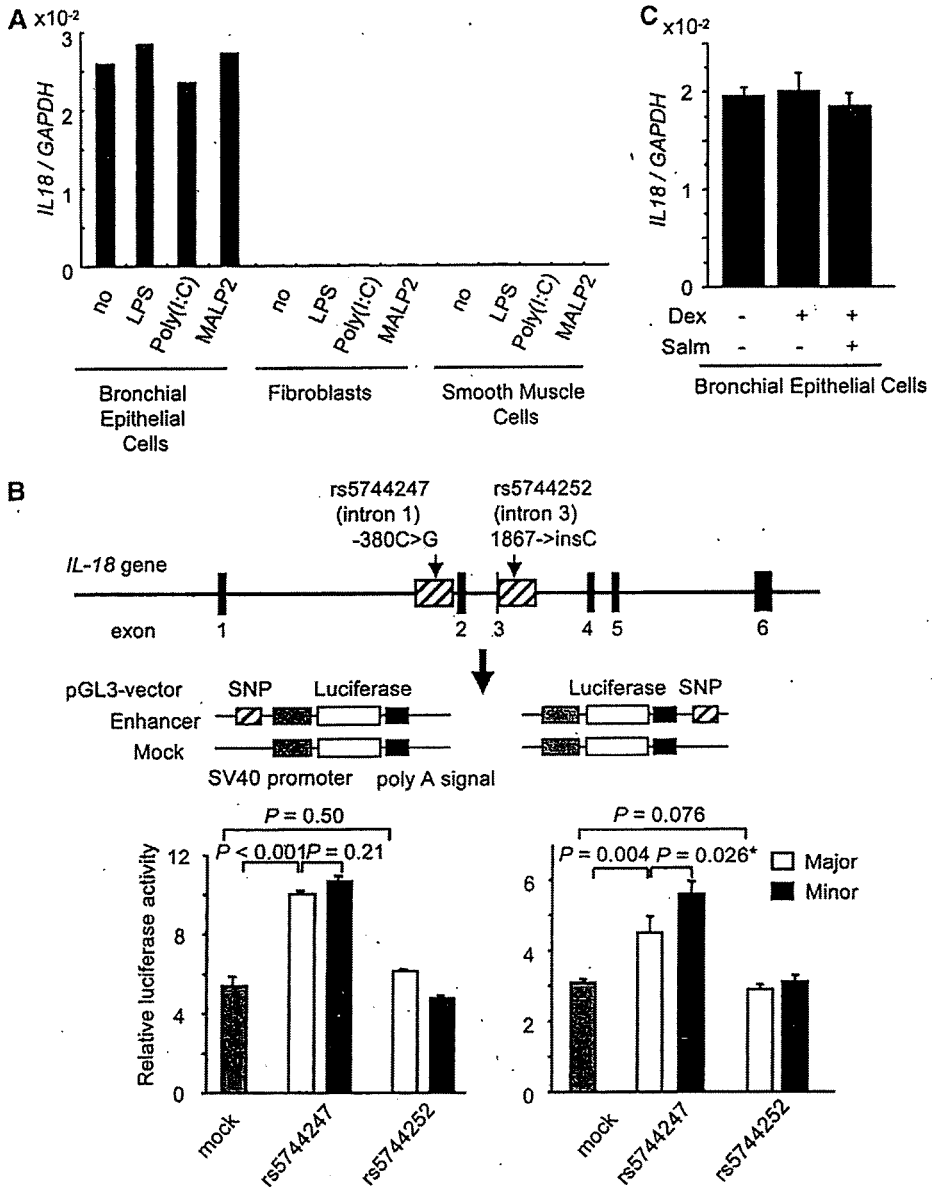

Figure 2. (*A*) *IL-18* mRNA expression analyses of normal human bronchial epithelial cells (NHBE), normal human lung fibroblasts (NHLF), and bronchial smooth muscle cells (BSMC) stimulated. with 100 ng/ml LPS, 10 μg/ml poly(I:C) or 1 μg/ml macrophage-activating lipopeptide-2 for 4 hours. NHBE, NHLF, and BSMC were each derived from one individual, respectively. Data represent means of duplicate samples. Two independent experiments were performed with similar results. (*B*) Enhancer activity of *IL-18* introns 1 and 3 in NHBE, containing rs5744247 and rs5744252 variants. Schematic representation of the reporter constructs and relative luciferase activities of the two SNPs. Values represent the means ± SD of three independent experiments. The asterisk (*) indicates a significant difference between minor and major alleles ($P = 0.026$) by Student *t* test. (*C*) Effects of dexamethasone (Dex) and salmeterol (Salm) on *IL-18* mRNA expression levels in NHBE. Cells were treated with dexamethasone and salmeterol for 0.5 hours before addition of 100 ng/ml LPS. Values represent the means ± SD using NHBE derived from four individuals. GAPDH = glyceraldehyde 3-phosphate dehydrogenase; MALP = macrophage-activating lipopeptide-2; SNP = single nucleotide polymorphism.

of subjects with severe asthma in the prospective cohort study appeared to be lower than that in our study using clinic-based samples, and the difference might have affected the results. In a Japanese population, an association study of the *IL-18* gene was conducted using three polymorphisms, rs1946518, rs187238, and rs549908 (Ser35Ser), with 221 children and 276 adults with asthma and 85 adult control subjects (20). The study screened only polymorphisms in the coding and promoter regions of the *IL-18* gene, and found a significant association between rs549908 (Ser35Ser) and asthma susceptibility. Among the three SNPs, we could not find any association between the *IL-18* variants and adult asthma susceptibility; however, we found a significant association between rs1946518 and asthma severity. Association between the SNPs and asthma severity was not examined in the earlier study (20), and sample size might account for the contradictory results. Furthermore, according to the data of Hapmap (www.hapmap.org), the allele frequencies of SNP rs5744247 in Japanese, Han Chinese, Yoruba people, and the Centre d'Etude du Polymorphisme Humain population are 34%, 53%, 1%, and 9%, respectively. Thus the functional effect of the rs5744247 variant on asthma severity might be specific to the Japanese population.

Recent studies have shown important roles of bronchial epithelial cells as both mediators and regulators of innate immune responses and adaptive immune responses (39), and constitutive expression of IL-18 protein was observed within airway epithelial cells (40, 41). In this study, human *IL-18* was highly expressed by airway epithelial cells, and we could confirm the enhancer-like effects of the rs5744247 variant and significantly greater transcriptional activity of the susceptible G allele in NHBE. Although the majority of individuals with asthma are well controlled with current therapies, the existent therapeutic strategies are inadequate for those with severe asthma (42). Approximately 5% to 10% of the asthmatic population is in the severe end of the disease spectrum and it is difficult to control the asthma with maximal inhaler therapy (43, 44). Combination therapy with long-acting β_2-agonists and inhaled corticosteroids reduces exacerbation frequency in asthma, and it is also efficacious as intervention therapy for exacerbation of the disease (33, 34, 45). The suppressive effect

