

Eosinophilic Inflammation, Remodeling of Lower Airway, Bronchial Responsiveness and Cough Reflex Sensitivity in Non-Asthmatic Subjects with Nasal Allergy

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Key Words

Nasal allergy · Eosinophilic inflammation · Lower airway remodeling · Bronchial responsiveness · Cough reflex sensitivity

Abstract

Background: It has been reported that nasal allergy influences the lower airway inflammation and functions. We elucidated whether nasal allergy would contribute to lower airway inflammation and functions. **Methods:** 266 subjects aged 21–39 years were interviewed with special emphasis on history of asthma and nasal allergies (perennial allergic rhinitis (PAR) and seasonal allergic rhinitis (Japanese cedar pollinosis; PO)). Symptomatic subject was defined when nasal symptoms were present during a 3-week study period. Pulmonary function, provocative concentration of methacholine causing a 20% fall in forced expiratory volume in 1 s (PC_{20}), capsaicin cough threshold defined as capsaicin concentration eliciting 5 or more coughs (C5) and eosinophil percentage in hypertonic saline-induced sputum were measured. **Results:** Based on the interview, 232 subjects without asthma were divided into symptomatic ($n = 25$) and asymptomatic ($n = 22$) PAR, PO on-season ($n = 15$) and off-season ($n = 36$), and non-nasal allergy subjects (control) ($n = 134$).

Sputum eosinophils were significantly greater in symptomatic PAR than another four groups ($p < 0.01$). FEV_1/FVC ratio was significantly lower in PAR than control ($p < 0.05$). Maximum mean expiratory flow was lower in PAR than control (asymptomatic: $p < 0.05$, symptomatic: $p = 0.06$). C5 was not different among groups. PAR tended to have a lower PC_{20} compared to control (symptomatic: $p = 0.078$; asymptomatic: $p = 0.086$). **Conclusions:** These results suggest that eosinophilic inflammation occurred in symptomatic period of PAR may contribute to development of lower airway remodeling and bronchial hyperresponsiveness. Reversely, PO may not be associated with lower airway eosinophilic inflammation or abnormal bronchial functions. Nasal allergy dose not influence the cough reflex sensitivity.

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Introduction

Asthma and allergic rhinitis (AR) are common comorbidities, suggesting the concept of 'one airway, one disease'. Epidemiological studies have shown that as many as 78% of asthma patients have nasal symptoms and approximately 38% of AR patients have asthma [1]. And in longitudinal follow-up, patients with AR are three times more

likely to develop asthma as compared with subjects without AR [2]. Eosinophilic inflammation has been demonstrated in the lower airways of symptomatic perennial AR [3] and seasonal on-season AR patients [4, 5] without asthma. Subjects with AR who never experienced symptoms of asthma have been shown to have increased bronchial responsiveness to pharmacological stimuli [6, 7].

In Japan house dust (HD), mite and Japanese cedar pollen are the main allergens to cause nasal allergy. HD and mite are important allergens of perennial AR and Japanese cedar pollen is the most important antigen of seasonal AR and cedar pollinosis. Nakamura et al. [8] mentioned that morbidity of perennial AR and Japanese cedar pollinosis were 19.8 and 17.3%, respectively, in Japan. It has been reported that the severities of laryngeal symptoms (e.g. persistent cough) varied directly with the Japanese cedar pollen counts [9].

To our knowledge, it is unknown whether Japanese cedar pollinosis without asthma contributes to the lower airway inflammation and function. Besides there is no report that to elucidate whether AR contributes to cough reflex sensitivity to capsaicin. Therefore, the present study was conducted to examine whether the two types of nasal allergy would contribute to lower airway inflammation, function and cough reflex sensitivity.

Methods

Subjects

A total of 266 students of Kanazawa University Graduate School of Medical Science, aged 21–39 years (mean age 24.1 years), were interviewed with special emphasis on history of asthma and nasal allergies (perennial allergic rhinitis (PAR) and seasonal allergic rhinitis (pollinosis; PO)). Symptomatic PAR was defined when nasal symptoms (rhinorrhea, nasal itching, sneezing and nasal blocking) were present during a three-week study period. Seasonal AR was defined as Japanese cedar pollinosis (PO). PO on-season was defined when nasal symptom was present during a 3-week study period. Diagnosis of asthma was done based on GINA guidelines [10]. 34 subjects (12.8%) had a present or past history of asthma (former asthma; 23 subjects (8.6%), current asthma 11 subjects (4.1%)). Asthmatic subjects were excluded from this investigation. The study was approved by the Ethics Committee of Kanazawa University, and informed consent was obtained from all subjects after the purpose of the test had been explained.

