times and the 50% inhibitory concentration (IC_{50}) was defined as the concentration of prednisolone that killed 50% of the cells.

Western blot analysis

Cells were washed twice with PBS and sonicated in RIPA buffer, which was PBS containing 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, and protease inhibitors (10 mg/ml Leupeptin, 10 mg/ml aprotinin, 1 mM phenylmethylsulphonyl fluoride (PMSF), and 1.8 mg/ml iodoacetamide). Lysates were boiled in SDS sample buffer for 3 min and electrophoresed through 12.5% SDS-polyacrylamide gels (30 μg per lane). The gels were transferred onto a nylon membrane (pore size 0.2 μM; Perkin-Elmer, Tokyo, Japan). After transfer, the nylon membrane was blocked with 5% skimmed milk in PBS and probed with 1 μg/ml rabbit anti-human Bax polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The blot was visualized with the labeled streptavidin–biotin method (Dako), according to the instructions of the manufacturer.

Analysis of apoptotic DNA fragmentation

Cells (5×10^{5}) were harvested and resuspended in 0.1 ml of lysis buffer (1 M Tris–HCl, 0.5 M EDTA, at 10% Triton X-100). After 10 min at 4°C, all tubes were centrifuged at 15,000 rpm for 20 min. Each supernatant was treated for 1 h at 37°C with 0.4 mg/ml RNase A and for 1 h at 37°C with 0.4 mg/ml proteinase K. DNA was extracted with 20 μl of 5 M NaCl and 120 μl of isopropyl alcohol overnight at −20°C and centrifuged at 15,000 rpm for 5 min. The DNA pellet was resuspended in 20 μl of TE buffer. The DNA was loaded onto a 5% acrylamide gel and electrophoresed. The gel was stained with ethidium bromide and the DNA fragments were visualized under ultraviolet light.

Statistical analysis

All in vitro determinations were made in triplicate, and the results were expressed as the mean ± SD (standard deviation). The significance of differences in cell viability between the absence and presence of prednisolone was determined with the Mann–Whitney U test. The significance of differences in cell viability among the three fibroblasts (Bax-NF, Neo-NF, and wt-NF) was determined by repeated measures ANOVA using Stat View software (Abacus Concepts, Berkeley, CA).

Results

Overexpression of Bax in transiently transfected human nasal fibroblasts

Human nasal fibroblasts were transiently transfected with the expression vector hBaxpcDNA3 (Bax-NF) or native pcDNA3 (Neo-NF). The extent of transfected Bax protein expression is as shown in Fig. 1. A 24-kDa band of exogenously transfected tagged-Bax was observed in Bax-NF. However, endogenous Bax protein was not detected in Neo-NF (Fig. 1). After hBaxpcDNA3 or naïve pcDNA3 was transfected, the viability and appearance of nasal fibroblasts did not differ from wild-type-nasal fibroblast (wt-NF) (data not shown). There was no difference in the value of OD among the three nasal fibroblasts after 72 h of culture in the absence of prednisolone, suggesting that the speed of proliferation in Bax-NF or Neo-NF is similar to that in wt-NF (data not shown).

Sensitivity to prednisolone in transfectant

Three types of nasal fibroblasts were cultured in conditioning medium for 24 h after transfection. After that, both transfectants (Bax-NF, Neo-NF) and wt-NF were treated with concentration of prednisolone for 72 h. The optimal culture period was determined as 72 h to show the apparent significance of cell viability of Bax-NF in the presence of prednisolone (data not shown).

The average IC-50 values of prednisolone against Bax-NF, Neo-NF, and wt-NF from 6 independent experiments were 12, 117, and 180 μg/ml, respectively. The cytotoxicity of prednisolone to Bax-NF was significantly higher than that to Neo-NF or wt-NF (Fig. 2, $p < 0.01$, respectively). Prednisolone at a concentration of 10 ng/ml decreased the viability of Bax-NF compared to that of Bax-NF in the

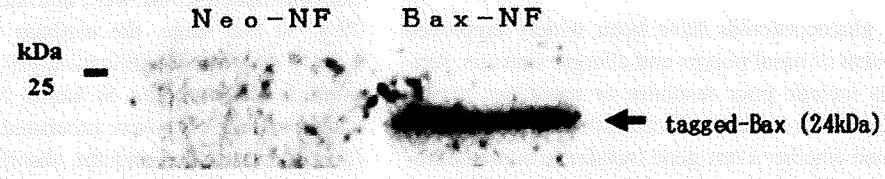

Fig. 1 Western blot analysis of Bax in nasal fibroblasts. Bax-NF was transiently transfected with hBaxpcDNA3. Neo-NF was transiently transfected with native pcDNA3. Equal amounts of cell extract (30 μg of protein) were loaded in each lane and blotted with anti-human Bax antibody. A band of Bax (24 kDa) was observed in Bax-NF

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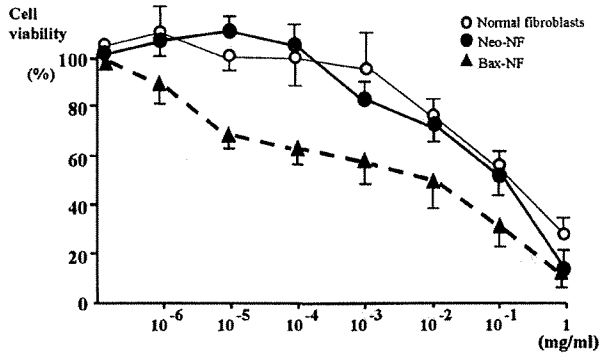

