflammation through the release of these cytokines. Another study has shown that IL-18 increases airway inflammation in sensitized mice by increasing antigen-induced TNF- α production [12].

It can be thought that IL-18 may be elevated as a cause of active disease state in pollen season. However, IL-18 level did not change significantly after pollen season when there is no rhinitis symptoms in SAR patients. In addition, rhinitis severity was not correlated with serum IL-18 levels in SAR patients and NAR patients. We included those SAR patients in whom BHR did not change during both seasons to minimize the effects of airway inflammation on the release of IL-18 that may be involved not only as a pathogenic mechanism but also as a result of underlying condition. One limitation of the study is the power of the study, which was 78% for those SAR patients with BHR had higher IL-18 levels than those without BHR during pollen season. This result suggested us that the 78% of studies would be expected to yield a significant effect, reflecting the null hypothesis that the two population (the patients with and without BHR) means are equal. This result is a little bit lower than the expected (80%).

The pathogenesis of allergic airway hyperresponsiveness is complex, involving variety of cytokines and chemokines interacting various cell types. The present study showed that IL-18 has a role on BHR during pollen season when SAR patients are exposed to allergen naturally. Although the patients had BHR during off-season, IL-18 does not seem to be related with BHR when they are not exposed to allergen. The results suggested that BHR in SAR patients during pollen season and off-season has different mechanisms probably as a result of allergen exposure.

References

- [1] Marple BF. Allergic rhinitis and inflammatory airway disease: interactions within the unified airspace. *Am J Rhin Allergy* 2010;24:249–54.
- [2] Slavin RG. The upper and lower airways: the epidemiological and pathophysiological connection. *Allergy Asthma Proc* 2008;29:553–6.
- [3] Greenberger PA. Allergic rhinitis and asthma connection: treatment implications. *Allergy Asthma Proc* 2008;29:557–64.
- [4] Kurt E, Bavbek S, Aksu Ö, et al. The effect of natural pollen exposure on eosinophil apoptosis and its relationship to bronchial hyperresponsiveness in patients with seasonal allergic rhinitis. *Ann Allergy Asthma Immunol* 2005;95:72–8.
- [5] Kurt E, Aktas A, Gulbas Z, et al. The effects of natural pollen exposure on inflammatory cytokines and their relationship with nonspecific bronchial hyperresponsiveness in seasonal allergic rhinitis. *Allergy Asthma Proc* 2010;31:126–31.
- [6] Wills-Karp M. Immunological basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999;17:255–81.
- [7] Cockcroft DW, Davis BE. Mechanism of airway hyperresponsiveness. *J Allergy Clin Immunol* 2006;118:551–9.
- [8] Kohno K, Kataoka J, Ohtsuki T, et al. IFN- γ -inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. *J Immunol* 1997;158:1541–50.
- [9] Wild JS, Sigounas A, Sur N, et al. IFN- γ -inducing factor (IL-18) increases allergic sensitization, serum IgE, Th2 cytokines, and airway eosinophilia in a mouse model of allergic asthma. *J Immunol* 2000;164:2701–10.
- [10] Fantuzzi G, Dinarello CA. Interleukin-18 and interleukin-1 β : two cytokine substrates for ICE (caspase-1). *J Clin Immunol* 1999;19:1–10.
- [11] Boraschi D, Dinarello CA. IL-18 in autoimmunity: review. *Eur Cytokine Netw* 2006;17:224–52.
- [12] Kumano K, Nakao A, Nakajima H, et al. Interleukin-18 enhances antigen induced eosinophil recruitment into the mouse airways. *Am J Respir Crit Care Med* 1999;160:873–8.
- [13] Sugimoto T, Ishikawa Y, Yoshimoto T, et al. IL-18 acts on memory Th1 cells to induce airway inflammation and hyperresponsiveness in a native host mouse. *J Exp Med* 2004;199:535–45.
- [14] Chai H, Farr RS, Froehlich LA, et al. Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975;56:323–7.
- [15] Bicakci A, Erken S, Malyer H. Airborne pollen grains of Eskisehir. In: 1st International symposium on protection of natural environment and Ehirami Karacam, 23–25th September 1999, Kutahya, Turkey, p. 315–20.
- [16] Boulet L, Turcotte H, Lampron Laviolette M. Influence of natural antigenic exposure in bronchoalveolar lavage in subjects with pollen induced rhinitis. *J Allergy Clin Immunol* 1990;86:19–25.
- [17] Foresi A, Leone C, Pelucchi A, et al. Eosinophils, mast cells and basophils in induced sputum from patients with seasonal allergic rhinitis and perennial asthma: relationship to metacholine responsiveness. *J Allergy Clin Immunol* 1997;100:58–64.
- [18] Izakovica HL. Interleukin-18 in asthma and other allergies. *Clin Exp Allergy* 2003;33:1023–5.
- [19] Tanaka H, Miyazaki N, Oashi K, et al. IL-18 might reflect disease activity in mild and moderate asthma exacerbation. *J Allergy Clin Immunol* 2001;107:331–6.
- [20] Ando M, Shima M. Serum interleukins 12 and 18 and immunoglobulin E concentrations and allergic symptoms in Japanese schoolchildren. *J Invest Allergol Clin Immunol* 2007;17:14–9.
- [21] Yoshizawa X, Nomaguchi H, Izaki S, et al. Serum cytokine levels in atopic dermatitis. *Clin Exp Dermatol* 2002;27:225–9.
- [22] Verhaeghe B, Gevaert P, Holtapples G, et al. Up-regulation of IL-18 in allergic rhinitis. *Allergy* 2002;57:825–30.
- [23] Nakajima H, Takatsu K. Role of cytokines in allergic airway inflammation. *Int Arch Allergy Immunol* 2007;142:265–73.
- [24] Kodama T, Matsuyama T, Kuribayashi K, et al. IL-18 deficiency selectively enhances allergen-induced eosinophilia in mice. *J Allergy Clin Immunol* 2000;105:45–53.