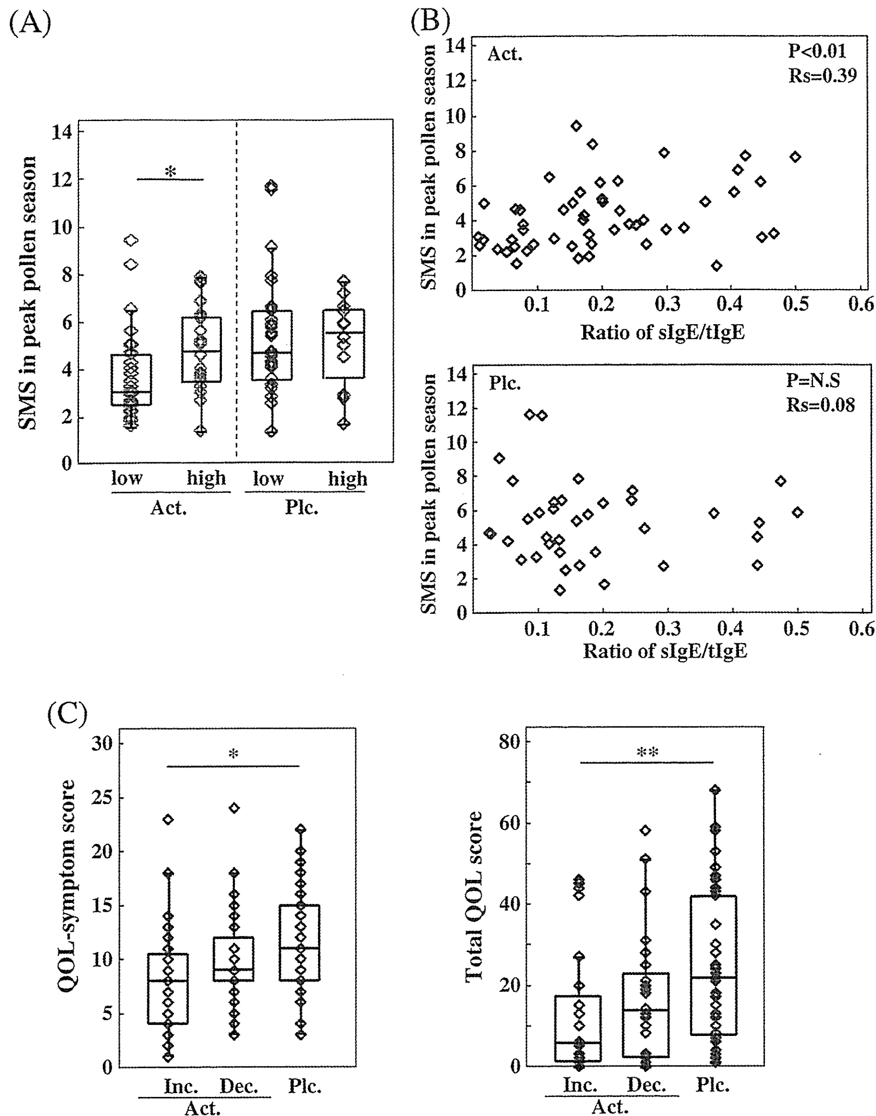

Figure 4 Biomarkers for positive therapeutic effects following SLIT. (A) SMSs in the 2008 peak pollen season for patients with low (low; N=28) and high (high; N=23) sIgE/tIgE ratios in the SLIT group (Act.), and for those with low (N=25) and high (N=12) sIgE/tIgE ratios in the placebo group (Plc.). *P<0.05. (B) Correlation between SMSs in the 2008 peak pollen season and sIgE/tIgE ratios before treatment in the SLIT (Act.; N=51) and placebo (Plc.; N=37) groups. Statistical data were obtained with Spearman correlation analysis. (C) QOL-symptom and total QOL scores from the QOL questionnaire plotted for a subgroup with increased Cry j 1-iTreg in the SLIT group (Inc.; N=24), a subgroup with decreased Cry j 1-iTreg in the SLIT group (Dec.; N=27), and the placebo group (Plc.; N=37) in the middle of the 2008 pollen season. Each diamond shows the value for an individual. *P<0.05, **P<0.01.

group (Fig. 3B). The SMS in all patients in the study correlated with the seasonal increases in IL4 (R=0.35, P<0.01), IL5 (R=0.35, P<0.01), and IL13 (R=0.36, P<0.01). The discrepancy in our current results and the results of previous studies with regard to downregulation of cytokine production from PBMCs may depend on the extent of the therapeutic effects achieved in each clinical trial.

Cry j 1-specific IgE production was not changed by treatment, even in the mild subgroup, as also found in our preliminary study [6]. We speculate that more time is required for changing antibody production following the changes of antigen-specific T cell profiles, because the alteration of T cell profiles strongly influences subsequent class switch recombination of B cells and antibody production. Another possibility is that the dose for SLIT used in this study was not high enough to alter the antibody profiles.

The sIgE/tIgE ratio has been found to be significantly higher in responders than in non-responders following 4-year immunotherapy [19]. In our trial, this ratio did not differ significantly between responders and non-responders (P=N.S.; Mann–Whitney U-test). However, subjects with a low sIgE/tIgE ratio before treatment were more likely to be responders to 2-year SLIT, and the ratio correlated with the SMS only in patients treated with SLIT (Fig. 4A, B). This suggests that SLIT was more effective in patients with a low sIgE/tIgE ratio than in those with a high sIgE/tIgE ratio. The range of total IgE levels for the participants were relatively wide (6.8–2090 IU/ml in all patients); however, the change of the total IgE for each

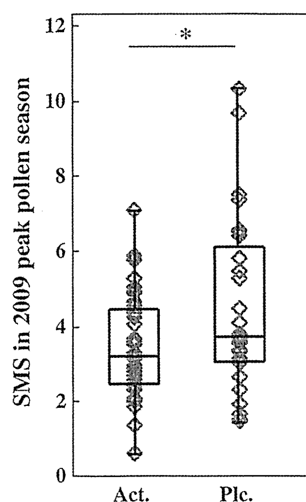

Figure 5 Carry-over effects following 2-year treatment with SLIT. SMSs in the 2009 peak pollen season were plotted for the SLIT (Act.; $N=36$) and placebo (Plc.; $N=27$) groups. Each diamond shows the value for an individual. Two-group comparisons were performed using an unpaired t-test.

individuals after 2-year treatment was not significantly different compared to before treatment (1.5±1.0 times higher, P=N.S.; paired t-test). Therefore, the wide range of total IgE levels was due to the variability on the allergic status for individuals, but not on method for measurement. The serum IgE level may affect the surface IgE level on effector cells such as mast cells and basophils, and Tregs can downregulate activation of mast cells and eosinophils [20,21]. We speculate that effector cells with a low specific IgE level are less likely to be activated by antigen crosslinking or are more susceptible to downregulation by Tregs than those with a high specific IgE level. It is also possible that the symptoms of patients with a low sIgE/tIgE ratio may be more readily attenuated by suboptimal potentiation of iTreg induced by SLIT.

We previously reported that an increased count of Cry j 1-iTregs was a candidate biomarker that could be used to distinguish between responders and non-responders to SLIT, as evaluated by the QOL-symptom score. In this report, the subgroup with increased Cry j 1-iTregs showed significant amelioration of the QOL-symptom and total QOL scores compared to the placebo group, while the subgroup with decreased Cry j 1-iTregs did not show this response (Fig. 4C). However, there was no significant difference in Cry j 1-specific cytokine production from PBMCs among patients with increased iTregs and decreased iTregs, and those in the placebo group (data not shown). Foxp3-expressing $CD25^+CD3^+$ cells and IL10-expressing $CD3^+$ cells, which are induced in the nasal mucosa after subcutaneous immunotherapy, have been linked to the clinical efficacy and suppression of seasonal inflammation [22]. Immunotherapy using an Amb a 1-immunostimulatory oligodeoxynucleotide conjugate also induced $CD4^+CD25^+$ T cells and IL10-producing cells in the nasal mucosa after the pollen season [23]. These data suggest that iTregs may downregulate effector cells at local sites of inflammation to suppress clinical symptoms. Induction of iTregs in the nasal mucosa and functional analysis of these cells may be necessary to determine the regulatory mechanisms affected by SLIT. Mucosal biopsy in the peak pollen season is useful for evaluation of local induction of iTregs and downregulation of effector cells. However, nasal biopsy in the pollen season significantly influences the daily SMS in the peak pollen season. Mucosal biopsy outside the pollen season after exposure using an artificial pollen chamber may be a powerful tool for evaluation of local regulatory mechanisms induced by SLIT [24]. Upregulation of iTregs in nasal mucosa may be difficult to determine since the evaluation may be painful for patients. However, upregulation of iTregs in peripheral blood is simple to analyze and may be a useful biomarker because an increase of peripheral Cry j 1-iTregs is correlated with QOL and QOL-symptom scores in the pollen season, as discussed here and elsewhere [6].