Study Design

A pulmonary function, the methacholine concentration producing a 20% fall in forced expiratory volume in 1 s (FEV_1) (PC_{20}), the capsaicin concentration causing 5 or more coughs (C5) and eosinophil percentage in hypertonic saline-induced sputum were measured within 3 weeks in a random order.

Measurement of Cough Reflex Sensitivity

Cough reflex sensitivity to capsaicin was measured by the method described previously by us [11]. Capsaicin (Wako Pure Chemical Industries Ltd., Tokyo, Japan) (30.5 mg) was dissolved in Tween 80 (1 ml) and ethanol (1 ml) and then in normal saline (8 ml) to make a stock solution of 3.05 mg/ml (1×10^{-2} M), which was stored at -20°C . This solution was diluted with physiological saline to give solutions of 0.49, 0.98, 1.95, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1,000 μM . Subjects inhaled progressively increasing concentrations of the capsaicin solution for 15 s by mouth tidal breathing every 60 s from a Bennett twin nebulizer (3012-60 ml, Puritan-Bennett Company, Carlsbad, Calif., USA), until they coughed at least five times. The nebulizer output was 0.21 ml/min. Number of coughs induced by each concentration of capsaicin solution was counted by two medical technicians. Cough threshold was defined as the lowest concentration of capsaicin (C5) that elicited five or more coughs as an index of cough reflex sensitivity.

Measurement of Bronchial Responsiveness

The methacholine provocation test was done according to a previously published method [12]. Methacholine chloride was dissolved in physiological saline solution to make concentrations of 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80 and 160 mg/ml. Saline and each methacholine solution were inhaled from a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, Pa., USA) operated by compressed air at 5 liters/min. The nebulizer output was 0.14 ml/min. Saline was inhaled first for 2 min and partial and full flow-volume curves were measured on a dry wedge spirometer (Chestac 11, Chest Co., Ltd., Tokyo, Japan). If the change in FEV_1 from the baseline value was 10% or less, inhalation of methacholine was started, and if the saline solution caused a change in $FEV_1 > 10\%$, the test was stopped or postponed. Methacholine was inhaled for 2 min by tidal mouth breathing with the patients wearing a nose clip, and this was followed immediately by three measurements of partial and full flow-volume curves at 1-min intervals; the curve with the largest FVC was retained for analysis. Increasing concentrations of methacholine were inhaled until a decrease of 20% or more in FEV_1 occurred. Methacholine provocative concentration producing a 20% fall in FEV_1 (PC_{20}) was calculated.

Sputum Induction

Induction of sputum was done according to a method described previously by us [13]. Hypertonic saline (5%) was nebulized with a compressor nebulizer (Omron, Mie, Japan) during 20–60 min. When subjects felt sticking in the throat, they were asked to try to cough sputum into a Petri dish. By this procedure most subjects successfully produced sputum. Subjects who had not been able to bring up material at the end of a 60-min period were released. The specimens were considered adequate if alveolar macrophages were detected and the percentage of squamous cells was less than 10% among nucleated cells. Sputum was transferred to slides in small amounts and finely distributed over two microscopic slides and smeared. Each smear was air-dried and stained by the May-Grünwald-Giemsa method. Differential cell counts were performed by an observer blind to the clinical characteristics of the subjects.

Data Analysis

Data except for C5 and PC_{20} were shown as mean $\pm$ SD. C5 and PC_{20} values were expressed as geometric means with the geometric standard error of the mean (GSEM) expressed as a factor. Logarithm

Table 1. Characteristics of subjects

	Control	Perennial allergic rhinitis subjects		Japanese cedar pollinosis subjects	
		Symptomatic	Asymptomatic	On-season	Off-season
Subjects	134 (57.8%)	25 (10.8%)	22 (9.5%)	15 (6.5%)	36 (15.5%)
Age, years	24.3 ± 2.3	23.6 ± 1.4	23.7 ± 1.8	23.9 ± 1.0	24.4 ± 2.9
Sex, male/female	93/41	17/8	18/4	13/2	29/7
Height, cm	168 ± 9.1	168 ± 7.2	169 ± 9.1	171 ± 6.2	170 ± 8.1
Body weight, kg	60.5 ± 11	61.2 ± 8.6	60.9 ± 8.5	60.3 ± 9.3	61.6 ± 5.5

Data are expressed as mean ± SD.