Fig. 2 Effect of Bax expression on the sensitivity of nasal fibroblasts to prednisolone in vitro. Bax-NF, Neo-NF, and wt-NF (wild-type) were incubated with various concentrations of prednisolone. Cell viability was assessed by the MTT assay. $*p < 0.05$, $**p < 0.01$, compared to cell viability in the absence of prednisolone. Bax-NF has significantly higher sensitivity to prednisolone than Neo-NF/wt-NF

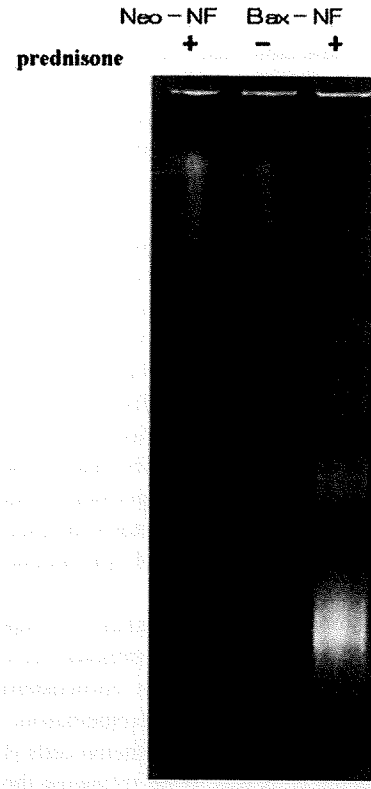

Fig. 3 Acrylamide gel electrophoresis of DNA. Bax-NF and Neo-NF were treated with prednisolone (1 μg/ml) for 72 h. A DNA ladder was seen in the prednisolone-treated Bax-NF

absence of prednisolone (66.8 ± 2.5%, $p < 0.01$). However, 10 ng/ml of prednisolone had no effect on the cell viability of Neo-NF and wt-NF (99.5 ± 4.5%, 112.3 ± 3.5%, respectively). Both Neo-NF and wt-NF were susceptible to 10 μg/ml of prednisolone (77.4 ± 4.9, 79.2 ± 5.1). Thus, the susceptibility of Bax-NF to prednisolone was about 1,000 times that of Neo-NF or wt-NF.

Overexpression of bax mediated apoptosis in prednisone-treated nasal fibroblasts

To confirm that the cell death caused by the treatment with prednisolone is apoptosis, we loaded the DNA into an acrylamide gel and electrophoresed it. Figure 3 shows the electrophoretic patterns of DNA extracted from prednisolone-treated (1 μg/ml, 37°C, 72 h) nasal fibroblasts. A DNA ladder appeared only in the prednisolone-treated Bax-NF (Fig. 3).

To quantify the extent of apoptosis occurring in nasal fibroblasts exposed to prednisolone, we tried to determine the fraction of cells expressing sub G1 DNA by using propidium iodide. Flowcytometric data could not be obtained, since the flowcytometer was affected by obstructions of the tube in the machine due to the large cell size of nasal fibroblasts.

Discussion

In this study, we found that nasal polyp-derived fibroblasts have no endogenous *bax* gene. The transfer of the *bax* gene to human nasal fibroblasts was successful. The transfer enhanced the induction of apoptosis by steroid-treatment. Therefore, we suggest that exogenous Bax protein expression by gene might be a useful strategy for the treatment of nasal polyps.

The pathogenesis of nasal polyps is largely unknown, but chronic inflammation of the nasal mucosa is thought to be an important factor. Glucocorticoids exert potent anti-inflammatory activity and are commonly used in the treatment of allergic rhinitis and asthma. Topical steroids have been administered to patients with allergic rhinitis and nasal polyp. Several studies have shown topical corticosteroids to be effective in reducing the size of nasal polyps [8, 9]. However, we have experienced serious problems including poor response or resistance to steroid therapy in some cases of nasal polyposis. The transfer of genes into tissues in order to increase sensitivity to drugs is an important approach in human gene therapy. We demonstrated previously that overexpression of Bax using gene technology enhanced the sensitivity of cancer cells to an antichemotherapeutic agent [14]. Recently, gene therapy has become a focus in the study of not only cancer but also lifestyle-related diseases. It has been shown that dexamethasone and prednisone are able to induce apoptosis among normal peripheral blood T-lymphocytes in a dose-dependent manner [6, 7]. In our in vitro study, prednisolone (1 μg/ml) did not cause apoptosis of normal fibroblasts. However, 40% Bax-NF was induced apoptosis with prednisolone (1 μg/ml). These results demonstrated that a

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combination of *bax* and prednisone might be useful for treating intractable nasal polyposis by promoting apoptosis.