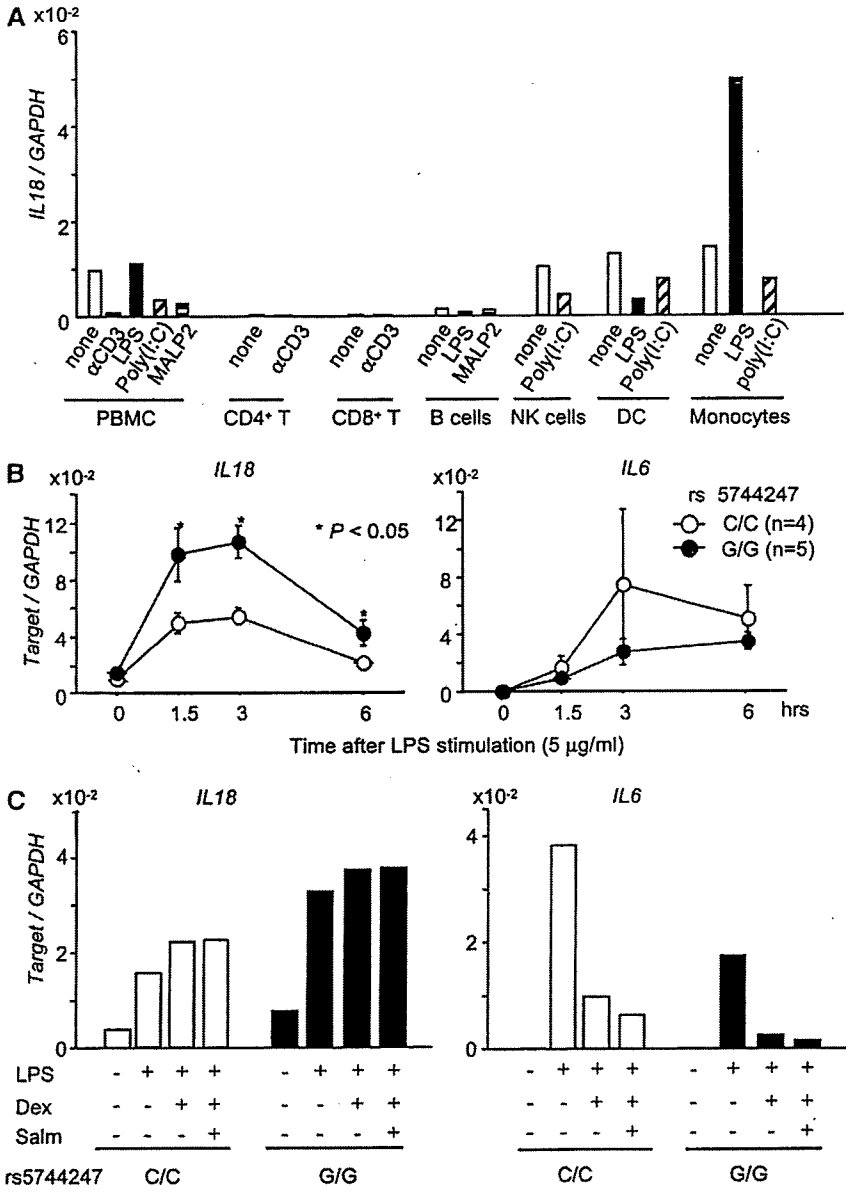

Figure 3. (*A*) Quantitative reverse transcriptase–polymerase chain reaction assays of *IL-18* in immune cells. The expression levels were normalized with glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) expression. Freshly isolated immune cells were stimulated using 1 μg/ml plate-bound anti-CD3 monoclonal antibody (mAb) with 1 μg/ml soluble anti-CD28 mAb, 5 μg/ml LPS, 25 μg/ml poly(I:C), or 1 μg/ml macrophage-activating lipopeptide (MALP)-2 for 5 hours. Data were averaged among duplicate samples and are representative of two independent experiments. Similar results were obtained using immune cells from two individuals. (*B*) Relationship of rs5744247 genotype to *IL-18* mRNA expression level. The *IL-18* and *IL-6* mRNA expression levels were measured in monocytes stimulated with 5 μg/ml LPS for the indicated time. The asterisks (*) represent $P = 0.014$, 0.014, and 0.033, respectively, by the Mann-Whitney *U* test. (C) Effects of dexamethasone (Dex) and salmeterol (Salm) on *IL-18* and *IL-6* mRNA expression levels in monocytes stimulated with 5 μg/ml LPS. Data are means in duplicate from an individual. Two independent experiments each using human monocytes, homozygous for C ($n = 4$) and homozygous for G ($n = 3$), were performed with similar results.

of steroids mediated by glucocorticoid response elements within the *IL-6* promoter has been reported (46). In the present study, we could confirm the effects of DEX and SAL on *IL-6* expression in LPS-stimulated monocytes; however, DEX and SAL were not able to suppress *IL-18* mRNA expression in either monocytes or NHBE cells. A recent study has shown that corticosteroid-resistant (CR) asthma is associated with classic antimicrobial activation of airway macrophages, and higher endotoxin levels are detected in bronchoalveolar lavage fluid from subjects with CR asthma (47). The study implies that prolonged exposure to LPS might contribute to CR asthma (47). In human monocytes, we here demonstrated that the mRNA expression of *IL-18* was highly induced by LPS and identified a -380C>G (rs5744247) SNP that had an allele-specific effect on mRNA expression. Primary monocytes from subjects homozygous for the susceptible -380G allele exhibited significantly high expression of *IL-18* mRNA in response to LPS. In addition, the induction of *IL-18* mRNA in monocytes was not suppressed by DEX and SAL. IL-18 is involved in severe asthma through functional polymorphism and might contribute to enhanced innate immunity and both Th1- and Th2-driven immune responses. It is likely that targeting IL-18 itself might be therapeutically efficacious as a new treatment for severe asthma.

Recent studies have reported elevated circulating levels of IL-18 in patients with allergic diseases (22–25). Serum IL-18 levels are higher in patients with acute asthma than in control subjects and the IL-18 level has a tendency to inversely correlate with peak expiratory flow (22, 23). In patients with atopic dermatitis, serum IL-18 levels are elevated and the levels are correlated with disease severity and with the number of eosinophils in peripheral blood (25). In another study, IL-18 secretion from mononuclear cells of patients with bronchial asthma and atopic dermatitis was significantly higher than that in nonallergic controls (48). In this study, we found a positive correlation between the serum IL-18 level and *IL-18* rs5744247 genotype. Although we randomly recruited 88 subjects with asthma who provided serum samples, in the subgroup examined for serum IL-18, asthma tended to be more severe than in the genotyped group. There might be a subpopulation selection bias with regard to disease severity, and the bias might influence the positive correlation between serum levels of the IL-18 protein

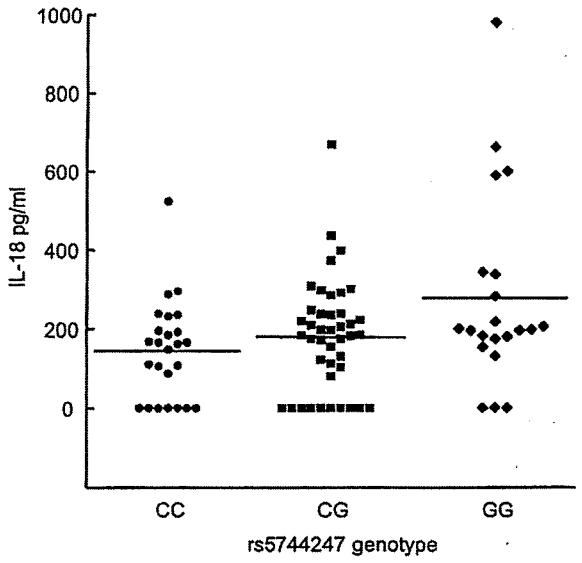

Figure 4. Relationship between rs5744247 genotype and serum IL-18 level. Serum levels of the IL-18 protein positively correlated with rs5744247 genotype by the Jonckheere-Terpstra trend test (P = 0.031).

and the *IL-18* genotype. Thus, validation studies of the connection between the serum IL-18 protein level and genotype are needed in a large number of samples.

We concluded that a genetic variant in the *IL-18* gene appears to influence the serum level of IL-18 and the asthma severity, putatively by altered enhancer activity in NHBE and increased *IL-18* mRNA expression in monocytes in response to LPS. Further investigations of IL-18 function would be helpful to understand the pathophysiology of inflammatory diseases whose development and progression are affected by microbial infections.

Conflict of Interest Statement: M.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.O. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. T.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. T.Y. received up to $1,000 from Boehringer Ingelheim in lecture fees and $5,001–$10,000 from Novartis Foundation Japan in grants. Y.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.D. received up to $1,000 from Ono Pharmaceutical Co., Ltd., up to $1,000 from Sanofi Aventis, and up to $1,000 from Kyorin Pharmaceutical Co., Ltd. in lecture fees. A.M. received up to $1,000 from GlaxoSmithKline and up to $1,000 from Banyu Pharmaceutical Co., Ltd. in lecture fees. K.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. T.E. received $1,001–$5,000 from Sanofi Aventis, $5,001–$10,000 from Kyowa Hakko Kirin Pharma, $1,001–$5,000 from Boehringer Ingelheim, $1,001–$5,000 from Dainippon Sumitomo Pharma, and up to $1,000 from Ono Pharmaceutical Co., Ltd. in lecture fees. M.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. N.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. Y.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.N. received $1,001–$5,000 in consultancy fees from Abbott, $1,001–$5,000 from Nippon Boehinger Ingelheim, $1,001–$5,000 from Nippon Boehinger Ingelheim, $1,001–$5,000 from Astellas Pharm, $1,001–$5,000 from Kyowa Kirin, and $1,001–$5,000 from Dainippon Sumitomo Seiyaku in lecture fees. Y.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.T. received up to $1,000 from Kyorin Pharmaceutical Co., Ltd. and up to $1,000 from GlaxoSmithKline in lecture fees.

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ORIGINAL PAPER Asthma and Rhinitis

Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis

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Summary

Background IL-33, an IL-1-like cytokine, is a ligand for IL1RL1, which is an important effector molecule of type 2 T helper responses. Although IL-33/IL1RL1 interaction has been suggested to be important in induction of allergic airway inflammation, serum levels of IL-33 and the genetic influences of the polymorphisms of *IL-33* in human allergic diseases are unclear.

Objective The aim of this study was to examine whether the serum IL-33 level and polymorphisms in *IL-33* are associated with Japanese cedar (JC) pollinosis, the most common form of allergic rhinitis, and a major public health problem, in Japan.

Methods We performed linkage disequilibrium (LD) mapping of the gene using the HapMap database, and two selected tag single nucleotide polymorphisms were genotyped. We conducted an association study of IL-33 (JC pollinosis, $n = 170$; normal controls, $n = 100$) and measured the IL-33 levels in sera of the 270 subjects by ELISA.