Table 2. Pulmonary function, methacholine bronchial responsiveness (PC₂₀), and capsaicin cough threshold (C5)

	Control	Perennial allergic rhinitis subjects		Japanese cedar pollinosis subjects	
		Symptomatic	Asymptomatic	On-season	Off-season
FVC, % predicted	112 ± 12.5	113 ± 12.4	111 ± 9.2	111 ± 9.5	111 ± 13.6
FEV ₁ , % predicted	101 ± 13.5	99 ± 11.0	99 ± 9.3	100 ± 7.8	105 ± 12.2
FEV ₁ /FVC, %	89 ± 6.2	85 ± 8.2*	85 ± 5.9*	88 ± 6.7	89 ± 7.1
%MMF	100 ± 20.4	92.2 ± 24.4**	89.1 ± 17.4*	98.8 ± 18.9	102 ± 16.4
PC ₂₀ , mg/ml	46.6 (1.14)	27.0 (1.39)	8.8 (1.63)	27.5 (1.57)	62.8 (1.30)
C5, μM	15.3 (1.12)	17.6 (1.29)	25.5 (1.29)	17.4 (1.52)	18.6 (1.23)

FVC = forced vital capacity; FEV₁ = forced expiratory volume in 1 second; MMF = maximum mid-expiratory flow rate.

PC₂₀ = provocative concentration of methacholine causing a 20% fall in FEV₁; C5 = provocative concentration of capsaicin eliciting five or more coughs.

Data are expressed as mean ± SD except for C5 and PC₂₀ which are expressed as geometric mean values with geometric standard error of the mean in parenthesis.

* p < 0.05 vs. control subjects, ** p = 0.06 vs. control subjects.

mically converted values of C5 and PC₂₀ were used in assessing the relationships and differences between these by simple regression analysis and analysis of variance (ANOVA), respectively. p < 0.05 was taken as significant.

Results

Subject Characteristics

Based on the interview, 232 subjects who had not experienced asthma, aged 21–39 years (mean age 24.2 years), were divided into symptomatic (n = 25) and asymptomatic (n = 22) PAR, PO on-season (n = 15) and off-season (n = 36), and non-nasal allergy (control) subjects (n = 134). A summary of characteristics of subjects is given in table 1.

Pulmonary Function Tests

As shown in table 2, no difference in FVC or FEV₁ was detectable between five groups. PAR subjects had a significant lower FEV₁/FVC ratio compared to control and PO off-season subjects (p < 0.05). PAR had a lower maximum mean expiratory flow (MMF) compared to control (asymptomatic vs. control: p < 0.05, symptomatic vs. control: p = 0.06).

Cough Reflex Sensitivity

There were no differences between the five groups in C5 (table 2).

Bronchial Responsiveness

PAR subjects tended to have lower PC₂₀ than control subjects (symptomatic PAR vs. control: p = 0.078, asymptomatic AR vs. control: p = 0.086). PC₂₀ was not different

Fig. 1. PC₂₀ in subjects with asymptomatic and symptomatic perennial allergic rhinitis, off-season and on-season Japanese cedar pollinosis and control (Mann-Whitney U test). Horizontal bars indicate median values.