Allergic rhinitis is apparently an IgE-mediated disease. High levels of antigen-specific IgE were detected in nasal lavage from patients with pollinosis [17]. An elevation of the IgE concentration in non-allergic nasal polyps has been described by several authors [18–20]. Notably, high levels of IgE antibodies to *Staphylococcus aureus* enterotoxins were detected in nasal polyps [21]. These results may point to local IgE synthesis. We and Cameron et al. [22, 23] demonstrated a local isotype switching to IgE in the nasal mucosa using molecular technology at the same time. IgE was produced by plasma cells that had differentiated from IgE-positive B-cells. Interestingly, plasma cells undergoing apoptosis were rescued by fibroblasts [24]. Fibroblasts are also efficient at maintaining germinal center B-cell survival [25]. Thus, induction of apoptosis in nasal fibroblasts might contribute to the decrease of IgE production in the nasal mucosa and polyps.

Although the Bax/Bcl2 balance is important in B-cells, T-cells, dendritic cells, and epithelial cells, susceptibility to the induction of apoptosis by corticosteroids may be cell-specific. Treatment with dexomethasone in vitro did not induce apoptosis in nasal epithelial cells [12]. No apoptosis of epithelial cells was found following the oral administration of prednisolone in vivo [10], suggesting that nasal epithelial cells may be resistant to corticosteroids. The susceptibility to gene transfer varies. Since fibroblasts are easily transfected with plasmid vectors, a number of laboratories prefer to use fibroblasts for experiments on gene transfer. It is in general, difficult to transfect lymphocytes with specific genes. When the *bax* gene is transferred in vivo into the human nose, transfection might be successful for only fibroblasts and epithelial cells. Although the possible adverse effects of *bax*-gene transfer should be thoroughly investigated, we expect any such effects to be weak. Continued development of replication-competent vectors will likely determine the cell-specificity and efficacy of gene transfer [26].

A potential phenotype of steroid-insensitivity is the overexpression of a splice variant of the glucocorticoid receptor (GR), designated GRbeta, in bronchial epithelial cells [27]. An inverse correlation was reported between baseline GRbeta expression and the anti-inflammatory effects of steroids [28]. Another strategy of gene therapy for nasal polyps is the use of antisense ologodeoxynucleotides or RNAi of the *grbeta* gene. Further studies are needed to determine the syngenetic effect of *bax*-gene transfer, GRbeta gene-diminishment, and steroid administration.

While failures are inevitable, it is highly likely that gene therapy approaches will be employed in the future treatment of nasal polyps.

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Prevalence of Allergic Rhinitis and Sensitization to Common Aeroallergens in a Japanese Population

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Key Words
Aeroallergen · Allergic rhinitis · Dust mite · Specific human IgE · Japanese cedar pollen

Abstract
Background: Allergic rhinitis (AR) is recognized as a major health problem worldwide, and its prevalence depends on the age range of the subjects. The aims of this study were to determine the current prevalence of AR, effects of age on the prevalence of IgE sensitization to inhalant allergens, and serum total IgE levels in Japanese subjects. ***Methods:*** We conducted a survey of 1,540 subjects between 20 and 49 years of age in 2006 and 2007 and examined the prevalence of AR and sensitization to 7 common aeroallergens. We measured serum total IgE and specific IgE to 7 aeroallergens. AR was determined based on symptoms, predominantly in the nose and eyes, caused by aeroallergens as mentioned in a questionnaire and sensitization to any of the 7 aeroallergens as assessed by measurement of serum specific IgE. ***Results:*** The prevalence of AR was 44.2% (681 of the 1,540 subjects) and there was no difference among age decades. Of the 1,540 subjects, 1,073 (69.7%) were sensitized to at least 1 of the 7 aeroallergens. The most common allergen in AR was Japanese cedar pollen (89.6%, 610 of the 681 with AR) in all the age decades examined. The sensitization rate to mites was significantly higher in the younger subjects. ***Conclusion:*** Our data suggest that the prevalence of AR between 20 and 49 years of age has increased by nearly 10% during the last 10 years. Cedar pollen and mites were predominant allergen sources among the 7 aeroallergens in the Japanese population.

Introduction

Allergic rhinitis (AR), the most common type of rhinitis, is a heterogeneous disorder that significantly impairs the patient's quality of life, and its prevalence has markedly increased in recent decades [1, 2]. Epidemiologic and serological studies have provided valuable information to develop effective strategies for the prevention and treatment of the disease [3–6]. Japanese cedar

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pollinosis (JCP) is a common allergic disease, and the increase in its prevalence is a major public health problem in Japan [7]. Several epidemiologic studies have been conducted on JCP [8–11]. Sakurai et al. [9] reported the prevalence and risk factors of AR and JCP among 2,307 Japanese men; the prevalence rates of AR, seasonal rhinitis and JCP were 35.5, 28.8 and 11.0%, respectively, in 1998. Kaneko et al. [10] conducted a meta-regression analysis of 38 population-based surveys in Japan. The prevalence of JCP among adolescents in the general population was estimated at 28.7% in metropolitan areas and 24.5% in urban areas in the year 2004. The study also reported that the prevalence of JCP increased 2.6-fold between 1980 and 2000. To monitor the prevalence of sensitization is useful for understanding AR and developing preventive measures.

In AR, an IgE-mediated response to allergens is triggered and characterized by type-2-helper-T-cell-dependent inflammation [12]. Allergen-specific IgE is a critical factor in the mechanism of AR. Serum allergen-specific IgE results closely correlate to those of skin tests and nasal challenges. Allergen-specific IgE tests are highly specific and sensitive. One of their advantages is that drugs and skin diseases do not influence the measurement [1].