Results Serum levels of IL-33 were significantly higher in patients with JC pollinosis ($P = 0.0018$) than in controls. In genetic association analysis, we found a positive association between the polymorphism and JC pollinosis ($P = 0.048$).

Conclusion Our results support a role for IL-33 in the pathogenesis of JC pollinosis.

Keywords association, IL-33, JC pollinosis, polymorphism, serum level
Submitted 28 August 2007; revised 3 February 2008, 31 July 2008; accepted 3 September 2008

Introduction

Allergic diseases are increasing world-wide, and Japanese cedar (JC) pollinosis, which is a disease of allergic rhinitis and allergic conjunctivitis caused by exposure to JC pollen, is one of the most common allergic diseases in Japan [1]. The increase of JC pollinosis in Japan has become a social problem, with a prevalence of > 16% [1]. Allergic rhinitis is the result of an inflammatory reaction triggered by type 2 T helper (Th2) cell-mediated immune responses against allergens [2]. Th2 cytokines induce B cells to produce high amounts of IgG4 and IgE in humans, and promote the growth and differentiation of mast cells and eosinophils [3, 4]. IL1RL1 belongs to the IL-1 receptor family and functions as an important effector molecule of Th2 responses [5–8]. IL-33, an IL-1-like cytokine, has been identified as a ligand for IL1RL1, and can be detected in epithelial cells from the bronchi and small airways, which indicates a possible role in the regulation of mucosal function [9]. It activates NF-κB and mitogen-activated protein kinases, and drives production of Th2-associated cytokines from *in vitro* polarized Th2 cells via IL1RL1 [9]. *In vivo* analysis has demonstrated that IL-33 strongly induces gene expression of Th2-associated cytokines such as IL-4, IL-5 and IL-13, and IL-33-treated mice have significantly higher serum levels of IgE. In addition, IL-33 induces pathological changes in mucosal organs such as the lung, resulting in hypertrophied epithelial lining of the airways with large amount of mucus,

and those changes are primarily restricted to the bronchi and larger bronchioles [9].

Although very little work has been done with IL-33, intensive studies of IL-33 receptor IL1RL1 have shown its regulatory functions in the development and effector phases of Th2 responses [10]. The *IL1RL1* gene encodes a soluble-secreted protein, IL1RL1, and a transmembrane protein, ST2L [11]. In murine models of allergic airway inflammation, increases in endogenous IL1RL1 protein after allergen exposure modulate Th2-mediated airway inflammation [12], and blockade of the binding of the ligand for ST2L using a recombinant IgG fusion protein inhibits allergic inflammation [6, 7]. Other studies have reported that ST2L is a reliable selective marker of both murine and human Th2 lymphocytes in allergic airway inflammation [7, 13]. Moreover, a study has revealed that soluble IL1RL1 acts as a negative regulator of Th2 cytokine production via IL-33 signalling in allergic airway inflammation. In asthmatic patients, serum levels of soluble IL1RL1 are markedly elevated during acute attacks and the magnitude of the elevation correlates with the reduction of pulmonary functions and increased levels of serum IL-5 [14]. These findings imply that IL-33 is a good candidate for involvement in JC pollinosis, an allergen-induced upper airway inflammation.

A large number of association studies using polymorphic markers have been performed to discover genetic components in the pathogenesis of allergic diseases [15–17]. Recently, we have reported that functional single nucleotide polymorphisms (SNPs) in the IL1RL1 distal promoter region are associated with atopic dermatitis. The genetic variants regulate IL1RL1 expression, and immunohistochemical staining of a skin biopsy specimen from an atopic dermatitis patient showed IL1RL1 staining in keratinocytes as well as in cells infiltrating the dermal layer [18]. However, there have been no genetic association studies with IL-33.

In this study, to test whether genetic variations of IL-33 contribute to susceptibility to JC pollinosis, we first selected a genetic polymorphism of IL-33 using HapMap linkage disequilibrium (LD) data and conducted association studies. In addition, we examined the associations between serum IL-33 levels and JC pollinosis and serum total IgE levels.

Methods

Study subjects

All subjects were recruited from residents of Eiheiji-cho, in Fukui prefecture, in the central area of Japan between May and June 2006. Because these participants were workers of the Fukui University hospital and students of nursing and medical colleges in Fukui, the number of females was higher than that of males. Specific IgE to seven aeroallergens, *Cryptomeria japonica, Dermatophagoides pteronyssinus, Dermatophagoides farinae, Candida albicans, Aspergillus fumigatus, Dactylis glomerata* and *Ambrosia*, were measured with a Pharmacia CAP System (Pharmacia CAP, Uppsala, Sweden) (Table 1). Positive sensitization refers to an allergen-specific serum IgE level >0.7 (CAP RAST score of 2). Diagnosis of JC pollinosis was confirmed by symptoms of allergic rhinoconjunctivitis during the JC pollinosis season and positive serum-specific IgE towards JC pollinosis. A total of 170 patients with JC pollinosis were recruited (Table 1). One hundred healthy subjects who had never had symptoms of allergic rhinitis and showed no sensitization to any of the seven aeroallergens were recruited as controls (Table 1). We recruited 29 subjects with infectious rhinitis who were diagnosed by otolaryngologists and showed no sensitization to any of the seven aeroallergens. All individuals were unrelated Japanese and gave written informed consent to participate in the study according to the rules of the process committees at the School of Medicine, University of Fukui, the Nippon Medical School and The Institute of Physical and Chemical Research.

Selection of polymorphisms for genotyping

Genomic DNA was prepared from peripheral blood samples, using standard protocols. There were 22 SNPs in the *IL-33* gene with a minor allele frequency (MAF) of $>10\%$ in the HapMap Japanese data set (URL: http://www.hapmap.org/index.html.en) (Table 2). Pairwise LD was calculated as r^2 by using the Haploview 3.2 program (http://www.broad.mit.edu/mpg/haploview/). Genotyping of SNPs was performed by the TaqMan™ allele-specific amplification (TaqMan-ASA) method (Applied Biosystems, Foster City, CA, USA). rs1929992 was genotyped by Custom TaqMan® SNP Genotyping Assay Service with primers 5′-GGAAAAAAACACATTTTCCCCCCAA-3′ and 5′-AAACCATCTTAACTACTACTTAAAATGTATAAAGTGT TAGAATTAT-3′. The probes used were VIC-TCATGGT CAAAATATTGAAAT and FAM-ATGGTCAAAATGTTGAA AT. rs10975519 was genotyped by TaqMan(R) Pre-Designed SNP Genotyping Assays, C___2762153_10.

Reagents for human interleukin-33

Recombinant human IL-33 (rhIL-33) and a rabbit-neutralizing anti-hIL-33 IgG antibody were made by Hokudo Co., Ltd. (Sapporo, Japan). Briefly, rhIL-33 (mature form) was amplified from human lung cDNA (BioChain Institute, Hayward, CA, USA) as a template, and subcloned into pET28a vector (Novagen, Madison, WI, USA). BL21 (DE3) RIL was transformed and the expressed recombinant protein was purified with Ni-NTA resin. Endotoxin was removed by filtration through Zetapor (Cuno, Meriden, CT, USA). For establishment of a polyclonal antibody to hIL-33, rabbits

were immunized with rhIL-33 (200 μg/body) with CFA, and boosted with rhIL-33 (200 μg/body) with IFA three times every 2 weeks. Seven weeks later, serum was collected and the antibody was purified using a Protein-A sepharose column. This IgG antibody (R2) was further purified with an rhIL-33 sepharose column and was biotinylated with NHS-biotin (Sigma, St Louis, MO, USA) in our laboratory. This purified anti-hIL-33 antibody could completely neutralize 50 ng/mL of IL-33 at the concentration of 10 μg/mL *in vitro*.

Table 1. Characteristics of the patients with Japanese cedar (JC) pollinosis and controls

Characteristics	Case	Control
Age (year, median with range)	30 (20–49)	32.5 (20–49)
Total subjects and sex (% male subjects)	170 (14)	100 (9.0)
Serum total IgE (IU/mL, mean±SEM)	280.2±879.2	42.9±51.5
Atopic sensitization (RAST) (number (%))		
Japanese cedar pollen positive	170 (100)	0 (0)
Dermatophagoides pteronyssinus positive	80 (47.1)	0 (0)
Dermatophagoides farinae positive	78 (45.9)	0 (0)
Candida albicans positive	10 (5.9)	0 (0)
Aspergillus positive	3 (1.8)	0 (0)
Dactylis glomerata positive	61 (35.9)	0 (0)
Ambrosia positive	23 (13.5)	0 (0)

Enzyme-linked immunosorbent assay of serum levels of interleulun-33

To elucidate the biological roles of the *IL-33* gene, we constructed an ELISA system to quantify human IL-33 protein in sera of subjects with JC pollinosis and controls. A 96-well plate (Costar, Cambridge, MA, USA) was coated with the anti-hIL-33 IgG antibody (R2) and blocked with StartingBlock™ blocking buffer (PIERCE, Rockford, IL, USA). Human IL-33 was detected with the biotinylated-anti-IL-33 antibody and streptavidin-HRP. The ELISA system was specific for hIL-33 and did not cross-react with other cytokines tested, which included IL-1β, IL-2, IL-4, IL-12, IL-18, TNF-α, IFN-γ and GM-CSF. Serum samples were collected, and then they were stored at −80 °C until measurement. IL-33 was assayed by ELISA with reference standard curves using known amounts of hIL-33. The lower limit of ELISA sensitivity for serum IL-33 was 30 pg/mL. A value of 0 was assigned to results that were below the assay's lower limit of detection for non-parametric statistical calculations in Fig. 2.