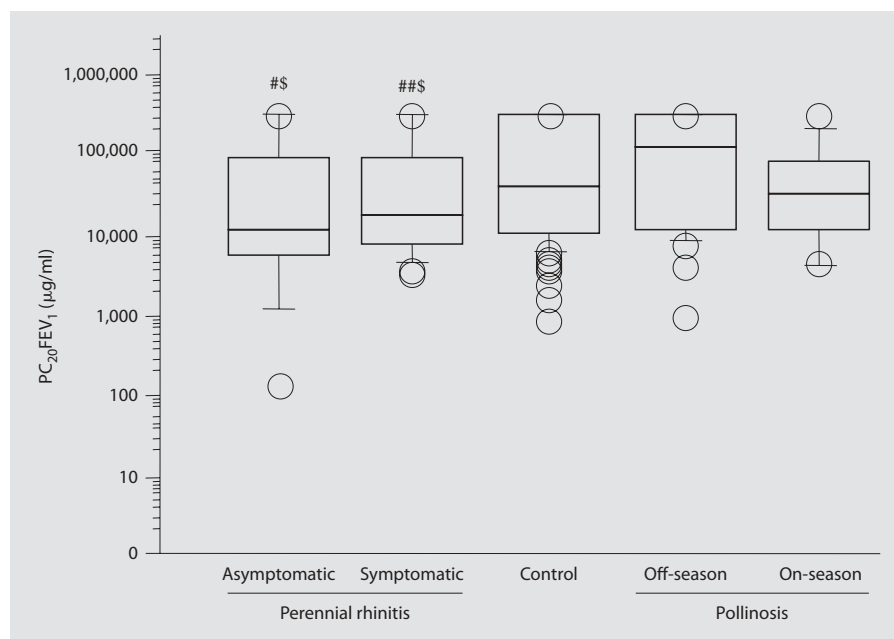
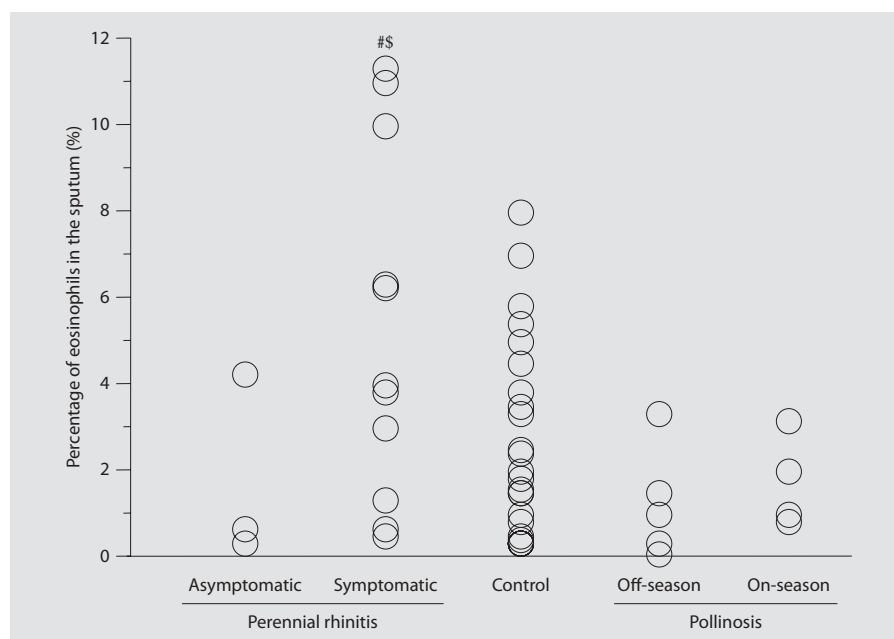


Fig. 2. Differences in eosinophil percentage in hypertonic saline-induced sputum among asymptomatic and symptomatic perennial allergic rhinitis subjects, off-season and on-season Japanese cedar pollinosis subjects and control subjects (Mann-Whitney U test). Horizontal bars indicate median values.



between PO on-season and off-season and control subjects (fig. 1).

Induced Sputum Eosinophils

Sputum eosinophils were significantly ($p < 0.01$) greater in symptomatic PAR than in asymptomatic PAR and control subjects. No difference was detected between asymptomatic PAR, PO on-season and off-season and control subjects (fig. 2).

Relationship between Sputum Eosinophils, Bronchial Responsiveness and Cough Reflex Sensitivity

In 232 subjects, we examined the correlation between sputum eosinophil ratio, log PC₂₀ and C5. The correlation between log PC₂₀ and percentage of eosinophils were significant ($r = -0.248$, $p < 0.01$) (fig. 3). On the other hand, there was no significant relationship between C5 and percentage of eosinophils ($r = 0.049$, $p = 0.55$) (data not shown).

Fig. 3. Correlation between eosinophil percentage in hypertonic saline-induced sputum and methacholine PC₂₀. r = Spearman's rank correlation coefficient.