Sensitization is an important risk factor for developing allergic disease [13]. Epidemiological investigation of AR is important to clarify its etiology and develop appropriate preventive and therapeutic techniques. There have been few epidemiological studies on the age effect on the prevalence of AR and IgE sensitization to inhalant allergens, and serum total IgE levels in Japanese subjects. Therefore, we conducted an epidemiological study on a total of 1,540 subjects aged 20–49 years. The protocol comprised a questionnaire, measurement of total serum IgE antibodies and allergen-specific IgE antibodies against 7 aeroallergens in 2006 and 2007. The major findings of this study are the prevalence of allergic sensitization and AR, the age effect on them, and total serum IgE and AR, and the related age effect.

Material and Methods

Study Subjects

A total of 1,540 subjects were recruited from residents of Eiheiji-cho and the cities of Fukui, and Echizen in Fukui prefecture, in the central Hokuriku area of Japan in May and June of both 2006 and 2007. In that area, Japanese cedar pollen counts are at the average level of the islands of Honshu, Shikoku and Kyushu [7]. The 1,540 subjects were workers of 4 hospitals and students of nursing and medical colleges in the University of Fukui. The number of females was higher than that of males (mean age, 32.1 years; range, 20–49 years; male:female ratio, 1.0:2.40; mean serum IgE level, 233.8 IU/ml; median serum IgE level, 73.5 IU/ml). The participants were recruited during their annual health check-up in 2006 or 2007; 13 subjects did not agree to participate in this survey. Reasons for nonparticipation were lack of interest or time. All of the 1,540 participants agreed to measurement of serum total IgE and specific IgE to 7 aeroallergens and to answer a questionnaire. Blood collection and the questionnaire survey were performed at the same time after informed consent was received. We did not conduct a follow-up survey in this study. The diagnosis of AR was confirmed by seasonal or perennial symptoms of rhinitis consisting of any combination of the following: nasal itching, sneezing, discharge and stuffiness caused by inhalation of aeroallergens, reported on a questionnaire. All of the subjects with AR were also positive for serum-specific IgE to 1 or more of the 7 aeroallergens. All individuals were unrelated Japanese individuals and gave written informed consent to participate in the study according to the rules of the ethics committees of the Faculty of Medical Science, University of Fukui and the Institute of Physical and Chemical Research (RIKEN).

Measurement of Serum Levels of Specific IgE Antibodies

Specific IgEs to 7 aeroallergens, *Cryptomeria japonica*, *Dermatophagoides pteronyssinus* (Der p), *Dermatophagoides farinae* (Der f), *Dactylis glomerata*, *Ambrosia artemisiifolia*, *Candida albicans* and *Aspergillus fumigatus* were measured with a Pharmacia CAP System (Pharmacia CAP, Upsala, Sweden) (table 1). Allergen sensitization was classified as positive if the allergen-specific serum IgE level was above 0.7 (CAP RAST score of 2).

Statistical Analysis

To clarify the age-specific prevalence of AR and sensitization to the 7 aeroallergens examined, patients were divided into 3 age groups, the 20s (20 to <30 years), 30s (30 to <40 years) and 40s (40 to <50 years). We then compared differences in frequencies of sensitization to each of the 7 aeroallergens among these age groups by using the Kruskal-Wallis test and then by individual testing using the Mann-Whitney U test if significant. Serum total IgE was analyzed at a quantitative level, and log-transformed individual serum IgE levels were used in the figures. Correlations of total IgE levels and age were analyzed by Spearman's test. $p < 0.05$ was considered statistically significant. Logistic regression analysis was implemented for the AR and sensitization to assess the effects of gender, age and total serum IgE (SPSS 14.0J, SPSS, Inc., Chicago, Ill., USA).

Results

Prevalence of Allergic Sensitization and AR

Positive sensitization refers to an allergen-specific serum IgE level >0.7 (CAP RAST score of 2). The prevalence of allergic sensitization to each allergen tested is presented in table 1. Of the 1,540 subjects, 1,073 (69.7%) exhibited positive sensitization to at least 1 aeroallergen (fig. 1). A total of 467 of the 1,540 subjects (30.3%) showed

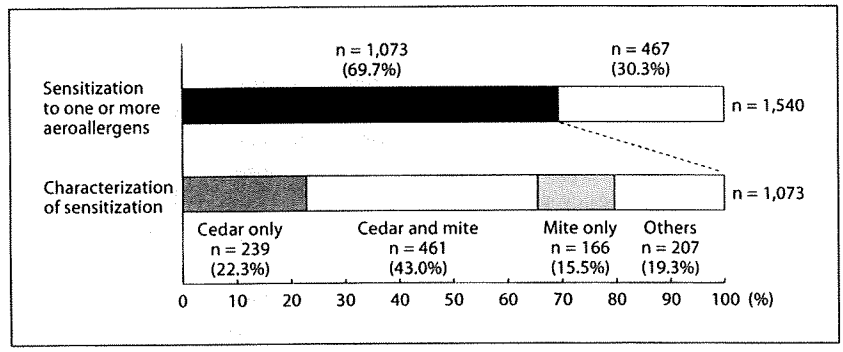

Fig. 1. The prevalence of sensitization to 7 test aeroallergens and characterization of sensitization.