Cry j 1-specific IgG4 production was not induced by SLIT in this study to the same extent as that in our previous study [6]. A clinical trial showing that daily 2500 SQ-T (14 μg Phl p 5 per 4 weeks) tablets failed to induce IgG production supports our current results [13]. A change in the immunoglobulin profile may require a higher allergen dose or longer duration of exposure. However, our study suggests that detectable quantitative changes in IgG4 are not essential for the amelioration of clinical symptoms.

In summary, we suggest that the sIgE/tIgE ratio and upregulation of iTregs may be considered as prognostic and response monitoring biomarkers, respectively, for SLIT. However, further investigation of induction of iTregs at local inflammatory sites and downregulation of inflammatory cells is needed. Furthermore, validation studies with larger sample size would be required before either biomarkers should be applied widely in the clinical management of pollinosis patients. Development of a more effective vaccine and better protocols may reveal more significant differences in the Cry j 1-specific cytokine profiles and iTreg induction, and these results may increase our understanding of the roles of iTregs or Tr1 in the therapeutic mechanisms underlying the efficacy of SLIT.

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3 years of life, but not thereafter. The observed sex differences were larger for asthmatic wheeze than for total wheeze, suggesting that sex differences are stronger for asthma than for transient symptoms.

Young boys are thought to have smaller airway diameters in proportion to their total lung volume than girls, predisposing them to airway obstruction and wheeze.[1,6] Our results suggest that sex differences in asthma may partly be explained by the higher prevalence of atopy in boys and cannot be explained by a stronger effect of perinatal risk factors in boys.

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Characterization of pollen antigen–induced IL-31 production by PBMCs in patients with allergic rhinitis

To the Editor:

Japanese cedar/cypress pollinosis (JCCP) is the major phenotype of allergic rhinitis in Japan and has a prevalence of 29.8%, with a substantial impairment of quality of life (QOL).[1] JCCP is mainly caused by exposure to Japanese cedar *(Cryptomeria japonica)* pollen and Japanese cypress *(Chamaecyparis obtusa)* pollen. The cypress pollen disperses after the cedar pollen in spring. Because cedar and cypress pollen contain several cross-reactive components, pollinosis-related symptoms can last for as long as 4 months, from February to May. On the other hand, species-specific components and epitopes for IgE, T cells, or both have been identified.[2]

IL-31 is a novel cytokine produced by $CD4^+$ T cells, particularly T_H2 cells and skin-homing $CD45RO^+$ cutaneous lymphocyte–associated antigen–positive cells.[3,4] Thus the role of IL-31 in patients with pruritic skin diseases, including atopic dermatitis, has been examined.[3-6] On the other hand, the role of IL-31 in the pathogenesis of respiratory allergic diseases remains unclear.[7-9] IL-31 enhances epidermal growth factor, vascular endothelial growth factor, and CCL2 production by human bronchial epithelial BEAS-2B cells.[8] However, a murine model of T_H2-biased pulmonary inflammation suggests that IL-31 is a negative regulator in this type of inflammation.[7]

In the present study we investigated the production of IL-31 in pollen antigen–stimulated PBMCs from subjects with and without JCCP. Details on the methods are available in the Methods section and Fig E7 of this article's Online Repository at www.jacionline.org.

PBMCs from the healthy control group did not produce IL-31 in response to pollen antigens. On the other hand, the JCCP group included both positive and negative responders. The detection limit of the ELISA (7.8 pg/mL) was used as a cutoff for discriminating $IL\text{-}31^-$ from $IL\text{-}31^+$ JCCP. Of PBMCs from patients with JCCP not treated with specific immunotherapy (SIT), 62.1% (P = .002 compared with control subjects, Fisher exact probability test), 63.0% (P = .002), and 34.6% (P = .060) produced IL-31 in response to Cry j 1, cedar crude antigen, and cypress crude antigen, respectively. This might be the first report of the induction of IL-31 protein production by means of allergen stimulation in human subjects. Among the SIT-treated patients with JCCP, only 25.0% (P = .011 compared with patients not treated with SIT), 21.1% (P = .005), and 16.7% (P = .166) of PBMCs produced IL-31 in response to Cry j 1, cedar crude antigen, and cypress crude antigen, respectively. Overall, the median amounts of IL-31 produced in response to Cry j 1 and cedar crude antigen, but not cypress crude antigen, were significantly higher in patients with JCCP not treated with SIT compared with those seen in healthy control subjects and SIT-treated patients with JCCP (Fig 1). Increased expression levels of IL-31 protein, mRNA, or both in sera, PBMCs, and inflamed tissues in other allergic diseases have been reported for both human subjects and mice.[4-6,9] The present results are consistent with the previous reports and suggest that the increased expression of IL-31 might be a common feature in patients with atopic allergic diseases.

The amounts of Cry j 1–induced, cedar crude antigen–induced, and cypress crude antigen–induced IL-31 production were significantly and positively correlated with the production of IL-5 and IL-13, but not IFN-γ, in response to the respective antigens in patients with JCCP without SIT treatment (see Fig E1 in this article's Online Repository at www.jacionline.org). In addition, PBMCs from patients who produced IL-31 in response to Cry j 1, cedar crude antigen, and cypress crude antigen produced significantly higher amounts of IL-5 and IL-13 by means of stimulation with the respective antigens compared with PBMCs from patients who did not produce IL-31 (see Fig E2, *A*, *B*, *D*, *E*, *G*, and *H*, in this article's Online Repository at www.jacionline.

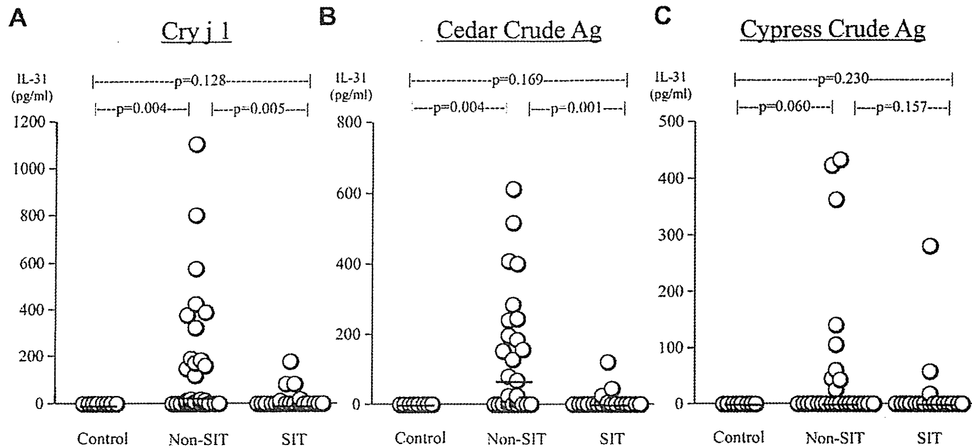

FIG 1. Production of IL-31 by PBMCs after stimulation with pollen antigens. PBMCs from control subjects *(Control)*, patients with JCCP without specific immunotherapy *(Non-SIT)*, and patients with JCCP with specific immunotherapy *(SIT)* were stimulated with Cry j 1 **(A)**, cedar crude antigen **(B)**, and cypress crude antigen **(C)**, and then the concentration of IL-31 in the supernatants was measured. The *horizontal line* indicates the median. *P* values were determined by using the Mann-Whitney *U* test. *Ag*, Antigen.

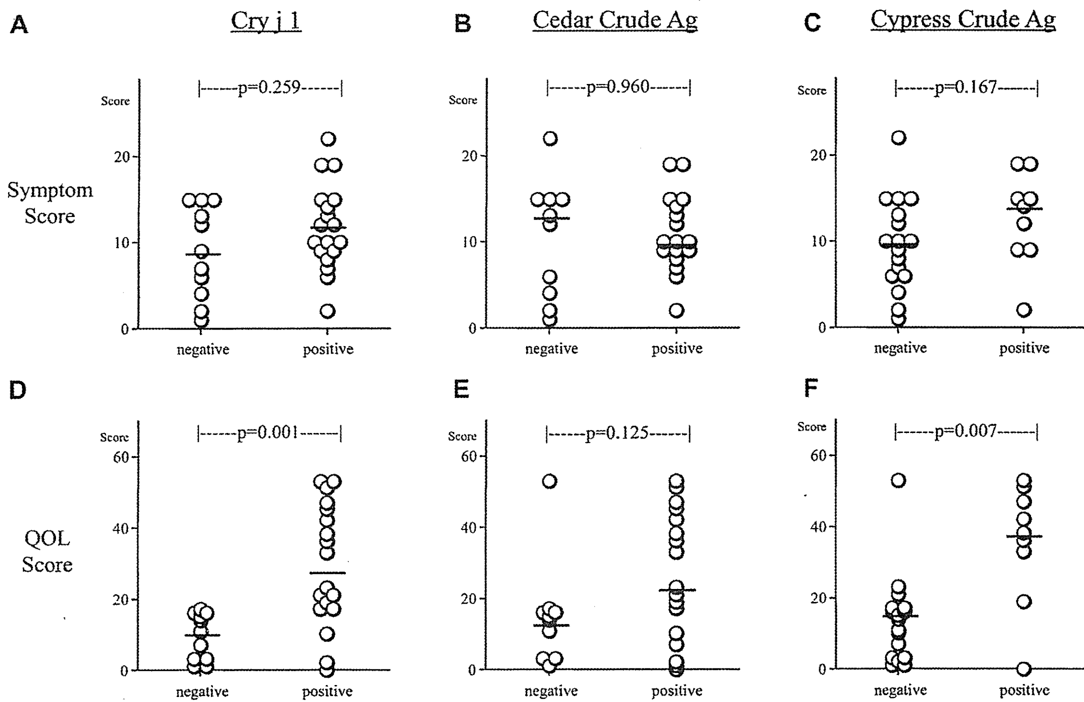

FIG 2. Comparison of naso-ocular symptoms **(A-C)** and QOL **(D-F)** during the peak season of cypress pollen dispersion between patients with positive and negative production of IL-31 in response to Cry j 1 (Fig 2, *A* and *D*), cedar crude antigen (Fig 2, *B* and *E*), and cypress crude antigen (Fig 2, *C* and *F*). The *horizontal line* indicates the median. *P* values were determined by using the Mann-Whitney *U* test. *Ag*, Antigen.