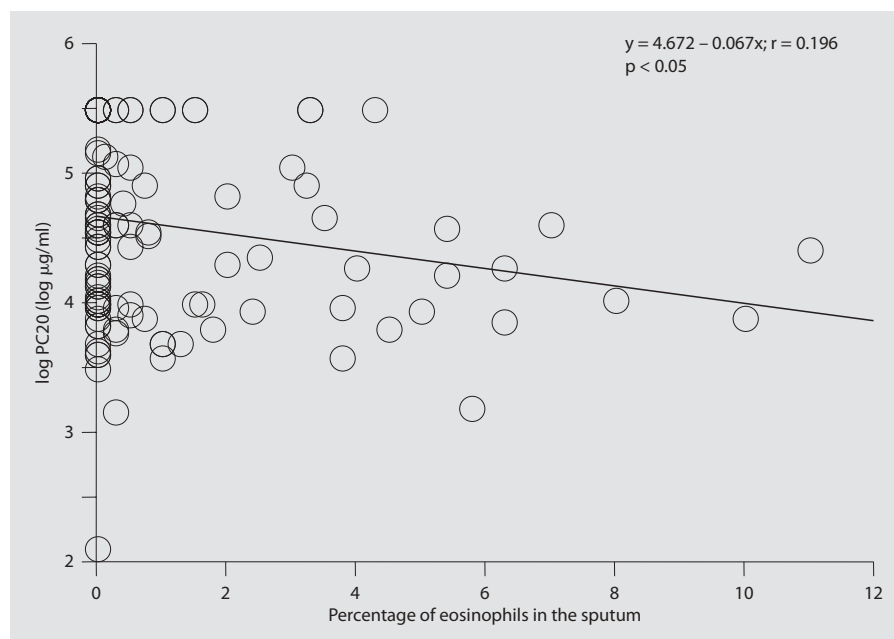
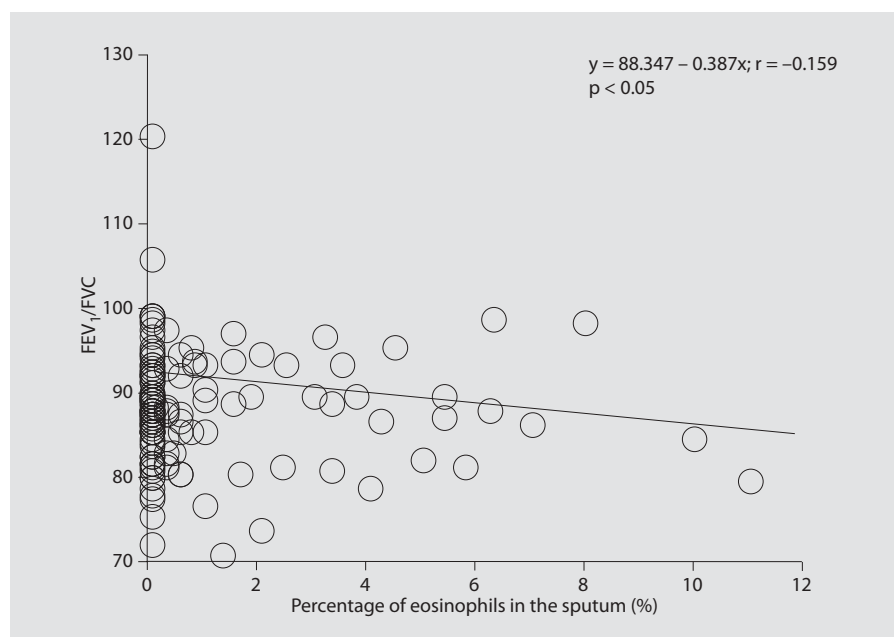


Fig. 4. Correlation between eosinophil percentage in hypertonic saline-induced sputum and FEV₁/FVC. r = Spearman's rank correlation coefficient.



Relationship between Sputum Eosinophils, FEV₁/FVC Ratio and MMF

In 232 subjects, we also examined the correlation between sputum eosinophil ratio, FEV₁/FVC ratio and MMF as percentage of predicted value (%MMF). There was weak relationship between percentage of eosinophils and FEV₁/FVC ratio ($r = -0.159$, $p < 0.05$) (fig. 4), but not between percentage of eosinophils and %MMF ($r = -0.116$, $p = 0.1655$) (data not shown).