Statistical analysis

We calculated allele frequencies and tested agreement with Hardy–Weinberg equilibrium using a χ^2 goodness-of-fit

Table 2. Locations and allele frequencies of polymorphisms in *IL-33* based on the HapMap JPT data set

SNP*	Location	Amino acid	MAF (%)‡	NCBI§
−5345 G/A	5′-Flanking region		0.477	rs928414
−5194 T/G	5′-Flanking region		0.477	rs4237164
−4432 G/A	5′-Flanking region		0.477	rs10975509
−1611 C/T	5′-Flanking region		0.466	rs7025417
−1037 T/C	Intron 1		0.467	rs10975511
1256 C/T	Intron 1		0.455	rs4742170
2241 C/G	Intron 1		0.455	rs7019575
4450 G/A	Intron 1		0.455	rs10975514
5999 G/A	Intron 1		0.443	rs10975516
9318 C/A	Intron 2		0.443	rs1317230
9813 G/T	Intron 3		0.455	rs1330383
9894 T/C†	Intron 3		0.455	rs1929992
11607 T/C	Intron 4		0.432	rs1113573
11877 C/T†	Exon 5	Tyr163Tyr	0.433	rs10975519
12016 G/C	Intron 5		0.422	rs10975520
12514 T/C	Intron 5		0.427	rs7044343
13206 A/G	Intron 6		0.487	rs7871381
13316 C/A	Intron 6		0.371	rs1412421
13625 G/A	Intron 6		0.422	rs7047921
14187 G/T	Intron 6		0.420	rs1332290
14598 G/A	Exon 7	3′-UTR	0.409	rs1048274
23562 G/C	3′-Flanking region		0.455	rs10815397

*Numbering according to the genomic sequence of IL-33 (AL353741.16) and position 1 is the A of the initiation codon. Major allele/minor allele.
†SNPs were genotyped in this study.
‡Minor allele frequencies
§NCBI, number from the dbSNP of NCBI (http://www.ncbi.nlm.nih.gov/SNP/).
SNP, single nucleotide polymorphisms; MAF, minor allele frequency.

test at each locus. We then compared differences in allele frequencies and genotype distribution of the polymorphism between case and control subjects by using a 2×2 contingency χ^2 test with one degree of freedom, and calculated odds ratios (ORs) with 95% confidence intervals (CIs). Serum total IgE and IL-33 levels were analysed as quantitative levels, and we investigated associations between these levels and genetic variations. Log-transformed individual serum IgE levels were analysed by one-way ANOVA. When the data for IL-33 levels were not distributed normally after log-transformation, they were analysed using non-parametric equivalents and summarized using the median. Multiple comparisons were first analysed by the Kruskal–Wallis test and then by individual testing by the Mann–Whitney *U*-test if significant. Correlations were analysed by Spearman's test. A *P* value of less than 0.05 was considered statistically significant.

Results

Linkage disequilibrium of the IL-33 *gene*

A total of 22 polymorphisms with a frequency >0.10 in IL-33 were contained in the public databases available at the NCBI dbSNP website (http://www.ncbi.nlm.nih.gov/SNP/) (Table 2). Two variants including a synonymous substitution (Tyr163Tyr) were in the exons, and four variants were in the 5′-flanking region of the *IL-33* gene. Pairwise LD among the 22 SNPs was measured by different parameters, r^2 using the Haploview 3.2 program (http://www.broad.mit.edu/mpg/haploview/) (Fig. 1), and all the 22 SNPs were in strong LD ($r^2>0.75$). We finally selected polymorphism rs1929992 and rs10975519 (Tyr163Tyr) for association studies using tagger in the Haploview 3.2 program, and these two SNPs captured 22 of 22 alleles with a mean r^2 of 0.95 ($r^2>0.91$).

Association between polymorphisms in the IL-33 *gene and susceptibility of Japanese cedar pollinosis*

The locus was in Hardy–Weinberg equilibrium in the entire group. To test the association between the SNP and JC pollinosis, we compared differences in the allele frequency and genotype distribution of each polymorphism between case and control subjects by using contingency chi-square tests with one degree of freedom. ORs with 95% CIs were also calculated. In the population genotyped in this study, the MAF of rs1929992 (C = 0.49) was higher than those in the HapMap JPT data set (C = 0.46). We found a significant association between rs1929992 (T>C) and JC pollinosis (TT+TC vs. CC: OR, 1.82; 95% CI, 1.00–3.31; $P=0.048$) (Table 3). The serum total IgE level was analysed as a quantitative level, and we investigated the association between this level and genetic variation. However, we could not find any association between the SNP and serum IgE level in this study ($P=0.46$ by ANOVA).

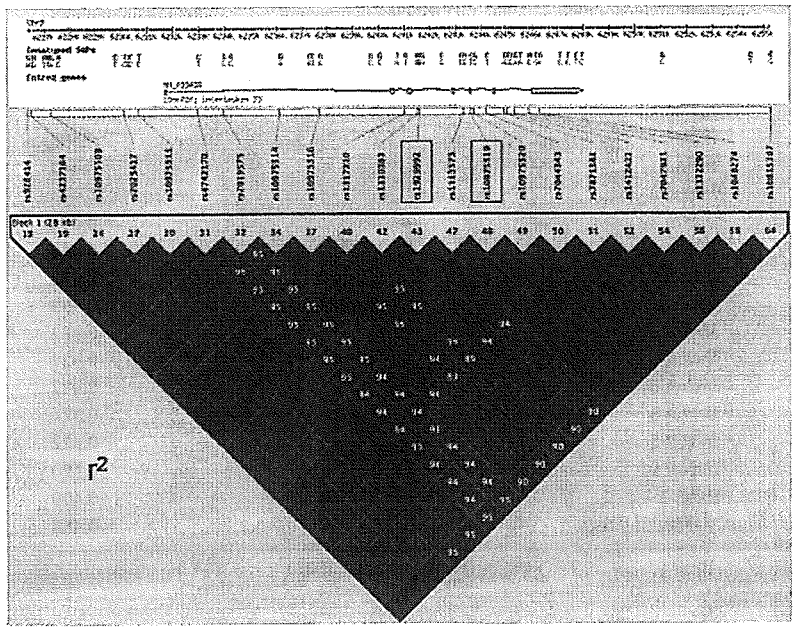

Fig. 1. Pairwise linkage disequilibrium between 22 SNPs as measured by r^2 estimated by the Haploview 3.2 program using the HapMap JPT data set. The boxed polymorphisms, rs1929992 and rs10975519, were genotyped in this study.

Patients with Japanese cedar pollinosis display higher interleukin-33 serum levels than healthy control subjects

To evaluate whether dysregulation at the IL-33 protein level might be a characteristic feature of JC pollinosis, we conducted ELISA assays of sera of patients with JC pollinosis ($n = 170$) and healthy control subjects ($n = 100$). Patients with JC pollinosis exhibited significantly higher serum levels of the IL-33 protein ($P = 0.0018$) (Fig. 2). The median serum IL-33 concentration of JC pollinosis patients was 549 pg/mL, compared with 361.8 pg/mL for controls. In addition, we examined the serum IL-33 level in infectious rhinitis as non-allergic rhinitis. The median serum IL-33 concentration of subjects with infectious rhinitis was 241.3 pg/mL. There was no significant difference of the serum IL-33 level between healthy control subjects and those with infectious rhinitis. Although total serum IgE and IL-33 levels were analysed as quantitative phenotypes, there was no significant association between the total serum IgE level and serum IL-33 level ($P = 0.095$ by Spearman's test). We also examined whether the *IL-33* genotype affected the serum level of IL-33, but we could not find any significant association between the genotype and serum IL-33 level ($P = 0.58$ by the Kruskal–Wallis test).