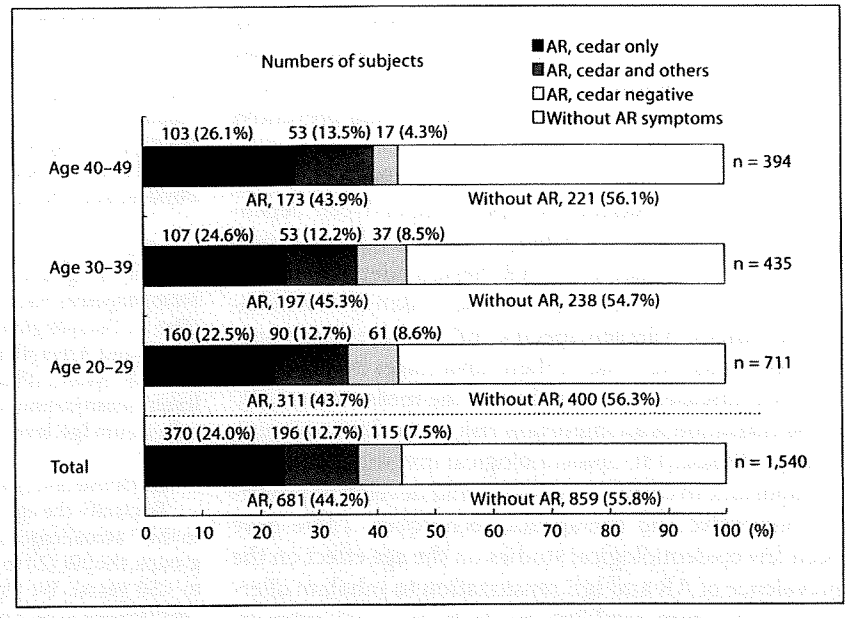

Fig. 2. Age effects on the prevalence of AR and sensitization to Japanese cedar pollen.

no sensitization to any of the 7 aeroallergens examined (fig. 1). Seven hundred subjects (45.3%) were sensitized to *C. japonica*, (Japanese cedar, JC) pollen, thus accounting for 65.3% of the 1,073 subjects with positive sensitization to aeroallergens. Of the 1,073 subjects, 627 (58.5%) were sensitized to mites. Thus, JC pollen and mites were the two predominant aeroallergens among the 7 tested aeroallergens (fig. 1).

Of the 1,540 participating subjects, 681 (44.2%) had symptoms of AR at the time of the survey (fig. 2). The prevalence of JCP was 36.7% (566 of the 1,540 subjects) in this study (fig. 2). The positive rates for specific IgE antibodies to Japanese cedar pollen were 89.6% (610 of 681) in the AR group and 28.5% (245 of 859) in the no-symptom group (fig. 3). Of the 681 AR subjects, 167 (24.5%) were sensitized to only Japanese cedar pollen (fig. 3).

Age Effect on the Prevalence of Allergic Sensitization and AR

We found significant associations between the allergic sensitization to the 7 aeroallergens and the age groups (table 1) ($p = 0.0019$ by the Kruskal-Wallis test). More subjects were sensitized to Japanese cedar pollen than to any other of the 7 tested allergens in each age group (table 1). The sensitization rates to Japanese cedar pollen were 59% (421 of 711 subjects), 52% (226 of 435) and 53% (208 of 394) for subjects in their 20s, 30s and 40s, respectively. We found a significant association between sensitization to Japanese cedar pollen and the age range of the subjects ($p = 0.015$ by the Mann-Whitney U test) (table 2). The sensitization rate against mites, Der p and/or Der f, was higher for those in their 20s (50%, 355 of 711 subjects), than for those in their 30s (41%, 179 of 435) and 40s

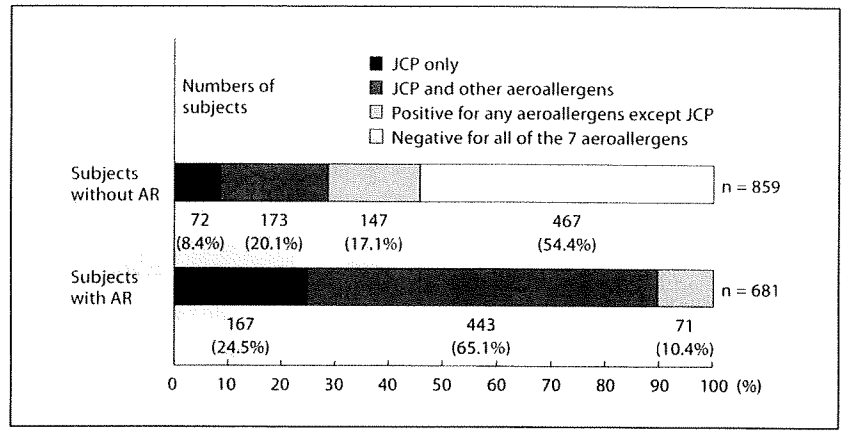

Fig. 3. Prevalence of AR and sensitization to Japanese cedar pollen.