org). The levels of IFN-γ production were not different between IL-31 producers and nonproducers, except for cedar crude antigen stimulation (see Fig E2, *C*, *F*, and *I*). These results suggest that antigen-induced IL-31 production is selectively associated with T_H2 responses in PBMCs.

Symptom scores in the peak season of cedar pollen dispersion were similar between IL-31 producers and nonproducers in response to Cry j 1, cedar crude antigen, and cypress crude antigen (see Fig E3, *A-C*, in this article's Online Repository at www.jacionline.org). QOL scores in the peak season of cedar pollen dispersion were also similar between IL-31 producers and nonproducers in response to cedar crude antigen and cypress crude antigen (see Fig E3, *E* and *F*). A trend in the exacerbation of QOL was seen in Cry j 1–induced IL-31 producers compared with nonproducers; however, this trend did not reach statistical difference ($P = .087$; see Fig E3, *D*).

Symptom scores in the peak season of cypress pollen dispersion were also similar between IL-31 producers and nonproducers in response to Cry j 1, cedar crude antigen, and cypress crude antigen (Fig 2, *A-C*). However, QOL scores, in which a high score means a low QOL, in the peak season of cypress pollen dispersion were significantly higher in IL-31 producers in response to Cry j 1 and cypress crude antigen, but not cedar crude antigen, compared with those seen in the respective nonproducers (Fig 2, *D-F*). Together with the finding that PBMCs that produced IL-31 in response to pollen antigens produced higher amounts of IL-5 and IL-13 in response to the respective antigens, these results suggest that the induction of IL-31 production might lead to a deterioration of JCCP.

The amount of IL-31 production in response to pollen antigens did not correlate with symptom or QOL scores in the peak season of cedar pollen dispersion (see Fig E4 in this article's Online Repository at www.jacionline.org). However, the amounts, especially in response to Cry j 1 ($\rho = 0.641$, $P < .001$) and cypress crude antigen ($\rho = 0.658$, $P = .002$), significantly and positively correlated with QOL scores in the peak season of cypress pollen dispersion (see Fig E5, *D-F*, in this article's Online Repository at www.jacionline.org). In addition, there was a trend for a positive correlation between cypress crude antigen–induced IL-31 production and symptom scores in the season ($\rho = 0.451$, $P = .070$; see Fig E5, *C*). In contrast, the amounts of IL-5 or IL-13 production after stimulation with pollen antigens did not correlate with the QOL scores (see Fig E6 in this article's Online Repository at www.jacionline.org). This result suggests that the induction of pollen antigen–induced IL-31 production by PBMCs is associated with the severity of allergic rhinitis. Detailed discussion is available in this article's Discussion section and Figs E8 and E9 in this article's Online Repository at www.jacionline.org.

The present study provides evidence that, unlike other T_H2-type cytokines, including IL-5 and IL-13, IL-31 displays a unique and independent role in the pathophysiology of allergic rhinitis. The amount of pollen antigen–induced IL-31 production by PBMCs is selectively associated with the severity of QOL in patients with JCCP. These observations might provide a basis for future therapeutic approaches targeting IL-31 in the management and alleviation of allergic rhinitis.

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Sequence variation in the *IL4* gene and resistance to *Trypanosoma cruzi* infection in Bolivians

To the Editor:

Chagas disease, caused by the parasite *Trypanosoma cruzi*, affects 10 to 12 million people each year in Latin America, with Bolivia having the highest prevalence of infection (see "Outlook: Chagas disease"[1] and references therein). In the chronic phase, Chagas infection may present as an indeterminate form in which 60% of infected individuals remain asymptomatic despite having positive serologic reactions for *T cruzi*. In the remaining 40% of patients with Chagas disease, tissue inflammation leads to organ damage, affecting the cardiac, digestive, or nervous systems up to 25 years after initial infection. Several studies identified genetic markers for disease establishment and progression in Venezuelans, Brazilians, Peruvians, Colombians, and Mexicans,[2] but no genetic studies have been conducted previously in Bolivians.

Cytokines produced in response to *T cruzi* infection appear to modulate disease progression by enhancing or inhibiting parasite replication in a variety of cell types. In particular, the T_H2 cytokine IL-4 maintains inflammation and parasite persistence in Chagas disease,[3] whereas T_H1 cytokines maintain control of parasitism[4] but can also contribute to the development of chronic myocarditis.[5]

To determine whether genetic variation at the *IL4* gene is associated with *T cruzi* infection in Bolivians, we performed a resequencing study of an approximately 12-kb region around the *IL4* locus, including 470 base pairs (bp) of coding (exon) sequence, 368 bp of 5′ untranslated region, 82 bp of 3′ untranslated region, and 11,453 bp of intronic sequence. The study included 110 individuals from the Department of Cochabamba, Bolivia, with infection status serologically confirmed by 2 different diagnostic tests (HAI Chagas Polychaco; Laboratorio Lemos, S.R.L., Buenos Aires, Argentina, and IFI Biocientifica S.A., Buenos Aires, Argentina). Each subject was classified according to the serologic results as a case (positive serology) or a control (negative

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ORIGINAL ARTICLE

Upregulation of *IL17RB* during Natural Allergen Exposure in Patients with Seasonal Allergic Rhinitis

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ABSTRACT

Background: Seasonal allergic rhinitis (SAR) to Japanese cedar (*Cryptomeria japonica*; JC) is an IgE-mediated type I allergy affecting the nasal mucosa. However, the molecular mechanisms that underlie SAR are only partially understood. The aim of the study was to identify novel genes related to SAR during natural exposure to pollens, by using microarray analysis.

Methods: Subjects were 32 SAR patients and 25 controls. Total RNA was extracted from $CD4^+$ T cells isolated from peripheral blood mononuclear cells and subjected to microarray analysis with Illumina Human Ref8 BeadChip arrays. The Mann-Whitney test was performed to identify genes whose expression was altered during allergen exposure. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed on samples collected from SAR patients and controls to verify the microarray results.

Results: Microarray analysis showed that the expression of 3 genes was significantly altered during allergen exposure. Among these 3 genes, the expression of interleukin 17 receptor beta (IL17RB) was confirmed to be upregulated in SAR patients compared to that of the *IL17RB* gene in healthy, non-allergic controls. The average fold change of *IL17RB* expression in the real-time RT-PCR experiment was 3.9 ($P = 0.003$).

Conclusions: The present study identified upregulation of *IL17RB* during natural allergen exposure in patients with SAR, which may further elucidate the molecular mechanisms underlying SAR.

KEY WORDS

allergen exposure, microarray, nasal mucosa, quantitative real-time RT-PCR, seasonal allergic rhinitis

INTRODUCTION

Allergic diseases such as asthma and allergic rhinitis are major causes of morbidity in developed countries, and their incidence is increasing. Seasonal allergic rhinitis (SAR) to Japanese cedar (*Cryptomeria japonica*) is an IgE-mediated type I allergy affecting the nasal mucosa. It is one of the most common allergic diseases in Japan, affecting 19.4% of the Japanese population,[1] and thus is a major public health issue. According to a national survey, the prevalence of rhinitis in Japan was 0.16 in 1992 and 0.21 in 2002.[2] We recently reported the prevalence of allergic rhinitis in an adult population of the Fukui area of Japan was 44.2% (681 of 1,540 subjects aged between 20 and 49 years), and the most common allergen in allergic rhinitis was Japanese cedar pollen (89.6%, 610 of 681 subjects with SAR).[3] SAR therefore contributes to the undermining of quality of life and decline in labor productivity.[4]

SAR is a chronic, inflammatory disease of the nasal mucosa caused by the infiltration of lymphocytes, mast cells, and eosinophils into the nasal mucosa. T-helper type 2 (Th-2) cytokines play a crucial role in orchestrating inflammatory responses. However, the molecular mechanisms that underlie SAR development are only partially understood. To understand the molecular basis of SAR, it would be helpful to examine the expression of genes in subjects with SAR during allergen exposure. Microarray techniques permit simultaneous analysis of the expression of many

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Table 1 Characteristics of the study population

	SAR (*n* = 32)	Control (*n* = 25)	*P* value
Age (year ± SD)	36.8±8.8 (25-50)	32.8±7.1 (18-47)	0.052
No. of Male/Female	16/16	12/13	0.334
Whole blood			
Neutrophil (%)	55.2 (range 39.5 to 76.0)	53.7 (range 36.3 to 76.0)	0.596
Lymphocytes (%)	33.7 (range 12 to 48.5)	37.4 (range 16.0 to 57.0)	0.154
Monotytes (%)	5.4 (range 3.3 to 8.0)	5.5 (range 3.6 to 7.3)	0.71
Eosinophils (%)	5.2 (range 0 to 18.0)	2.8 (range 0.3 to 7.3)	0.008
Basophils (%)	0.6 (range 0 to 1.5)	0.6 (range 0 to 1.8)	0.92
Total serum IgE (IU/mL)	50.1 (range 7 to 880)	28.4 (range 5 to 160)	0.049
JC-specific IgE (U_A/mL)	8.08 (range 0.79 to 86.1)	0.11 (range <0.34 to 0.56)	<0.00001

genes. Therefore, large-scale gene expression analysis by microarray may clarify which novel molecules are related to SAR.