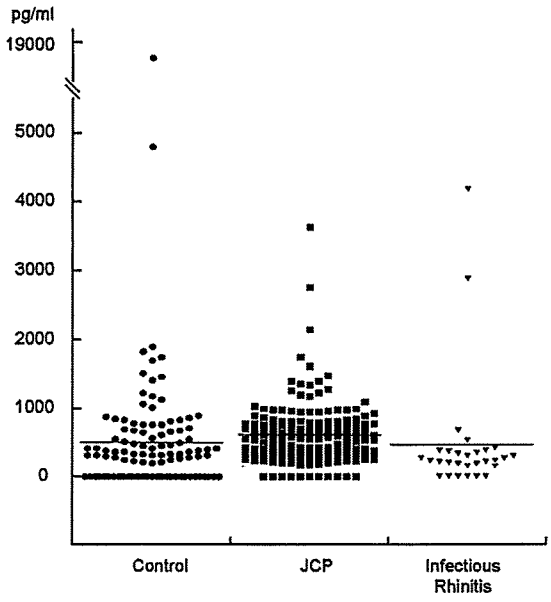

Fig. 2. Comparison of serum IL-33 levels among controls, patients with JC pollinosis and those with infectious rhinitis. Horizontal bars indicate the median value of each group. JCP, Japanese Cedar pollinosis.

Discussion

To determine the role of the *IL-33* gene in the pathogenesis of JC pollinosis, we conducted an association study using the sequence variation of the *IL-33* gene and compared serum IL-33 levels between subjects with JC pollinosis and controls. We found a significant association between JC pollinosis susceptibility and IL-33 polymorphism and higher serum IL-33 levels in subjects with JC pollinosis. Although IL-33 has been thought to play an important role in allergic diseases, this is the first study providing evidence for its involvement in such a disease. We consider the results to be hypothesis generating as the findings in this study need to be confirmed in another population with a larger size.

Recent studies have reported important roles of non-lymphoid cell-derived cytokines such as IL-33 and TSLP in the induction of Th2 differentiation [9, 19]. IL-33 is highly expressed in normal human bronchial epithelial cells and airway smooth muscle cells [9]. It induces Th2-type responses and Th2-associated cytokines IL-4, IL-5 and IL-13 by signalling through IL1RL1 [9, 20]. A recent study has shown that IL-33 induces IL-13 production by mast cells independently of IgE–FcεRI signals in mice. These findings suggest important roles for IL-33 in mast cell- and Th2 cytokine-associated immune disorders [21].

Table 3. Association between polymorphisms of *IL-33* and Japanese cedar (JC) pollinosis

Genotype	Cases ($n = 170$)	Controls ($n = 100$)	Allele	Cases ($n = 170$)	Controls ($n = 100$)	Genotype *P*	Dominant* *P*	Recessive† *P*	Allelic‡ *P*
rs1929992									
TT	44 (26.0)	32 (32.3)	T	162 (47.9)	112 (56.6)	0.13	0.27	0.048	0.053
TC	74 (43.8)	48 (48.4)	C	176 (52.1)	86 (43.4)				
CC	51 (30.2)	19 (19.2)							
rs10975519									
CC	52 (30.6)	36 (36.0)	C	177 (52.1)	119 (59.5)	0.20	0.36	0.074	0.093
CT	73 (42.9)	47 (47.0)	T	163 (47.9)	81 (40.5)				
TT	45 (26.5)	17 (17.0)							

*Dominant model (TT vs. CC+TC in rs1929992, CC vs. CT+TT in rs10975519).

†Recessive model (TT+TC vs. CC in rs1929992, CC+CT vs. TT in rs10975519).

‡Allelic model (T allele vs. C allele in rs1929992, C allele vs. T allele in rs10975519).

 Clinical and Experimental Allergy, 38 : 1875–1881

Structurally, IL-33 is related to IL-18, and intensive studies of the relationship between allergic inflammation and IL-18 have been conducted. IL-18 was the first cytokine demonstrated to activate T cells to produce abundant IFN-γ without T cell receptor (TCR) engagement [22]. Furthermore, genetic association studies of the *IL-18* gene have provided evidence for an association with atopic diseases [23–26]. Verhaeghe et al. reported the up-regulation of IL-18 in nasal secretions in allergic rhinitis and the persistence of elevated IL-18 concentrations until after the season [27]. Increased IL-33 concentrations were observed in subjects with JC pollinosis in the present study; however, there was no significant difference in the serum IL-33 level between controls and subjects with infectious rhinitis. Up-regulation of the IL-33 level appears to be characteristic of JC pollinosis. Further analyses of the involvement and interactions of those structurally similar cytokines in allergic inflammation should also be conducted.

Recent reports have shown that IL1RL1 is a reliable marker of Th2 lymphocytes in allergic airway inflammation [7, 13, 28]. Elevated levels of the soluble form of IL1RL1 in the circulation of patients with asthma with acute exacerbation have been reported [14]. The study has also shown that a differential rise of serum IL1RL1 level that correlates well with the severity of asthma exacerbation [14]. In a murine model of allergic airway inflammation, serum murine (m) IL1RL1 protein levels increased after allergen exposure, and pre-treatment with soluble mIL1RL1 protein significantly inhibited the Th2 cytokine production [12]. Other studies have shown that administration of either a monoclonal antibody against IL1RL1 or a recombinant IL1RL1 fusion protein attenuates eosinophilic inflammation of the airways and suppresses IL-4 and IL-5 production *in vivo* following adoptive transfer of Th2 cells [6, 7]. These findings suggest that blocking IL1RL1 pathways would be therapeutically efficacious as a new treatment for allergic diseases, and expression of soluble IL1RL1 could serve as a physiological mechanism to down-regulate Th2-driven immunopathology [10]. In this study, we did not measure the serum soluble IL1RL1 levels, and further examination of the relationship between serum IL-33 and soluble IL1RL1 is needed to clarify their functions in Th2 inflammation. The genetic factors of the *IL-33* gene or serum IL-33 level might provide valuable information for selecting appropriate therapeutic options.

We showed here a significant association between susceptibility to JC pollinosis and a polymorphism. In this study, we selected polymorphisms using HapMap information, and did not examine the functional effects of polymorphisms in strong LD with the related variant. Previous studies have shown that polymorphisms in exons often contribute to their transcript stability [29, 30]. Variants rs10975519 (Tyr163Tyr) and rs1048274 in the exon might affect the expression level or mRNA stability of the *IL-33* gene. In addition, four genetic variations were in the 5′-flanking region, which is often involved in transcriptional regulation of the gene. Several transcription factors are involved in asthmatic inflammation, including NF-κB, activator protein-1 (AP-1), nuclear factor of activated T cells (NF-AT), cyclic AMP response element-binding protein (CREB) and signal transduction-activated transcription factors (STAT) [31]. Using the TRASFAC system, we surveyed whether SNPs in the 5′ region of the *IL-33* gene create transcription factor binding sites. However, we could not find any SNP that changed the affinity of those transcription factors. The functions of these linked polymorphisms remain to be elucidated. Demonstrating the alteration of gene functions as the result of polymorphisms is necessary to further validate the involvement of the *IL-33* gene in the pathogenesis of JC pollinosis. Furthermore, there were gender differences in the population in this study, and several studies have suggested that sex affects the asthma phenotype, possibly via hormone-related events [32, 33]. If there is a sex-related difference in the association of IL-33 with JC pollinosis, looking at females only might be informative.

Our data strongly support the important role of IL-33 in JC pollinosis. Further investigation of the connections between genotypes and the functional role of IL-33 during allergic events may provide additional targets for therapeutic interventions and would be helpful to clarify the aetiology of allergic diseases.

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2009年におけるスギ花粉症に対する第2世代抗ヒスタミン薬による初期療法の有用性 －JRQLQ No.1を用いたQOL評価－

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Summary 2009年におけるスギ花粉症患者に対してエピナスチン塩酸塩を花粉飛散開始前から投与した初期治療群と，飛散後から投与した発症後治療群において，日本アレルギー性鼻炎標準QOL調査票(JRQLQ No.1)を用いたQOL評価を行った。花粉飛散開始時において，初期治療群のほうが鼻の症状スコアおよびQOLスコアが有意に低く，花粉飛散期を通しても初期治療群で低い傾向が認められた。本試験により，エピナスチン塩酸塩を用いたスギ花粉症患者への初期療法は，花粉の本格飛散期における症状改善およびQOLの維持効果をもたらし，ベースライン治療として第2世代抗ヒスタミン薬による初期療法の有用性が，あらためて裏付けられた。

Key Words：スギ花粉症／初期療法／エピナスチン塩酸塩／鼻噴霧用ステロイド薬／日本アレルギー性鼻炎*QOL*調査票(*JRQLQ No.1*)

はじめに

スギ花粉症は，本邦の季節性アレルギー性鼻炎の代表的な疾患であり，くしゃみ，鼻漏，鼻閉，流涙などの症状により，患者のQuality of Life (QOL)は著しく阻害され，日常生活に支障をきたすことも多い。スギ花粉症の全国平均有病率は，1998年には16.2%であったが，2008年には26.5%に増加したことが報告されている[1]。花粉症の治療に関しては，鼻アレルギー診療ガイドライン[2]に，「重症度に応じた花粉症に対する治療の選択」の表が示されており，花粉の本格飛散期における症状軽減を目的とした治療法のひとつに，花粉飛散予測日または症状が少しでも現れた時点で第2世代抗ヒスタミン薬の投与を行う初期療法が推奨されている。