Discussion

In this study, we demonstrated that eosinophils were detected in the induced sputum of nonasthmatic subjects with symptomatic PAR. Subjects with PAR had a lower FEV₁/FVC ratio and MMF and tended to have increased bronchial responsiveness compared to control subjects. On the other hand, these findings were not investigated in PO on-season or off-season subjects. Thus, the present

study showed that variability of lower airway eosinophilic inflammation, airway narrowing, and bronchial hyperresponsiveness were present between PAR and PO subjects (Japanese cedar pollinosis) in the same Japanese population. There were no differences in cough reflex sensitivity to capsaicin among PAR subjects, PO subjects and control subjects. Furthermore, there were significant relationships between sputum eosinophils, bronchial responsiveness to methacholine and FEV₁/FVC ratio.

It has been previously reported that a large number of eosinophils are present in the lower airway of nonasthmatic patients with on-season seasonal AR [4, 5], off-season seasonal AR [14] and symptomatic perennial AR [3]. Gibson et al. [15] exhibit allergic rhinitis as the cause of eosinophilic bronchitis. Structural changes of the lower airways, i.e. basement membrane thickness and epithelial desquamation in symptomatic perennial AR patients [3] and subepithelial airway collagen deposition [16, 17], were observed in off-season seasonal AR patients. Many of the subjects with perennial AR show reduced MMF value [18], which is considered a marker of small airways impairment in mild asthmatics with normal FVC values [19]. And it is known that bronchial hyperresponsiveness is present in on-season seasonal AR [4, 20] and perennial AR [21] without asthma. Several studies have reported the relationship between sputum eosinophils and bronchial responsiveness to methacholine [22, 23], and FEV₁/FVC ratio [24] in asthmatic subjects. These reports suggest that the airway remodeling partly caused by allergic inflammatory process including eosinophilic inflammation may contribute to the development of bronchial hyperresponsiveness both in seasonal AR and perennial AR patients as well as asthma. But, in our PO subjects, sputum eosinophilia, lower airway remodeling and bronchial hyperresponsiveness were not demonstrated. Most of previous reports targeted seasonal AR patients who were sensitized to *Parietaria*, grasses, olive tree, birch, hazel tree and ragweed pollen, etc. Generally, the diameter of Japanese cedar pollen is about 30 µm that is larger in size than above pollens. In addition, the pollen count is small in Hokuriku area located in the Japan Sea side compared with the Ocean Sea side of Japan. Therefore, in our study, the Japanese cedar pollen could not induce the eosinophilic inflammation in the lower airway. On the other hand, because perennial antigens, i.e. house dust or mite, are small enough to reach the lower airways and exposed continuously, eosinophilic inflammation could be induced in the lower airways of PAR subjects.

Forty to seventy percent of Japanese cedar pollinosis patients complain of laryngeal symptoms (e.g. persistent

cough and foreign body sensation). The severity of cough changes in parallel with seasonal change of cedar pollen counts. Cough might be one of the symptoms of laryngeal allergy induced by cedar pollen [9]. It appears that there is considerable overlap between laryngeal allergy and atopic cough [25]. The defining physiological feature is increased cough reflex sensitivity without bronchial hyperresponsiveness in atopic cough [25]. In our study, cough reflex sensitivity to capsaicin was not different between symptomatic and asymptomatic PAR, PO on-season and off-season and control subjects even though the counts of pollen is small in our area. And there was no relationship between sputum eosinophil percentage and cough reflex sensitivity to capsaicin in subjects without asthma in our study. It has been reported that no significant relationship between cough receptor sensitivity and eosinophilic inflammation existed in subjects with non-productive cough [13] or mild allergic asthma [26]. Therefore, eosinophilic inflammation might not be a critical factor for regulating airway cough receptor sensitivity to capsaicin in nasal allergy. There is no report about cough reflex sensitivity in laryngeal allergy. Further investigation is required to elucidate whether allergic inflammation of the larynx actually increases cough reflex sensitivity.

In conclusion, the results of this study suggest that eosinophilic inflammation of lower airway may contribute to development of lower airway remodeling and bronchial hyperresponsiveness in symptomatic perennial AR. On the other hand, seasonal AR may not be associated with lower airway eosinophilic inflammation or functions. Nasal allergy does not influence the cough reflex sensitivity to capsaicin.

Acknowledgement

This study was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture (17607003) by the Japanese Government.

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