Table 1. Prevalence of sensitization to 7 aeroallergens according to age group

	Total (n = 1,540)	20s (n = 711)	30s (n = 435)	40s (n = 394)
Cryptomeria japonica	855 (56)	421 (59)	226 (52)	208 (53)
Dermatophagoides pteronyssinus	625 (41)	345 (49)	174 (40)	106 (27)
Dermatophagoides farinae	622 (40)	342 (48)	168 (39)	112 (28)
Dactylis glomerata	352 (23)	198 (28)	90 (21)	64 (16)
Ambrosia artemisiifolia	137 (9)	67 (9)	45 (10)	25 (6)
Candida albicans	82 (5)	43 (6)	24 (6)	15 (4)
Aspergillus fumigatus	34 (2)	25 (4)	8 (2)	1 (0.3)

Figures in parentheses are percentages.

Table 2. Age effects on sensitization to JCP, dust mites and *Dactylis glomerata*

Aeroallergen	Sensitization	20s (n = 711)	30s (n = 435)	40s (n = 394)	p value
Cryptomeria japonica	positive	421 (59)	226 (52)	208 (53)	0.015
	negative	290 (41)	209 (48)	186 (47)	
Dust mites	positive	355 (50)	179 (41)	115 (29)	3.9×10^{-11}
	negative	356 (50)	256 (59)	279 (71)	
Dactylis glomerata	positive	198 (28)	90 (21)	64 (16)	4.8×10^{-6}
	negative	513 (72)	345 (79)	330 (84)	

Figures in parentheses are percentages. p value as obtained by the Mann-Whitney U test.

(29% 115 of 394), ($p < 0.001$ by the Mann-Whitney U test) (table 2). The prevalence of sensitization to *D. glomerata* was also higher in those in their 20s (28%, 198 of 711 subjects) than in those in their 30s (21%, 90 of 435) and 40s (16%, 64 of 394) ($p < 0.001$ by the Mann-Whitney U test) (table 2). AR was confirmed in 311 of the 711 subjects (43.7%) in their 20s, 197 of the 435 (45.3%) in their 30s and 173 of the 394 (43.9%) in their 40s (fig. 2). There was no significant difference in the prevalence of AR among the age groups.

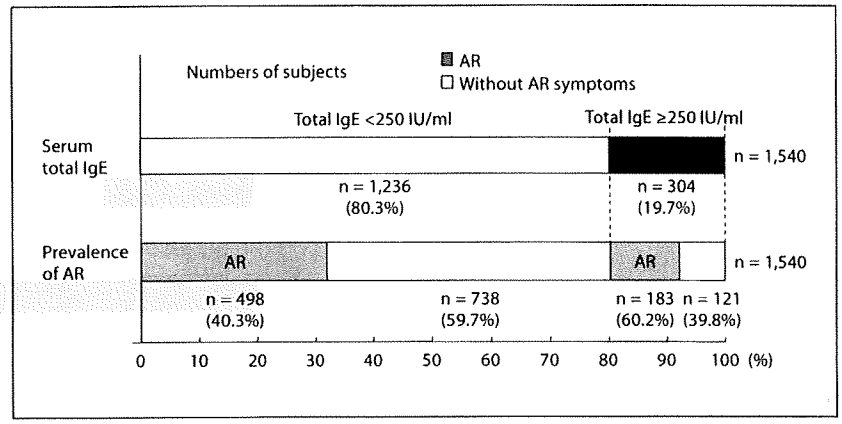

Fig. 4. Serum total IgE levels and prevalence of AR.

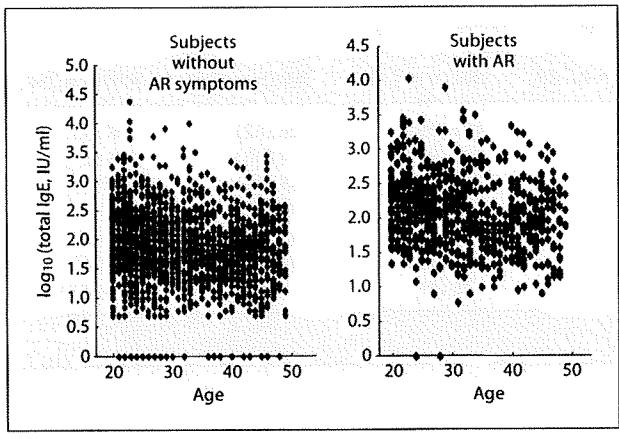

Fig. 5. Age effects on serum total IgE levels in subjects with AR and non-AR.

Total Serum IgE and AR, Sensitization, and Age Effect

There were 304 subjects (19.7%) who had high total IgE levels (≥250 IU/ml), and the prevalence of AR in this group was 60.2% (183 of the 304). However, the prevalence of AR of subjects with normal total IgE (<250 IU/ml) was 40.3% (498 of 1,236) (fig. 4).

The serum total IgE level was analyzed at a quantitative level (fig. 5). The means of $\log_{10}$ [total IgE (IU/ml)] and standard deviations of all 1,540 subjects, subjects without AR and subjects with AR were 1.87 [= $\log_{10}$ (74.1 IU/ml)] ± 0.65, 1.69 [= $\log_{10}$ (49.0 IU/ml)] ± 0.67 and 2.09 [= $\log_{10}$ (123.0 IU/ml)] ± 0.53, respectively.