今回我々は，第2世代抗ヒスタミン薬であるエピナスチン塩酸塩(アレジオン® 錠)を試験薬として，スギ花粉飛散前から投与した群(初期治療群)と飛散後から投与した群(発症後治療群)における，患者の自覚症状およびQOLの改善効果について，日本アレルギー性鼻炎QOL調査票(JRQLQ No.1)[3]を用いて評価を行い，スギ花粉症に対する抗ヒスタミン薬による初期療法の有用性を検証した。

Ⅰ．対象と方法

1．対象

本試験は，岡山県の9施設（小野田耳鼻咽喉科医院，小山医院駅前耳鼻咽喉科，さいとう耳鼻科，

耳鼻咽喉科菅田医院，てしま耳鼻咽喉科，服部耳鼻咽喉科医院，ばば耳鼻咽喉科，藤原耳鼻咽喉科医院，本荘耳鼻咽喉科医院）および，東京都北区の1施設（いがらしクリニック）の計10施設において，それらの施設の耳鼻科専門医を試験担当医師として実施した。

2009年1～5月に，試験協力施設を受診した15歳以上のスギ花粉症患者（性別不問）を対象とした。ただし，患者の選択基準を，過去3年以内に実施したスギ花粉症に対する血液中特異的IgE (immunoglobulin E) 抗体定量がクラス2～6を示す陽性であること，本試験内容の説明を受け同意が得られた患者とした。患者が20歳未満の場合には，保護者などの代諾者にも説明を行った。なお，本試験は通常の治療によって得られるデータに基づく調査であるため，試験担当医師が被験者に対して本試験の説明を行い，その後の被験者の調査票への回答をもって，同意確認の記録とした。

また，以下の症例は，本試験の対象から除外した。すなわち，①アレルギー性鼻炎以外の鼻・眼粘膜表面に病変が認められた患者，②本試験開始前6カ月以内に，ステロイド薬の注射を受けた患者，③気管支喘息などの下気道疾患を有する患者，④アナフィラキシーの既往を有する患者，⑤本試験薬に対して，過去に過敏症の既往のある患者，⑥妊娠，授乳婦，妊娠している可能性のある患者，あるいは試験中に妊娠を希望する患者，⑦その他，試験担当医師が本研究の参加に不適切と判断した場合。

なお，本試験は当該試験実施施設の外部に設置された倫理委員会で審査・承認された後に，施設長の許可を得るとともに，ヘルシンキ宣言に基づく倫理的原則，「臨床研究に関する倫理指針」（2004年12月28日全部改正，厚生労働省）を遵守して実施した。

2．投与方法

スギ花粉飛散前から受診し，試験薬投与を開始した被験者を初期治療群，飛散後に本格的な症状が出てから試験薬投与を開始した被験者を発症後治療群とした。

各試験協力施設を受診し，同意の得られた被験者に，受診時毎（約4週間毎）にJRQLQ No.1への記入を依頼した。試験担当医師は，記載もれのないことを確認した上で，当日回収した。受診後，被験者は試験薬であるエピナスチン塩酸塩（アレジオン® 錠，以下，試験薬）20 mg 1日1回の服用を開始した。スギ花粉飛散開始日以降は，症状に応じて鼻噴霧用ステロイド薬の併用を許可した。

試験期間中には，本試験の評価判定に影響を及ぼす可能性のある薬剤，すなわち試験薬以外の抗ヒスタミン薬，遊離抑制薬，ロイコトリエン受容体拮抗薬，プロスタグランジンD_2・トロンボキサンA_2受容体拮抗薬，経口ステロイド薬などの併用は，原則として禁止した。

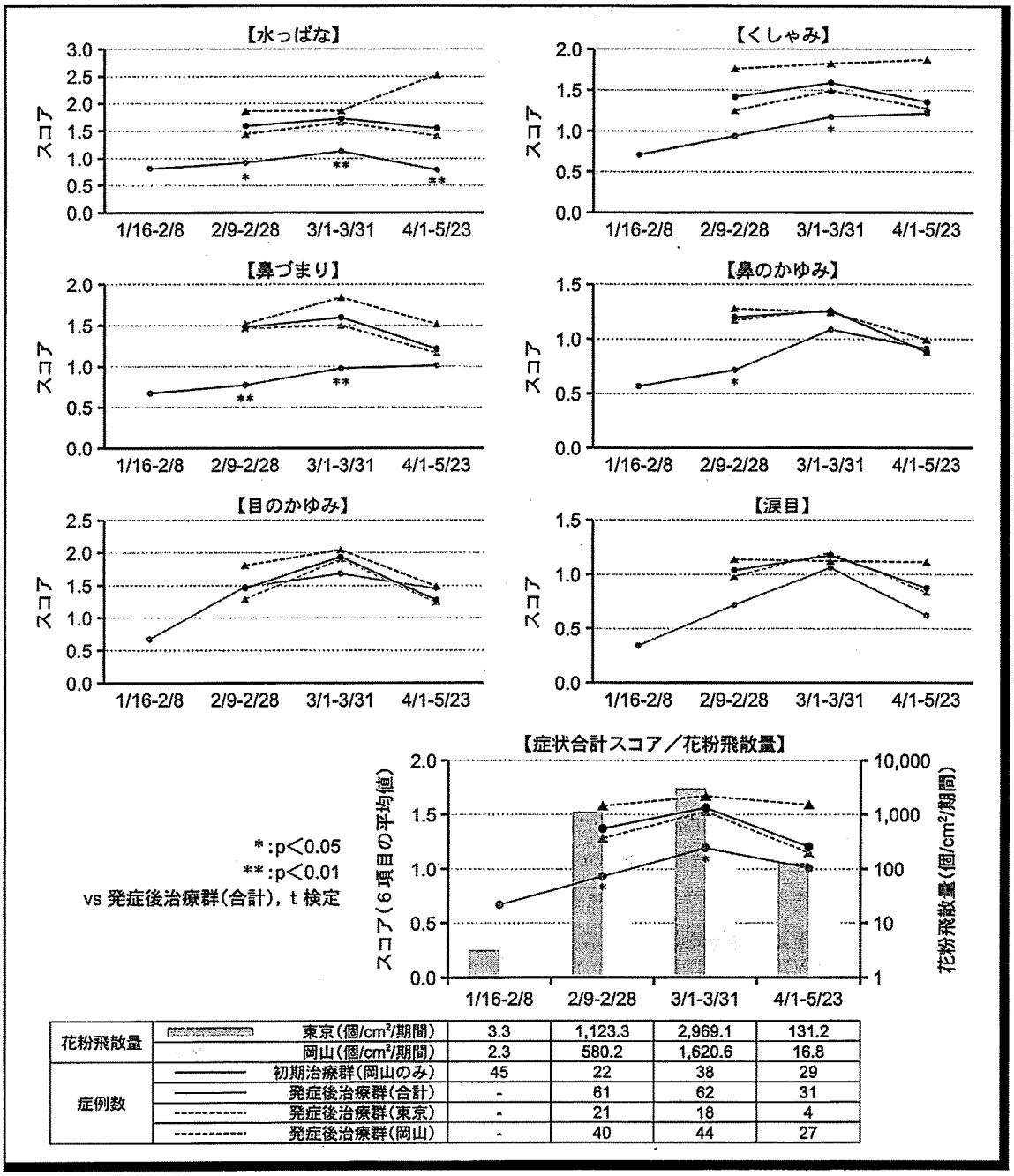
3．評価方法

JRQLQ No.1は，計量心理学的手法を用いて妥当性が証明されており，日本のアレルギー性鼻炎患者を対象に標準化されたものである。評価項目は，パートⅠ：自覚症状（水っぱな，くしゃみ，鼻づまり，鼻のかゆみ，目のかゆみ，涙目の全6項目），パートⅡ：QOL（日常生活，戸外活動，社会生活，睡眠，身体機能，精神生活の6領域からなる全17項目），パートⅢ：総括的状態（1項目）から構成される。被験者は，調査日前1～2週間の状態について，パートⅠ，Ⅱは，0（なし）～4（非常に重い，ひどい）の5段階から選択し，パートⅢは，1（晴ればれ）～5（泣きたい）の5つのface scaleから選択する。