Two types of tissues have been used for human microarray studies of allergic rhinitis. One is tissue from the nose, such as nasal polyps and nasal mucosa. Zhang *et al.*[5] performed microarray studies with nasal mucosa obtained from subjects with and without allergic rhinitis and found that several chemokine genes such as CC chemokine receptor (CCR) 2, CCR3, CCR5, CCR8, and CX3 chemokine receptor 1 were highly expressed in the nasal mucosa of subjects with allergic rhinitis compared to the expression of these genes in the mucosa of subjects without allergic rhinitis.

The other type of tissue is peripheral blood obtained from patients and controls. Larsson *et al.*[6] examined the transcriptional profiles of dendritic cells (DCs) after stimulation with grass pollen allergens and co-culture with autologous $CD4^+$ memory T cells. This study found a distinct T-cell-induced DC profile in atopic individuals, suggesting that T cells have a key instructive role in educating DCs in Th2-type responses. Benson *et al.*[7] performed microarray analysis using allergen-challenged $CD4^+$ T cells from patients with SAR and compared the expression level of $CD4^+$ T cells challenged with diluents only. This study found that tumor necrosis factor receptor superfamily member 4 (TNFRSF4), which is related to apoptosis, is significantly upregulated in allergen-challenged $CD4^+$ T cells. Allergic diseases are thought to be involved in the dysregulation of T cells, including $CD4^+$ lymphocytes. Therefore, examining changes in gene expression levels in $CD4^+$ T cells from SAR patients and healthy, non-allergic controls may improve our understanding of the molecular mechanism underlying SAR.

In the present study, we performed microarray analysis to identify changes in gene expression that reflect the status of SAR during natural allergen exposure and found that interleukin 17 receptor B (*IL17RB*) is upregulated during natural allergen exposure in SAR patients.

METHODS

SUBJECTS

Between 2003 and 2007, 1575 hospital workers and university students were invited to participate in an epidemiological survey of allergic rhinitis. All participants were of Japanese origin and were residents of Fukui Prefecture, Japan. The characteristics of the study population have been described in detail previously.[3] Total and specific IgE (produced in response to Japanese cedar, Dermatophagoides, *Dactylis glomerata*, *Ambrosia artemisiifolia*, *Candida albicans*, and *Aspergillus*) were measured using the CAP-RAST method (Pharmacia Diagnostics AB, Uppsala, Sweden).

We invited 56 of the 1575 survey subjects to participate in a gene expression analysis study and collected a 150-ml blood sample from each subject between February and April 2009, the time during which subjects were naturally exposed to Japanese cedar pollens. We also collected blood samples from the same individuals between November and December 2008, when they were not exposed to Japanese cedar pollens. Cases of SAR due to Japanese cedar polinosis (SAR group) were diagnosed on the basis of a positive history of rhinitis between February and April and high levels of Japanese cedar-specific IgE antibodies in the serum (RAST score ≥ class 2). We included only those SAR patients who were sensitized to Japanese cedar (i.e., no detectable allergen-specific IgE against dust mites, *D. glomerata*, *A. artemisiifolia*, *C. albicans* or *Aspergillus*; RAST score ≤ class 1). Subjects without allergies (control group) had to satisfy the following criteria: (1) no symptoms or history of allergic diseases, (2) no detectable, specific IgE antibodies against 6 common inhalant allergens (RAST score ≤ class 1), and (3) total serum IgE levels below the general population mean. The characteristics of subjects are listed in Table 1 and Table 2. The sample from one SAR patient (No. 19) was used only for realtime RT-PCR analysis because cRNA amplification for microarray experiment was not successful.

All participants provided written informed consent

Table 2 Treatment of SAR patients during natural pollen exposure

Sample No.	Age	Sex	Treatment
2	45	Female	Pranlukast Bepotastine besilate
3	41	Female	Epinastine hydrochloride
4	47	Female	Epinastine hydrochloride
11	27	Female	Olopatadine hydrochloride
12	30	Male	No treatment
14	25	Female	No treatment
15	28	Female	Fexofenadine hydrochloride
19	30	Male	Epinastine hydrochloride
23	30	Female	No treatment
24	47	Male	No treatment
25	27	Male	No treatment
30	50	Male	Olopatadine hydrochloride
32	49	Female	Epinastine hydrochloride
34	31	Male	No treatment
36	27	Male	No treatment
39	43	Male	Cetirizine hydrochloride
40	49	Male	No treatment
41	44	Female	No treatment
45	45	Male	Fexofenadine hydrochloride
53	45	Female	Epinastine hydrochloride
55	26	Female	Epinastine hydrochloride
56	28	Female	Epinastine hydrochloride
57	44	Male	Cetirizine hydrochloride
58	37	Male	Bepotastine besilate
59	25	Female	No treatment
60	25	Female	No treatment
65	44	Male	Epinastine hydrochloride
68	32	Male	Olopatadine hydrochloride
69	45	Female	Cetirizine hydrochloride
73	42	Female	No treatment
75	38	Male	Cetirizine hydrochloride
81	30	Male	Bepotastine besilate Olopatadine hydrochloride

to participate in the study. The study was approved by the ethical committees of the University of Tsukuba and the University of Fukui, Japan.

RNA EXTRACTION

Peripheral blood (150 ml) was taken from each subject. Peripheral blood mononuclear cells (PBMCs) were purified with Ficoll-Paque™ gradient (GE Healthcare, Piscataway, NJ, USA). $CD4^+$ T cells were isolated from PBMCs with a human CD4 Isolation kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's instructions. RNA was extracted from PBMCs with an RNeasy Mini Kit (Qiagen K.K., Tokyo, Japan) according to the manufacturer's instructions.

We used the Illumina BeadArray with single-color array (Illumina, San Diego, CA, USA) as a microarray platform. For the Illumina BeadArray assay, cRNA was synthesized with an Illumina® RNA Amplification Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. In brief, 500 ng of total RNA from $CD4^+$ T cells were reverse transcribed to synthesize first- and second-strand cDNA, purified with spin columns, and then *in vitro* transcribed to synthesize biotin-labeled cRNA. A total of 750 ng biotin-labeled cRNA was hybridized to each Illumina Human Ref8 BeadChip array (Illumina) at 55℃ for 18 h. The hybridized BeadChip was washed and labeled with streptavidin-Cy3 (GE Healthcare) and then scanned with the Illumina BeadStation 500 System (Illumina). The scanned image was imported into BeadStudio software (Illumina) for analysis. Twenty-two thousand transcripts representing 8 whole-genome samples can be analyzed on a single Bead-

Table 3 Genes related to SAR identified by microarray

Gene Name	Description	Microarray		Real-time PCR		Accession †
		Fold change	*q*-value	Fold change	*P*-value	
ARID4B	AT rich interactive domain 4B	-1.6	0.049	1.1	0.204	NM_016374.5
SERPINE2	serpin peptidase inhibitor, member 2	-1.5	0.049	1	0.104	NM_006216.2
IL17RB	interleukin-17 receptor B	1.6	0.049	3.9	0.003	NM_018725.3

† GenBank accession numbers.

Chip. We included at least 1 technical replicate (i.e., the same cRNA sample) for each BeadChip. The correlation coefficients for identical RNAs were 0.995 to 0.996 (r^2) in the present study.

QUANTITATIVE REAL-TIME REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (RT-PCR)

$CD4^+$ T cells from the subjects in each group were purified by Ficoll-Paque™ gradient (GE Healthcare) and human CD4 T cell Isolation kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). The purity of the isolated CD4 was over 98% in the present study. Total RNA was extracted from PBMCs with an RNeasy Kit (Qiagen). Quantitative real-time RT-PCR was performed with TaqMan Universal Master Mix and an Assay-on-Demand Gene Expression Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The endogenous control GAPDH was used to normalize the sample with the $\Delta\Delta C_T$ method for relative quantification with SDS software 2.2.0 (Applied Biosystems).