We investigated the correlation between this level and age using Spearman's rank correlation coefficient (fig. 5). Although we could not find any significant correlation between the serum total IgE level and the age range of the 1,540 subjects, an inverse correlation was found between the total IgE level and age in the AR group ($rs = -0.21$, $p < 0.01$) (fig. 5). Total IgE levels were higher in younger subjects than in older subjects in the AR group. The results of the stepwise logistic regression analysis for positive sensitization to 1 or more of the 7 aeroallergens showed significant effects of total IgE (Wald statistic = 153.5, d.f. = 1, $p < 0.001$) and age (Wald statistic = 9.5, d.f. = 1, $p = 0.002$), but no effect of gender. There was no significant effect of age, gender, or total IgE on AR by logistic regression analysis.

Discussion

Estimates of the latest prevalence provide valuable information to develop effective strategies for the prevention and treatment of disease. We conducted an epidemiologic survey of AR and examined the sensitization rates against 7 aeroallergens by measuring the serum-specific IgE of 1,540 subjects aged between 20 and 49 years in a Japanese population in 2006 and 2007. The population aged between 20 and 49 years represented 38.8% of the population of Japan in 2008 according to current population estimates by the Ministry of Internal Affairs and Communications (http://www.stat.go.jp/english/data). We also examined the role of age effects on the prevalence. In this study, 681 of the 1,540 subjects (44.2%) were diagnosed as having AR. Increases in prevalence of AR and asthma have been reported by studies of relatively large populations in the United States, Great Britain, Australia and New Zealand, with cross-referenc-

es to earlier relevant studies, and the recent AR prevalence in these studies ranged from 23 to 28% [14]. The International Study of Asthma and Allergies in Childhood in 1997 reported that the prevalence of rhinoconjunctivitis varied across centers from 0.8 to 14.9% in 6-to 7-year-olds and from 1.4 to 39.7% in 13- to 14-year-olds [15]. In an Aberdeen population study on 3,537 subjects, the prevalence of hay fever increased significantly from 1994 (13%) to 1999 (15%) [16]. In Japan, Sakurai et al. [9] showed that the prevalence rates of AR, seasonal rhinitis and JCP were 36, 29, and 11%, respectively, and age was a negative risk factor for all allergic conditions. The subjects of the study consisted of 2,307 male railway employees who underwent a health examination from February to May 1995 (mean age, 41.4 years; range, 19–65 years). In the study, AR was determined from self-reported AR or from the seasonal nasal symptoms, and JCP was defined as the presence of cedar-specific IgE positivity among subjects with seasonal rhinitis. The prevalence of AR in this study was 44.2% (681 of the 1,540 subjects), which is higher than in previous reports. However, there was no difference of prevalence between 20 and 49-year-olds. Interestingly, the prevalence of AR in subjects aged 30–39 years was 42.7% in a study conducted in 1995 [9]. These subjects aged 30–39 years in 1995 were 40–49 years old in 2005. The prevalence of AR in this study for subjects from 40 to 49 years of age was 43.9%, and there was no difference in the prevalence between the studies. The prevalence among this age group did not markedly increase during the last 10 years. Further etiological studies in independent populations or those aged less than 20 years and elderly populations are needed to determine the effects of age on the susceptibility to AR.

In the present study, a total of 859 subjects (859/1,540, 55.8%) had no symptoms of AR; however, among them, 392 subjects (392/859, 45.6%) were already sensitized to one or more of the 7 test aeroallergens. It is generally recognized that sensitization to any allergen is an important risk factor for developing allergic diseases; however, those sensitized subjects had no symptoms of AR.

The present study has shown that a total of 167 of 681 subjects with AR (24.5%) were sensitized to JC pollen and not to the other 6 test aeroallergens. Allergen-specific immunotherapy is established as an effective treatment for patients with IgE-mediated reactions, and it has been widely used as a desensitizing therapy for AR [17, 18]. Specific immunotherapy retrospectively reduces new sensitization in monosensitized subjects suffering from AR [19]. Subjects with monoallergen sensitization appear to be good candidates for immunotherapy.

Among the 681 subjects with AR, 451 (66.2%) were sensitized to multiple (two or more) aeroallergens, and 385 (56.5%) were sensitized to dust mites. Although our data strongly indicated an important role of JCP in AR, a significantly higher prevalence of sensitization to dust mites was observed in younger subjects. Dust mites, an indoor allergen, have a predominant impact on asthma, and a recent population-based study has shown that dust mite sensitization is a significant risk factor for developing the disease [20]. Another recent study, a long-term (23-year) follow-up study of university students, has shown that sensitization to pollen leads to an increased risk of developing asthma [21]. A limitation of our study was the lack of longitudinal data. To clarify factors that increased the risk of developing new AR or bronchial asthma, further cohort analyses should be conducted regarding the involvement of the sensitized allergens in airway allergic inflammation.

A recent etiological study in an unselected rural Chinese population tested sensitization to 14 allergens, including 5 aeroallergens (dust mite, cockroach, *Alternaria tenuis*, dog epithelia, and cat hair) by skin prick tests. 2,118 subjects whose ages ranged from 11 to 71 years were tested (43.3% were children between 11 and 17 years old) [22]. The study showed that 41.1% of the children were sensitized to 1 or more aeroallergens, and 36.5% of the adult subjects aged ≥18 years were sensitized [22]. The most common sensitizing aeroallergen in the Chinese study was dust mites (30.6%) [22]. In meta-analyses using data from 12,687 subjects aged 20–44 years in the European Community Respiratory Health Survey conducted in 2002, the highest prevalence of sensitization was found for the house dust mite (20.2%) [23]. In the present study, of the 1,540 subjects, 1,073 (69.7%) were sensitized to at least 1 of the 7 aeroallergens, and 855 (55.5%) and 649 (42.1%) were sensitized to Japanese cedar pollen and dust mites, respectively.