統計解析にはt検定を用い，有意水準は5％未満とした。

Ⅱ．結果

花粉飛散開始日は，1 cm^2あたり1個以上の花粉が2日連続して観察された最初の日とされてお

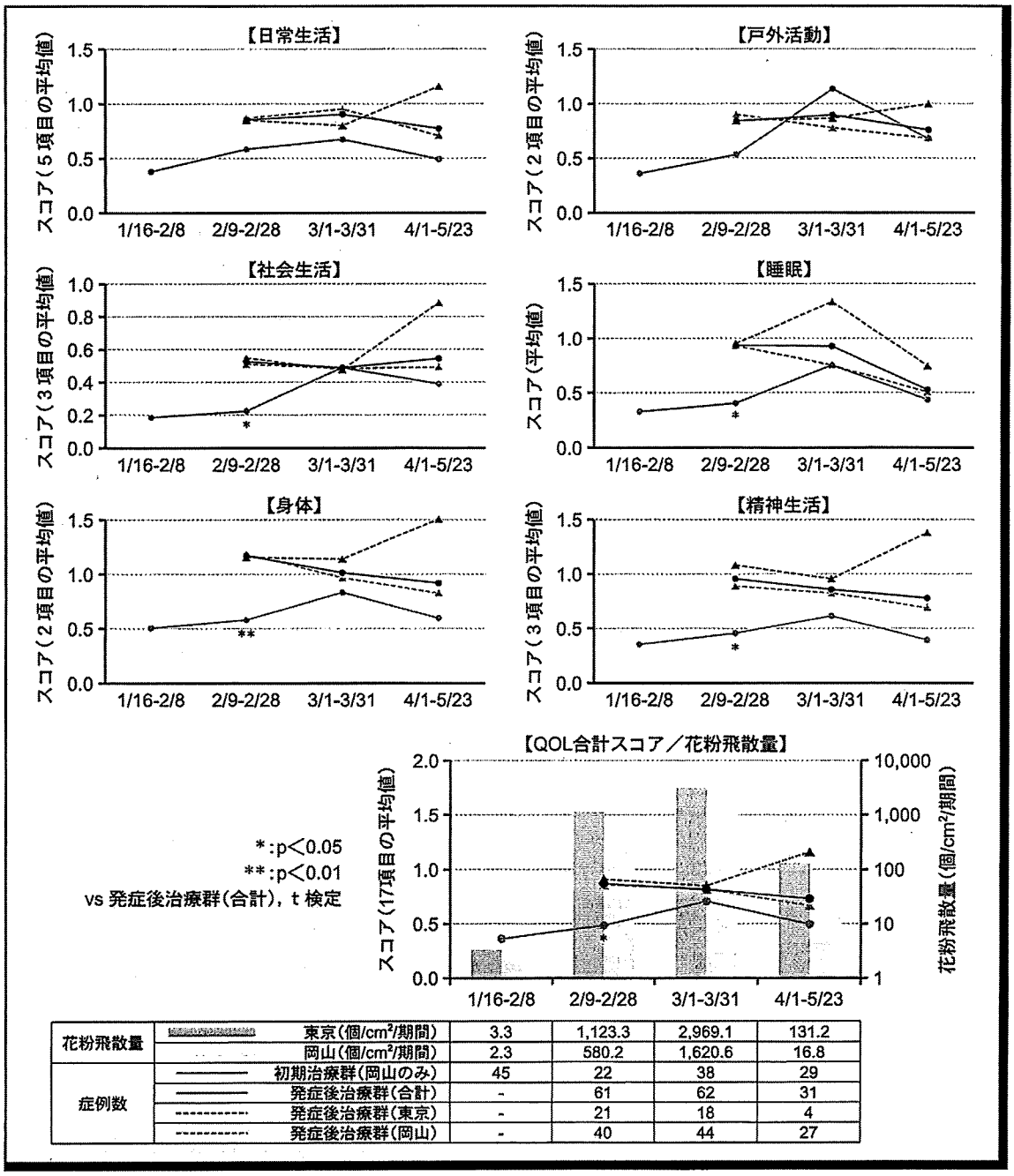


花粉飛散量	東京（個/cm²/期間）	3.3	1,123.3	2,969.1	131.2
	岡山（個/cm²/期間）	2.3	580.2	1,620.6	16.8
症例数	初期治療群（岡山のみ）	45	22	38	29
	発症後治療群（合計）	-	61	62	31
	発症後治療群（東京）	-	21	18	4
	発症後治療群（岡山）	-	40	44	27

図1　JRQLQ No.1　Ⅰ．鼻・眼の症状の比較

花粉飛散開始時（2/9～2/28）において，水っぱな，鼻づまり，鼻のかゆみ，および症状合計スコアの平均スコアは，発症後治療群よりも初期治療群のほうが有意に低かった．

り，環境省花粉情報サイトによると，2009年のスギ花粉飛散開始日は，岡山県では2月10日，東京都北区では2月9日であった。したがって，本試験において，1月16日から2月8日までに受診した被験者を初期治療群，2月9日以降に本格的な症状を訴えてから受診した被験者を発症後



花粉飛散量	東京(個/cm²/期間)	3.3	1,123.3	2,969.1	131.2
	岡山(個/cm²/期間)	2.3	580.2	1,620.6	16.8
症例数	初期治療群(岡山のみ)	45	22	38	29
	発症後治療群(合計)	-	61	62	31
	発症後治療群(東京)	-	21	18	4
	発症後治療群(岡山)	-	40	44	27

図2　JRQLQ No.1　Ⅱ. QOL 質問項目の比較

花粉飛散開始時(2/9～2/28)において，社会生活，睡眠，身体，精神生活，およびQOL合計スコアの平均スコアは，発症後治療群よりも初期治療群のほうが有意に低かった．

治療群とした。初期治療群として 45 例，発症後治療群として 86 例，合計 131 例が本試験に登録したが，初回受診後 4 週間毎に来院しない症例があったため，各期間中に来院し JRQLQ No.1 に記入した症例を対象として統計解析を行った（図に症例数を記載）。

1．自覚症状

JRQLQ No.1 の鼻・眼の症状の程度に関する結果を図1に示す。花粉飛散開始時（2/9～2/28）の調査において，水っぱな，鼻づまり，鼻のかゆみ，および症状（合計スコア）の平均スコアは，発症後治療群よりも初期治療群のほうが有意に低かった。花粉飛散最盛期（3/1～3/31）の調査においては，水っぱな，くしゃみ，鼻づまり，および症状（合計スコア）の平均スコアが，初期治療群で有意に低かった。花粉飛散後期（4/1～5/23）の調査においては，水っぱなの症状スコアのみに有意差が認められた。

2．QOL

QOL に関する結果を図2に示す。花粉飛散開始時の調査において，社会生活，睡眠，身体，精神生活の4領域の QOL スコアおよび，QOL（合計スコア）の平均スコアは，発症後治療群よりも初期治療群のほうが有意に低かった。花粉飛散最盛期および花粉飛散後期の調査においては，両群に有意差は認められなかった。

3．総括的状態

face scale の平均スコアを図3に示す。いずれの期間においても，総括的状態スコアについて，両群間の有意差は認められなかった。

Ⅲ．考案

2009年版の鼻アレルギー診療ガイドライン[2]が発刊され，改訂第6版となった。今回の改訂により，重症度に応じた花粉症に対する治療の選択に関して，初期療法に用いる治療薬に，プロスタグランジン D_2・トロンボキサン A_2 受容体拮抗薬および Th2 サイトカイン阻害薬が加えられた。花粉症の初期療法では，花粉が本格的に飛散する前の無症状あるいは軽症の段階で薬剤の服用を開始するため，副作用が少なく，服用回数が少ないコンプライアンスの良い薬剤のほうが，患者の負担が軽いと考えられる。本試験薬のエピナスチン塩酸塩は，第2世代抗ヒスタミン薬のなかでも特

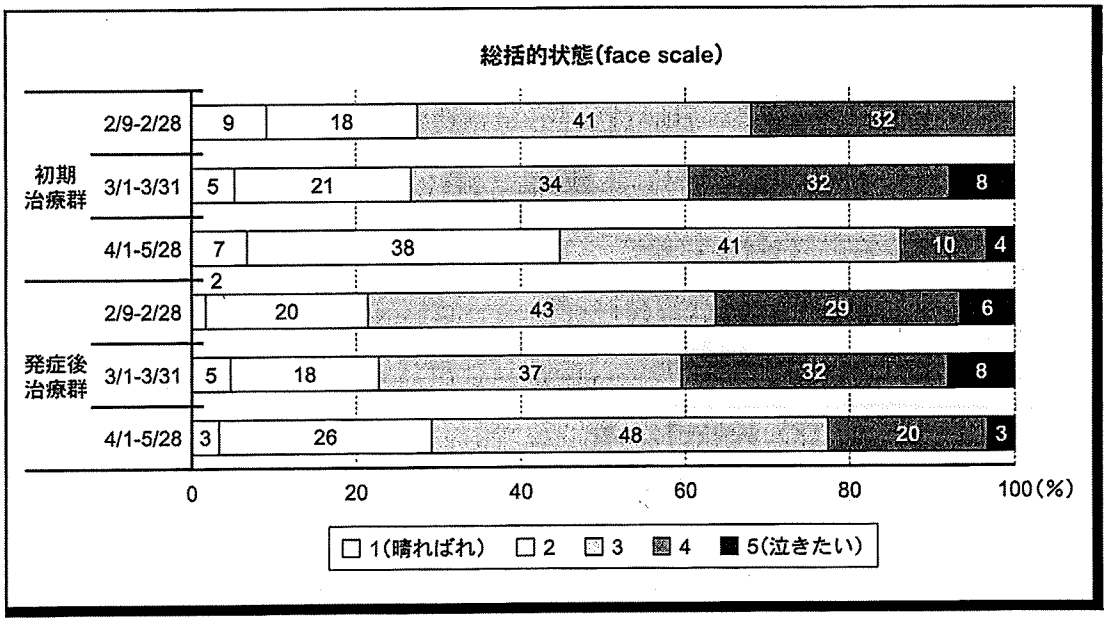


図3　JRQLQ No.1　Ⅲ．総括的状態（face scale）の比較
花粉飛散開始時（2/9～2/28）において，両群間に有意差は認められなかった．

に中枢への移行が少なく[4]，鎮静作用の少ない薬剤であり[5,6]，その持続性により1日1回投与で臨床的有効性が認められている。エピナスチン塩酸塩を用いた花粉症の初期療法に関する報告は過去にもなされ，初期療法が花粉症の症状軽減に有用であることが示されている[7,8]。