STATISTICAL METHODS

For the microarray analysis, background-corrected values for each probe on the BeadChip array were extracted using BeadStudio version 2.0 (Illumina). The detection limit corresponding to a 0.01 detection *p*-value was determined by using a normal model of intensities of 20 negative control probes that had no corresponding target in the sample.[8] The extracted values were exported to the software GeneSpring version 10 (Silicon Genetics, Redwood, CA, USA), and per chip and per gene normalizations were performed. The statistical significance of the microarray data was calculated using the Mann-Whitney test, and multiple tests were corrected by the Benjamini and Hochberg false discovery rate.[9]

Statistical significance of real-time RT-PCR was calculated by using the Wilcoxon signed rank-sum test (paired samples) and the Mann-Whitney U test (2 independent samples). In the paired samples, each sample from SAR patients and controls exposed to pollens was normalized to the sample from the same individual not exposed to pollens (sample-specific normalization). The correlation between normalized values of the microarray and those of the quantitative PCR experiments were performed with Spearman's rho test. Significance was defined as $P < 0.05$.

RESULTS

We first selected transcripts that were expressed by at least half (i.e., 28 samples) of the 56 samples on the Human Ref8 BeadChip arrays with detection P values < 0.01. Among the 10,477 expressed transcripts, those satisfying all the following criteria were selected as up- or downregulated transcripts in the microarray analysis: (1) more than 1.5-fold increase/decrease on average and (2) transcripts showing statistically significant differences between the SAR and control groups ($q < 0.05$).

A total of 4 transcripts were upregulated, and 15 transcripts were downregulated at the 1.5-fold level (19 transcripts total). The change in the expression of 3 (1/2 = up-/downregulated) transcripts was statistically significant ($q < 0.05$); 1 was up-regulated, and 2 were down-regulated. Genes that were up- and downregulated in $CD4^+$ T cells between the SAR and control groups are listed in Table 3.

We then performed quantitative real-time RT-PCR to verify the results of the microarray analyses. Significant correlations between the microarray results and the results of the quantitative real-time RT-PCR were observed for *IL17RB* (Spearman's rho = 0.815, $P < 0.0001$) and *SERPINE2* (Spearman's rho = 0.877, $P < 0.0001$). However, no correlation was observed for *ARID4B* (Spearman's rho = -0.063, $P = 0.58$). The average fold change for *IL17RB* in the real-time RT-PCR experiment was 3.9 ($P = 0.003$, Table 3). Although good correlation was observed between the microarray results and the results of the quantitative real-time RT-PCR for *SERPINE2*, the average fold change for *SERPINE2* in the real-time PCR experiment was 1.0 ($P = 0.10$, Table 3), because of the existence of outliers.

In the quantitative real-time RT-PCR results of paired (exposed versus non-exposed) samples, *IL17RB* expression was elevated during natural pollen exposure in SAR patients but not in the controls (Fig. 1). *IL17RB* expression in SAR patients did not differ from that in controls when they were not exposed to cedar pollens ($P = 0.93$).

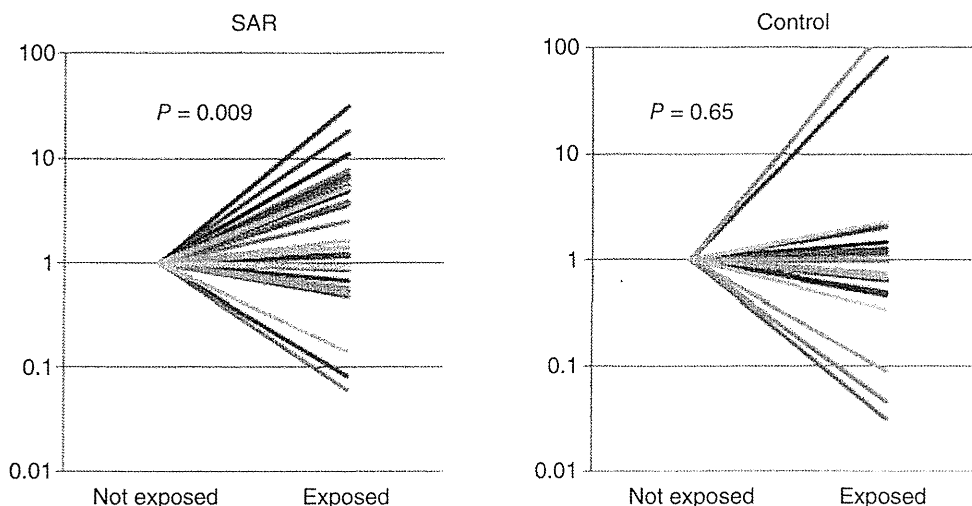

Fig. 1 Quantitative real-time RT-PCR validation of microarray data. Each sample from SAR patients and control individuals exposed to pollens was normalized to the sample from the same individual not exposed to pollens (sample-specific normalization). P values were calculated with Wilcoxon signed rank-sum test.

DISCUSSION

In the present study, we performed microarray analyses to identify genes related to SAR and identified 3 up- or downregulated genes related to SAR. Among these 3 genes, *IL17RB* was confirmed to be upregulated in real-time quantitative PCR analysis.

In our study, the number of genes that were significantly altered was small compared with those in other microarray studies, which used CD4+ cells stimulated with allergens. One possibility for this difference is that gene expression change during natural allergen exposure in vivo is more subtle. Hansel *et al.*[10] performed microarray analyses using CD4+ T cells from 84 subjects and did not find a dominant allergy-associated profile in CD4+ T cells between allergic and non-allergic subjects. Because distinct CD4+ T-cell-induced DC profiles were reported in atopic individuals,[6] changes in CD4+ T cell profiles are likely to influence subsequent allergic responses, leading to the development of SAR.

Microarray experiments are now widely used to simultaneously analyze the expression of tens of thousands of genes. Quantitative real-time PCR is a commonly used method for validating microarray experiments. However, microarray and quantitative real-time PCR results sometimes disagree. In general, it has been reported that correlations increase with increasing degrees of change.[11] Dallas and colleagues reported poorer correlations between microarray expression scores for genes that exhibited fold-change differences of <1.5 compared with fold-change differences of >1.5.[12] We observed poor correlation for *ARID4B*. The poor correlations may be due, in part, to the existence of alternative, cross-hybridizing transcripts differentially recognized by the oligonucleotide probe sets and qRT-PCR probes, because several splice variants exist in *ARID4B*.

IL-17RB is the receptor for IL-17B and IL-17E (also known as IL-25).[13,14] IL-25 has been shown to induce Th-2 responses, and recent studies revealed that IL-17RB was highly expressed on a subset of naive and activated CD4+ invariant natural killer (NK) T cells, but not on activated T cells and that IL-17RB+ invariant NKT cells produced large amounts of Th-2 cytokines that were substantially increased by IL-25 stimulation.[15,16] It has also been reported that *IL17RB* knockout mice did not exhibit histological signs of lung inflammation, while marked infiltration of inflammatory cells were observed in wild-type mice.[17] Recently, Wang *et al.* performed microarray experiments using PBMCs stimulated with allergens or diluents in vitro, and reported that *IL17RB* was the most significantly upregulated gene on allergen stimulation in SAR patients when compared with that in controls. They also observed a significant increase in *IL17RB* gene expression from microarray data of allergen-challenged CD4 cells from SAR patients as compared to diluent-challenged cells. This is in agreement with our findings, which reveal upregulation of *IL17RB* in SAR patients naturally exposed to pollens, although the degree of changes in gene expression that we observed was lower than that in the study by Wang *et al.* Therefore, it is speculated that the differential gene expression pattern of *IL17RB* in SAR patients during allergen exposure may be related to the development of SAR.

In conclusion, the present study identified upregulation of *IL17RB* during natural allergen exposure in patients with SAR. Because the blockade of

IL-17RB has been reported to prevent IL-25-induced lung inflammation and Th-2 type cytokine secretion,[17] antagonists for IL-17RB could be a novel therapeutic target for allergic diseases.

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Ⅱ．耳鼻科における皮下アレルゲン免疫療法

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Summary
鼻アレルギー診療ガイドラインでは，皮下免疫療法はアレルギー性鼻炎の病型および重症度に関わらず基盤療法の1つとして位置づけられている。アレルギー性鼻炎はⅠ型アレルギー疾患であり，原則的に6歳以上で全身的に重篤な疾患を持たない患者には適応がある。ダニや花粉など多くの吸入アレルゲンに関して，プラセボ対照二重盲検試験でアレルギー性鼻炎の症状およびQOLに対するSCITの有効性が示されており，特に成人季節性鼻炎(花粉症)についてはメタ解析によってその有効性が実証された。皮下免疫療法は様々な免疫担当細胞に働き免疫寛容を誘導するが，特に制御性T細胞や特異的Th2細胞に対する調節作用が重要である。

Key Words
皮下免疫療法／アレルギー性鼻炎／メタ解析／QOL

はじめに

アレルゲン免疫療法は，約100年の歴史を有する，アレルギー性鼻炎の治癒あるいは長期寛解が期待できる治療法である[1]。稀に全身性の副反応を生じることや，即効性に乏しいことなどが普及を妨げているが，WHO見解書や鼻アレルギー診療ガイドラインなどにより標準的かつ実用的な治療法が示され，また作用機序の解析が進み，有効性と安全性が理解されるようになった[2)~4)]。さらに，薬物療法では得がたいアレルギー疾患の自然経過（natural course）を修飾する作用も明らかとなり，鼻アレルギー診療ガイドラインではアレルギー性鼻炎に対する基盤療法の1つとして位置づけられている[3]。本稿では，アレルギー性鼻炎に対する皮下免疫療法（subcutaneous immunotherapy；SCIT）の実際と効果および限界，さらに作用機序のアップデートについて，諸家の報告に自験例を交え概説する。

Ⅰ．SCITの実際

1．適応

鼻アレルギー診療ガイドラインで触れられているように，原則的に6歳以上で全身的に重篤な疾患をもたず，全身性ステロイド薬や抗癌薬など免疫調節されていない患者が適応である[3]。小児でも実施は可能であり，年齢の上限はない[4]。特に，薬物療法でのコントロールが不十分なもの（無効

SCIT（subcutaneous immunotherapy；皮下免疫療法）

例，副作用が強い例，薬物療法を希望しない例）や長期寛解を望むものには良い適応である。

一方，βブロッカー投与例では副反応を生じた際に救急用エピネフリンを用いることができないため禁忌である。活動性喘息を合併する例，特に1秒率が70％未満の患者はSCITにより重篤な喘息発作を生じうるので禁忌である。妊婦に対しては，維持療法を継続することは可能だが，導入療法は避ける[2)〜4)]。

2．アレルゲンエキス

力価の安定した標準化エキスを用いることが望ましい[2)]。我が国で標準化された治療用エキスはスギのみである。スギ以外の花粉エキスではブタクサおよびアカマツが市販されている。通年性鼻炎に使用しうる治療用エキスとしては，ハウスダストエキスおよび真菌エキス（アスペルギルス，アルテルナリア，カンジダ，クラドスポリウム，ペニシリウム）がある。これらのエキスはいずれも標準化されていない。動物毛垢などのエキス，あるいは標準化エキスの使用を望む場合は，日本アレルギー協会・抗原研究会に輸入代行を依頼し，所定の手続きを経て米国ホリスター社製のエキスを入手できる。

3．導入療法

SCITを実施するにあたっては，根治や長期寛解が期待しうる治療法であるが即効性に乏しいこと，継続した治療が必要であること，稀に副反応を生じうることなどを説明する。初回注射量は，順次10倍希釈した治療エキスに対する皮内反応閾値（注射15分後の紅斑が20 mm以上となる最大希釈濃度）を参考にする。我々は，喘息既往例では閾値の1/10より，その他は閾値より導入療法を開始している。

原法では50％増量法で週に1〜2回の実施が勧奨されている。しかし本法では維持量に達するまで長期間を要し脱落しやすい傾向がある。我々は，注射15分後の紅斑が60 mmを超えない範囲で，かつ最高濃度（例：標準化スギ花粉エキスなら2,000 JAU/mL）でなければ100％増量法を行っている（表1）。WHO見解書では，副反応を予防する手段として副腎皮質ステロイド薬，長時間持続型テオフィリン，あるいは抗ヒスタミン薬による前処置を示している[2)]。最近では抗IgE（immunoglobulin E）抗体による前処置の有効性も報告されている[5)]。我々は注射当日に抗ヒスタミン薬を内服させている。抗ヒスタミン薬による前処置は安全性のみならず有効性を向上できることが示されている[6)]。また注射後の局所反応を観察・記録し次回注射量への参考とすること，さらに帰宅後の局所や気道症状の有無を次回注射前に問診することが重要である。

4．維持療法

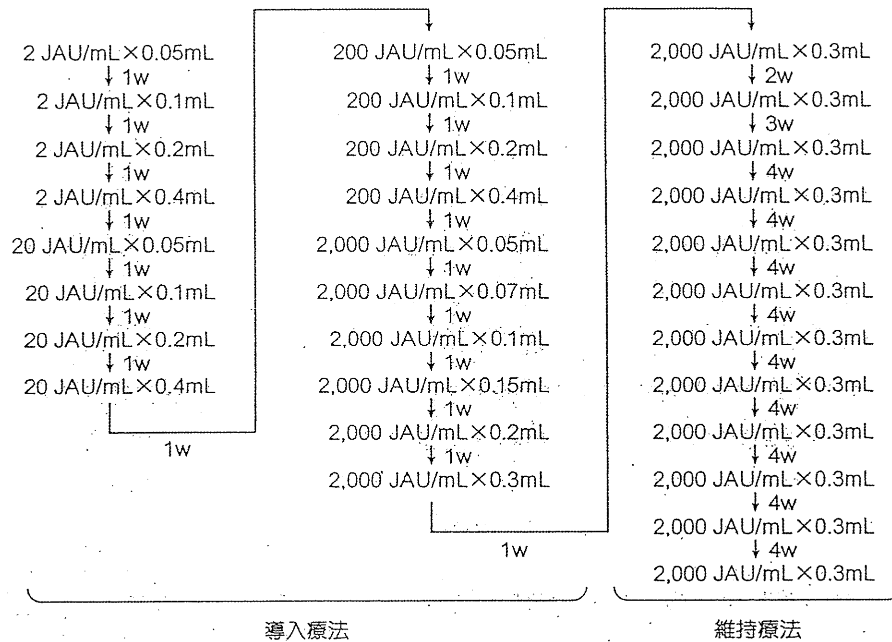
注射15分後の発赤が60 mmを超えるようであれば増量を終了し，維持量としている。維持療法の最適な継続期間は不明であるが，良好な効果を示した報告の多くは3〜5年間の治療を要している[2)〜4)]。後述のように，寛解に到達した患者では治療を終了した後もその効果が持続する[7)]。治療終了後に寛解が持続する期間は，免疫療法の治療期間と関連することを示す報告もある[8)]。従って，なるべく長期に継続治療するように患者を指導し，また予約診療を行うなど，治療脱落を防ぐ配慮が望ましい。

5．急速免疫療法

導入療法を頻回に行い，数日間で維持量に到達させる方法である。通常は入院下で行う。ハウス

IgE (immunoglobulin E)

表1　標準化スギ花粉エキスを用いた治療スケジュール例（通常法）



（筆者作成）

ダストエキスを用いた投与プロトコールを表2に示す。全身的な副反応の出現頻度は通常法よりも高い[5]。我々は抗ヒスタミン薬にて前処置することにより，現在までのところ蕁麻疹以上の全身副反応の経験はない。

注射後には皮膚反応や胸部聴診を行い，副反応の前駆症状をチェックする。急速免疫療法という名前から，患者によっては入院下での注射で治療が終了すると誤解するものがいる。通常法と同様に，退院後は維持療法が必要であることを説明し同意を得ておく。

6．副反応とその対応

局所反応と全身反応がある[2]。局所反応としては注射部位の腫脹や蕁麻疹があり，また全身反応としては喘息やアナフィラキシーショックなどがある。標準化したイネ科花粉エキスやダニアレル

表2　ハウスダスト（HD）エキスを用いた治療スケジュール例（急速法）

日程	時間	抗原	濃度・投与量
1日目	9：00	HD	100,000倍×0.1 mL
	10：00	HD	100,000倍×0.3 mL
	11：00	HD	10,000倍×0.1 mL
	13：00	HD	10,000倍×0.3 mL
	15：00	HD	1,000倍×0.1 mL
2日目	9：00	HD	1,000倍×0.3 mL
	11：00	HD	100倍×0.1 mL
	13：00	HD	100倍×0.2 mL
	15：00	HD	100倍×0.4 mL
3日目	9：00	HD	100倍×0.4 mL
	11：00	HD	10倍×0.05 mL
4日目	9：00	HD	10倍×0.05 mL
5日目	9：00	HD	10倍×0.05 mL
	11：00	退院	

（筆者作成）

表３　岡山大学病院耳鼻咽喉科における副反応の発生頻度（2009年）

		導入療法		維持療法	全体
		通常法	急速法		
患者数		11例	3例	94例	98例
受診回数（入院1日は1回と算定）		144受診	15受診	774受診	920受診
注射回数		282回	39回	1,098回	1,419回
副反応の回数	局所	38回	2回	15回	55回
	全身	1回	0回	0回	1回
局所副反応の頻度（%）	患者ベース	54.5%	66.7%	4.2%	12.2%
	注射ベース	13.5%	5.1%	1.4%	3.9%
全身副反応の頻度（%）	患者ベース	9.0%	0%	0%	1.02%
	注射ベース	0.4%	0%	0%	0.07%