本試験では，JRQLQ No.1を用いた評価により，自覚症状だけでなく，患者のQOLに対する効果についての検討も行った。まず，自覚症状については，花粉飛散開始時において，初期治療群のほうが発症後治療群よりも，水っぱな，鼻づまり，鼻のかゆみ，合計の症状スコアが有意に低く，飛散前に試験薬の投与を始めることで，症状発現が抑制されていることが示された。さらに調査期間を通して，初期治療群の症状スコアは，発症後治療群よりも低値で推移し，スギ花粉飛散最盛期の3月でも，水っぱな，くしゃみ，鼻づまり，合計の症状スコアが有意に低かった。スギ花粉飛散後の治療では，初期治療群・発症後治療群ともに症状スコアの差は徐々に小さくなったが，初期療法群では全経過を通じて全ての症状スコアが1.0前後，すなわち「軽い」で推移している。ガイドラインの推奨する初期療法が，症状の軽減に寄与していることが示唆された。

QOLに関しては，花粉飛散開始時において，社会生活，睡眠，身体，精神生活，合計のQOLスコアが，初期治療投与群のほうが有意に低く，これまでの報告と同様に抗ヒスタミン薬による初期療法の飛散開始時におけるQOL維持効果が確認できた。さらに合計のQOLスコアをみると，初期治療群のほうが低値で推移しており，抗ヒスタミン薬による初期療法は花粉飛散期全般を通して，QOLの維持のために有効であると考えることができた。

また，スギ花粉飛散後の治療では症状に応じて鼻噴霧用ステロイド薬の併用を許可していたことより，初期治療群・発症後治療群ともに症状スコア及びQOLスコアが徐々に低下していたことから，発症後治療のひとつとして抗ヒスタミン薬と鼻噴霧用ステロイド薬による併用療法の有用性が示唆された。

本試験により，エピナスチン塩酸塩を用いたスギ花粉症患者に対する初期療法は，花粉の本格飛散期における症状軽減と，QOLの維持効果をもたらすことが示され，特に花粉飛散開始時において，患者の鼻の症状（水っぱな，鼻づまり，鼻のかゆみ，合計）の軽減，およびQOL（社会生活，睡眠，身体，精神生活，合計）の維持に有効であり，あらためて，ガイドライン推奨の第2世代抗ヒスタミン薬服用による初期療法の有用性が裏付けられた。

利益相反なし。なお，本試験の調査主体は財団法人予防医学事業中央会であり，その依頼により本研究を行った。

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本試験の実施に際し，ご参加いただいた施設の先生方に深謝いたします。

いがらしクリニック：五十嵐利一・白坂邦隆，小野田耳鼻咽喉科医院：小野田友男，小山医院駅前耳鼻咽喉科：遠藤龍太郎，さいとう耳鼻科：齋藤稚里，耳鼻咽喉科菅田医院：菅田研一，てしま耳鼻咽喉科：手島裕之，服部耳鼻咽喉科医院：服部謙志，ばば耳鼻咽喉科：馬場雄三，藤原耳鼻咽喉科医院：渡辺　徹，本荘耳鼻咽喉科医院：本荘智康（五十音順・敬称略）

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分担研究報告書

リアルタイムモニター飛散数の情報のあり方の研究と舌下ペプチド・アジュバント療法の臨床研究

スギおよびヒノキ抗原に対する末梢血単核細胞の IL-5/IL-10/IL-13/IL-17/IL-18/IL-31/IL-33/IFN-γ産生と免疫療法による制御

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研究要旨
今回我々は、スギ花粉症患者末梢血単核細胞（PBMC）のスギおよびヒノキ抗原に対するサイトカイン産生を検討した。スギ花粉症患者の PBMC は、健常者と比較してスギおよびヒノキ抗原のいずれに対しても IL-5、IL-13 を有意に産生した。IFN-γ産生に関しては両群で差を認めなかった。IL-10/IL-17/IL-18/IL-33 に関しては両群共に有意な産生を示さなかった。IL-31 産生に関しては、スギ粗抗原においてスギ花粉症患者で有意に亢進した。ヒノキ飛散期においては症状スコアとヒノキ粗抗原特的 IL-31 産生との間に相関傾向を認め、さらに QOL スコアとヒノキ粗抗原特的 IL-31 産生との間に有意な正の相関がみられ、IL-31 産生はヒノキ花粉症のバイオマーカーになりえる可能性が示唆された。免疫療法施行群では非施行群と比較してスギ粗抗原に特異的な IL-5/IL-13/IL-31 産生が有意に抑制され、また IFN-γ 産生も抑制される傾向を認めた。ヒノキ粗抗原に特異的なサイトカイン産生に関しては免疫療法の有無で有意な差を認めなかった。

A. 研究目的

これまでにスギ花粉症患者の末梢血単核細胞(PBMC) はスギ抗原刺激により IL-5 を産生し、その産生は免疫療法で抑制されることを報告した。一方、PBMC による他の Th2 タイプあるいは Th1/Th17/Treg タイプのサイトカイン産生に関しては未明な点が多い。そこで今回は、スギおよびヒノキ抗原に対する PBMC の IL-5/IL-10/IL-13/IL-17/IL-18/IL-31/IL-33/IFN-γ 産生を測定し、こられのサイトカインがスギ・ヒノキ花粉症の病態を反映するバイオマーカーとなりうるのか、さらに免疫療法により制御されうるのか検討した。

B. 研究方法

2009 年 5 月前後に免疫療法施行 (n=19) および非施行 (n=29) のスギ花粉症患者、あるいは健常人 (n=8) より PBMC を採取した。10 μg/ml の Cry j 1、スギ粗抗原あるいはヒノキ粗抗原にて刺激し、培養 72 時間後に上清を回収し上記のサイトカインを ELISA にて測定した。スギおよびヒノキ飛散期の症状および QOL は JRQLQ を用いてスコア化した。(倫理面への配慮)

ボランティアからの検体（末梢血）採取に関しては、学術的な意義について十分な説明を行い、同意・協力が得られた上で採取保存する。

C. 研究結果

スギ花粉症患者の PBMC は、健常者と比較してスギおよびヒノキ抗原のいずれに対しても IL-5、IL-13 を有意に産生した。IFN-γ 産生に関しては両群で差を認めなかった。IL-10/IL-17/IL-18/IL-33 に関しては両群共に有意な産生を示さなかった。IL-31 産生に関しては、スギ粗抗原においてスギ花粉症患者で有意に亢進した。スギ花粉症患者で産生を認めた IL-5/IL-13/IL-31/IFN-γ に関してスギおよびヒノキ最大飛散期における症状および QOL スコアとの相関を検討した。スギ飛散期においてはこれらのサイトカイン産生とスコアとの間には有意な相関を認めなかったが、ヒノキ飛散期においては症状スコアとヒノキ粗抗原特的 IL-31 産生との間に相関傾向を認め、さらに QOL スコアとヒノキ粗抗原特的 IL-31 産生との間に有意な正の相関がみられた。免疫療法施行群では非施行群と比較してスギ粗抗原に特異的な IL-5/IL-13/IL-31 産生が有意に抑制され、また IFN-γ 産生も抑制される傾向を認めた。ヒノキ粗抗原に特異的なサイトカイン産生に関しては免疫療法の有無で有意な差を認めなかった。

D. 考察

スギ花粉症患者と健常人との間で IFN-γ /IL-10/IL-17/IL-18/IL-33 産生に差を認めないことは、PBMC レベルではスギ・ヒノキ花粉症の病態に Th1/Th17/Treg の関与は少ないと思われた。またスギ花粉飛散期の症状/QOL スコアとサイトカイン量との間に有意な相関がみられない要因のひとつとして採血時期が考えられる。

E. 結論

スギ花粉症患者の PBMC はスギ抗原に対して Th2 タイプのサイトカインである IL-5/IL-13/IL-31 を選択的に産生し、これらのサイトカイン産生は免疫療法で有意に抑制される。さらにヒノキ粗抗原に特異的な IL-31 産生はヒノキ花粉飛散期の症状および QOL を反映するバイオマーカーになりえる可能性が示唆された。

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G. 知的財産権の出願・登録状況(予定を含む)

1. 特許取得
   なし
2. 実用新案登録
   なし
3. その他
   なし