（筆者作成）

ゲンを用いたSCITでの副反応の発生率は，局所反応で10.5%，全身反応で4.8%，また注射回数では0.37%の頻度で全身反応を来したとの報告がある[9]。特に標準化していないエキスを用いる場合は，ロットによるアレルゲン含有率に差を生じうるため，濃度上昇時やロット変更時には注意が必要である。一般に，副反応は導入療法の際に生じることが多く，特に開始時や濃度上昇時に生じやすい。しかしながら維持療法においても，患者の状態（睡眠不足，感冒，発熱，生理など）により全身副反応を認めることがあり，注射前の問診や診察が重要である。我々の2009年における調査では，98例の患者に計1,419回のSCITが施行されたが，局所副反応は55回（患者ベースで12.2%，注射ベースで3.9%），全身副反応は1回（患者ベースで1.02%，注射ベースで0.07%）であった（表3）。患者の選択や抗ヒスタミン薬による前処置，注射後の皮膚反応の観察と記録，および注射前の問診や診察などを適切に行うことにより，全身副反応は軽減できると思われる。

Ⅱ．SCITの鼻炎症状への効果

ダニ，真菌，ネコ，花粉などの吸入アレルゲンに関して，これまでに多くのプラセボ対照二重盲検試験でアレルギー性鼻炎に対するSCITの有効性が示されている[10〜13]。例えば中等症以上の成人鼻炎患者にヤケヒョウヒダニエキスを用いたSCITを1年間行ったところ，有意な鼻症状の改善とともに，薬剤使用頻度の有意な減少，さらにはダニに対する皮膚反応の有意な低下がみられる[12]。

季節性アレルギー性鼻炎（花粉症）への効果はより顕著である。樹木，イネ科花粉およびキク科花粉による季節性アレルギー性鼻炎に対するSCITの効果を検討した51件の二重盲検試験（n＝2,871）をメタ解析した結果，プラセボと比較し有意な（$p < 0.00001$）症状および薬物スコアの軽減がみられている（表4）。また救急用アドレナリンの使用頻度は注射ベースで0.13%（プラセボは0.01%）で，致死例は認めなかった[13]。

表4　メタ解析による季節性アレルギー性鼻炎（花粉症）の症状スコアに対するSCITの効果

Study or subgroup	Treatment N	Mean(SD)	Control N	Mean(SD)	Weight	Std. Mean Difference Ⅳ, Random, 95% CI
Balda 1998	49	656(10.43)	56	9.07(8.19)	9.3%	−0.27[−0.65, 0.12]
Bodtger 2002	16	22(1)	17	3.3(1.4)	5.7%	−0.88[−1.60, −0.16]
Bousquet 1990	20	63.6(32.5)	18	108.6(33.2)	5.8%	−1.34[−2.05, −0.63]
Brewczynski 1999	10	59.5(32.6)	8	122.4(85.13)	3.8%	−0.98[−1,97, 0.02]
Corrigan 2005	77	1,665(114.93)	77	218(135.39)	10.1%	−0.41[−0.73, −0.09]
Drachenberg 2001	74	0.75(0.44)	50	0.95(0.41)	9.6%	−0.46[−0.83, −0.10]
Ferter 2005	22	0.44(0.32)	20	0.8(0.54)	6.5%	−0.81[−1.44, −0.17]
Frew 2006	187	3.31(2.42)	89	4.59(2.93)	10.8%	−0.49[−0.75, −0.24]
Jutel 2005	29	3.93(3.28)	28	5.82(3.44)	7.6%	−0.55[−1.08, −0.02]
Meriney 1986	10	3.51(2.97)	10	8.43(4.24)	3.9%	−1.29[−2.27, −0.30]
Ortolani 1984	8	2.01(0.57)	7	5.86(1.63)	1.8%	−3.06[−4.69, −1.43]
Ortolani 1994	18	0.61(0.12)	17	2.3(0.98)	4.5%	−2.40[−3.29, −1.51]
Varney 1991	19	1,531(1,875)	16	2,230(856)	6.1%	−0.46[−1.13, 0.22]
Walker 2001	17	−1,212(2,632)	13	−115(1,159)	5.6%	−0.50[−1.24, 0.23]
Zenner 1997	41	8,224(64.38)	40	115.98(83.67)	8.6%	−0.45[−0.89, −0.01]
Total(95% CI)	**597**		**466**		**100.0%**	**−0.73[−0.97, −0.50]**

Heterogeneity. $Tau^2=0.12$: $Chi^2=38.05$, df=14(P=0.00051) : $I^2=63\%$
Test for overall effect Z=6.10(P<0.00001)

Std. Mean Difference Ⅳ, Random, 95% CI
−4 −2 0 2 4
Favours treatment　Favours control

（文献13より）

スギ花粉症に関しては，SCITの効果は花粉飛散量に依存するが，初期治療と同等の効果がみられ，さらに初期療法と併用することにより有意な上乗せ効果がみられる（図1）[14]。日本アレルギー性鼻炎標準QOL調査票（JRQLQ）を用いた我々の2009年での解析では，初期治療を行わなかったSCIT施行患者のスギ花粉飛散期の鼻眼症状は，初期療法と有意差はないものの，レスキュー療法群と比較すると有意に軽減していた（図2）。さらにSCIT施行例の約半数は薬物療法を必要としなかったことから，メタ解析の結果と同様にスギ花粉症においてもSCITは薬物使用量を軽減できることが期待できる。

西日本ではヒノキの植林が多くなされていることから，スギ花粉飛散後のヒノキ花粉症が問題となっている[14]。スギエキスを用いたSCITは，ヒノキ花粉飛散量の多いシーズンではヒノキ花粉飛散期に効果が減弱する（図1）[15]。この要因の1つとして，末梢血単核細胞のヒノキアレルゲンCha o 1に対するIL（interleukin）-5産生は免疫

JRQLQ（日本アレルギー性鼻炎標準QOL調査票）　　IL（interleukin）

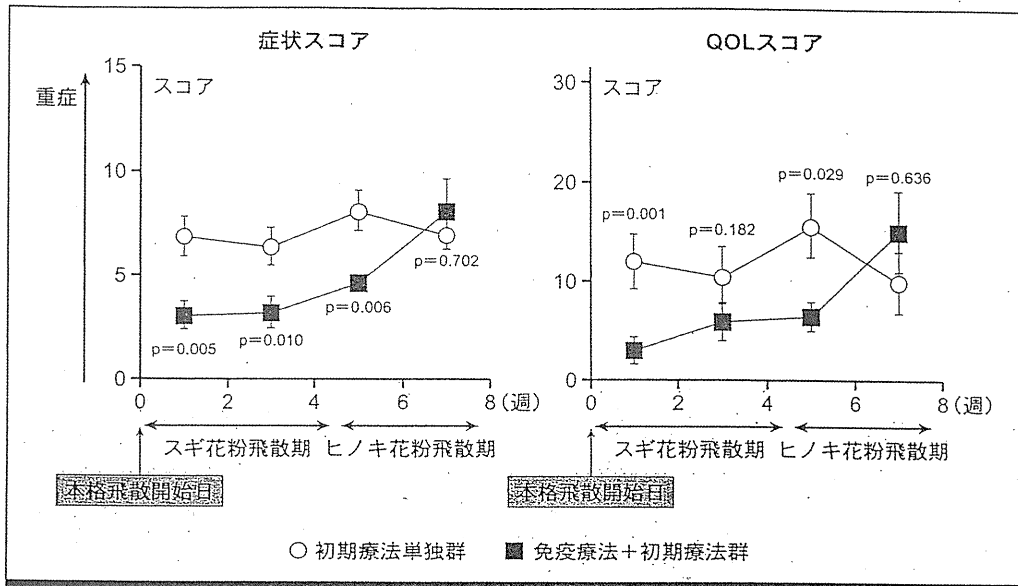


図1　2006年（ヒノキ花粉大量飛散年）におけるSCITの上乗せ効果

スギ花粉エキスによるSCITは初期療法と併用することにより，特にスギ花粉飛散期に有意な上乗せ効果がみられる.

（文献15より）

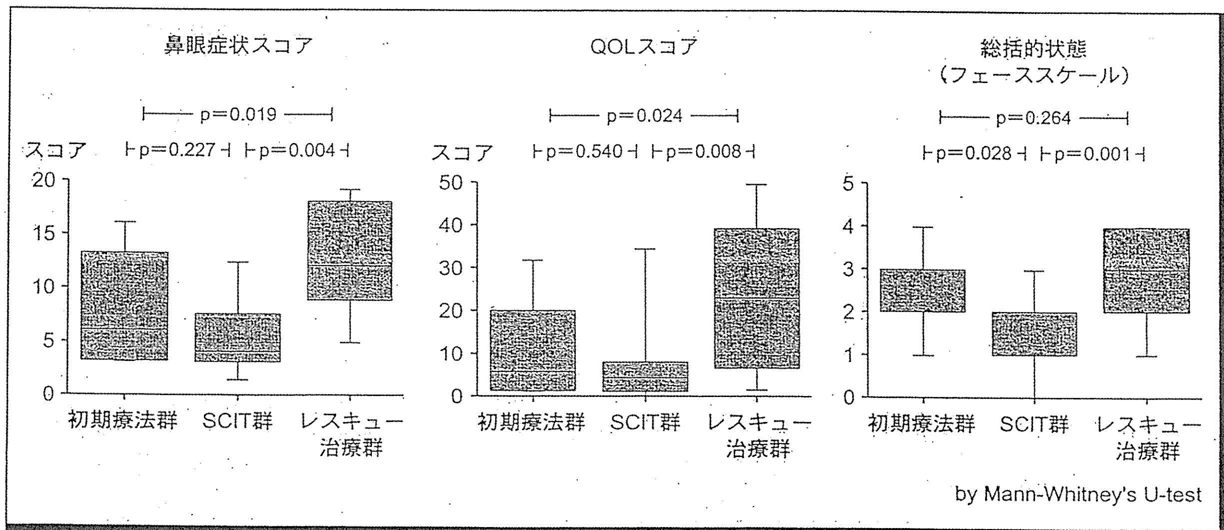


図2　初期療法非施行SCITのスギ花粉症への効果（2009年）

治療効果のよいSCIT患者では初期療法を行わなくても，初期療法と同等以上の効果を示す.

（筆者作成）

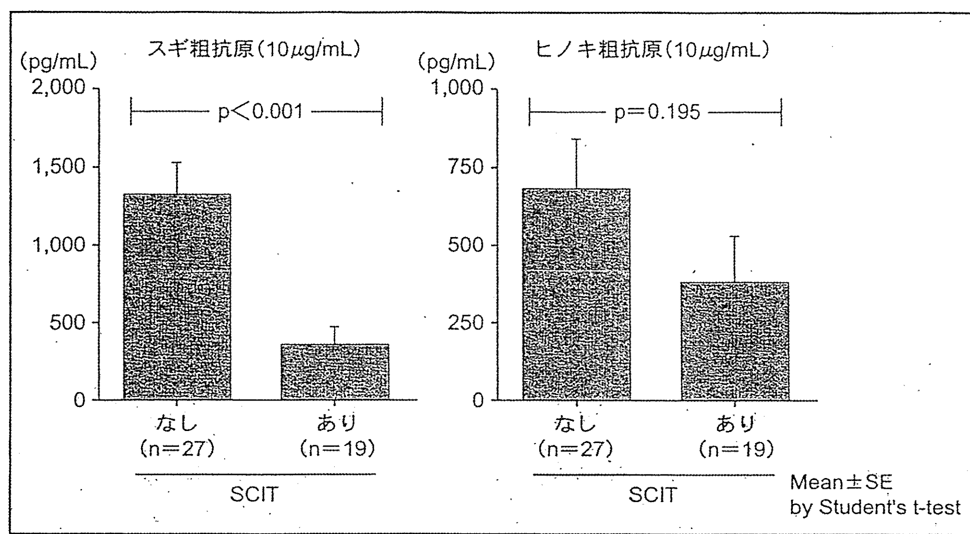


図３　スギ特異的SCITの有無による抗原特異的なIL-5産生量の比較

スギ特異的SCITを行った患者では，非施行患者と比較してスギ粗抗原特異的なIL-5産生は有意に抑制されるが，ヒノキ粗抗原特異的なIL-5産生抑制は有意でない．（筆者作成）

療法患者で有意に抑制されるが，一方でヒノキ粗抗原に対するIL-5産生は免疫療法群では低いものの，非免疫療法患者との間には有意な差を認めなかった(図３)。すなわち，スギ花粉エキスを用いた免疫療法ではスギ花粉と交叉反応性のないヒノキ抗原に対する免疫寛容を充分には誘導できない可能性が示唆された。事実，ヒノキ主要アレルゲンの１つであるCha o 2のなかには，対応するスギアレルゲンCry j 2と交叉反応しないT細胞エピトープが存在することが報告されている[16]。スギ・ヒノキ花粉症の根治を目指すのであれば，今後は診断および治療法ヒノキ花粉エキスの開発も視野におく必要がある。

Ⅲ．SCITのQOLへの効果

アレルギー性鼻炎は致死的な疾患ではないが，quality of life（生活の質；QOL）が著しく障害される。SCITは通年性鼻炎および季節性鼻炎（スギ花粉症，イネ科花粉症，ブタクサ花粉症，ロシアアザミ花粉症など）におけるQOLの改善に寄与することが示されている。

国際的なアレルギー性鼻炎疾患特異的QOL調査票であるRQLQを用いた検討では，小児通年性アレルギー性鼻炎における最低２年間のSCITは，薬物治療に比較して疾患特異的なQOLを有意に改善する。さらにQOLの改善度は免疫療法の治療期間と相関することや，薬物療法に関するコストを有意に削減することが示された[17]。

我々は，標準化スギ花粉エキスを用いたSCITがスギ・ヒノキ花粉症のQOLにインパクトを与えるのかJRQLQを用いて検討を続けている。鼻眼症状と同様に，SCITは初期治療と同等の効果がみられ，さらに初期療法と併用することにより少量飛散年以外のシーズンでは有意な上乗せ効果

QOL（quality of life；生活の質）

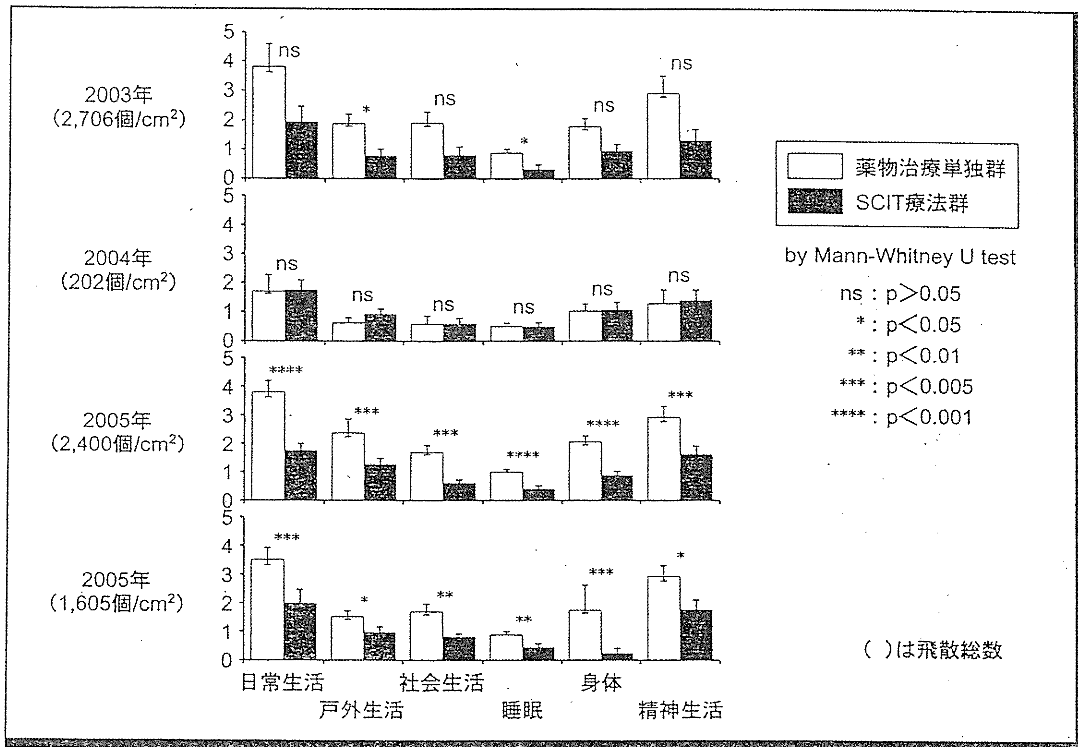


図４　QOLスコアの領域別比較（本格飛散開始後８週間の合計）
「睡眠」や「戸外活動」では，SCITの上乗せ効果が複数年に渡り観察される．

（筆者作成）

がみられる（図１，２）[14]。さらにフェーススケールで示される総括的状態は初期療法と比較しても有意に優れており，患者満足度が高い治療法であることが示唆される。JRQLQを構成する６つの領域別に解析すると，「睡眠」や「戸外活動」はSCITの上乗せ効果が複数年に渡りよくみられる領域であることが示された（図４）。「睡眠」は薬物療法では改善の乏しいQOL領域であり，スギ花粉症に伴う「睡眠」に悩む患者に対して，SCITは有効な治療法となりうる可能性が示唆された。その他，JRQLQを用いた検討で，スギ花粉症に対する急速免疫療法でもQOLの改善効果が示されている[18]。

Ⅳ．SCITの自然経過への効果

アレルギー性鼻炎に対するSCITの最も重要な意義は，本治療法は一般的な薬物治療と異なりアレルギー疾患の自然経過（natural history）を修飾しうることである。予防作用（preventive effect）とも換言できる。予防作用は，新規アレルゲン感作を予防する作用，気管支喘息など他のアレルギー疾患の発症を予防する作用，ならびに免疫療法終了後も再燃を予防する作用（効果継続），の３点に集約できる。例えば小児イネ科花粉症に対する３年間のSCITは終了６年後において症状スコア，症状薬物スコア，新規アレルゲン感作，皮膚反応，下気道症状を有意に抑制することが報告