Several limitations of this survey should be mentioned. The survey is likely to be fraught with a certain recruitment bias. In general, individuals affected by a specific disease are more willing and interested in a study. However, only 13 subjects (0.84%) did not agree to participate in this survey whereas 1,540 subjects agreed to assays of serum total IgE and specific IgE for the 7 aeroallergens and to answer the questionnaire in the present study. Hospital workers, nursing and medical students might not be representative of the general population and there might have been a population selection bias with regard to socioeconomic status and higher education. Previous studies in various countries have reported an increased

occurrence of asthma among specific groups of healthcare workers [24–26]. Thus, selection bias might have had an influence on the higher prevalence of sensitization to 1 or more aeroallergens (69.7%) and of AR (44.2%) in our study.

Although a population selection bias might reduce the generalizability of the study, we showed here that the prevalence of AR has increased and that Japanese cedar pollen and dust mites were the predominant allergen sources among the 7 tested allergen sources in the Japanese population. However, further study is needed using larger, more representative samples.

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Efficacy of Oral Administration of a Heat-Killed *Lactobacillus gasseri* OLL2809 on Patients of Japanese Cedar Pollinosis with High Japanese-Cedar Pollen-Specific IgE

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A randomized, double-blind, placebo-controlled clinical trial was conducted to determine whether oral administration of heat-killed *Lactobacillus gasseri* OLL2809 would affect the immune response and reduce the symptoms of Japanese cedar pollinosis (JCP) in subjects with JCP. Following a 1-week pre-observation period, the subjects were randomly divided into two groups and were orally administered a placebo or tablets containing 100 mg of *L. gasseri* OLL2809 per d for 8 weeks during the pollen season in 2007. The results showed no obvious differences between the groups. Supplementary subgroup analysis revealed that the OLL2809 subgroups with CAP-RAST scores of 4 or 5 exhibited improvement in nasal symptoms scores and serum allergy-related items, including Japanese cedar pollen-specific IgE levels. *L. gasseri* OLL2809 was found to be effective in reducing symptoms in subjects with a high predisposition to allergies by modulating systemic immune systems.

Key words: Japanese cedar pollen; Japanese cedar pollinosis; *Lactobacillus gasseri*; probiotics; Th1/Th2

In the past few decades, the incidence of Japanese cedar pollinosis (JCP) has been increasing, and a current paper suggests that at least one-sixth of the Japanese population is affected by this allergic disease.[1] Thus far, several medicines, including antihistamines, leukotriene inhibitors, anti-inflammatory cytokines, and corticosteroids, have been developed that greatly contribute to reduction of the symptoms of allergic disease.[2–7] However, because allergic diseases are chronic, continuous treatment is required, and the cost spent on treatment is enormous. In addition, most of the available treatments are symptomatic.[8] Therefore, in view of the high prevalence of JCP and the adverse effects that accompany long-term treatment,[4,9] more effective treatment methods are required.

The increase in the prevalence of allergic diseases has been explained by the hygiene hypothesis proposed by Strachan.[10] Strachan suggested that limited exposure to bacterial and viral pathogens during early childhood leads to insufficient stimulation of the Th1 direction of the immune system and primes an overactive Th2 reaction, leading to allergic disease. The mechanisms underlying this phenomenon are considered to include defective maturation or an absence of regulatory T cells and an inappropriate Th1/Th2 balance.[11] A number of reports suggest correlations between the incidence of allergic diseases and intestinal microbiota, which might serve as stimuli to develop appropriate immune systems.[12,13]

Lactobacilli are gram-positive anaerobic bacteria commensal to humans and animals.[14] For many years, they have been consumed worldwide through foods such as fermented milk. Consequently, they are known to be very safe microorganisms. Moreover, a recent double-blind placebo-controlled clinical study revealed that *Lactobacillus rhamnosus* GG administration suppressed the incidence of atopic diseases in high-risk children by approximately 50%.[15] Hence, the use of lactobacilli might be an easy and effective way to prevent or treating allergies without any side effects.

L. gasseri OLL2809, which was isolated from a human subject, has been selected from approximately 300 *Lactobacillus* strains on the basis of its immunoregulatory effect.[16] We have found using mouse experimental allergy models that when orally administered, heat-killed *L. gasseri* OLL2809 exhibit suppressive effects on antigen-specific IgE and eosinophilia *via* modulation of the Th1/Th2 balance.[16–18] This suggests that heat-killed *L. gasseri* OLL2809 can reduce the clinical symptoms of JCP. In this study, we examined the clinical efficacy of heat-killed *L. gasseri* OLL2809 as to JCP.

Methods

Subjects. Subjects ($n = 107$) aged 20 to 50 years were enrolled in the clinical study. Subjects with JCP who fulfilled the following criteria were enrolled: (i) subjects who experienced JCP symptoms for

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Abbreviations: JCP, Japanese cedar pollinosis; QOL, quality of life; SMS, symptom medication score; IFN, interferon; IL, interleukin

over 2 years, (ii) subjects with serum cedar pollen-specific IgE levels at scores 2 to 5 by the CAP-radioallergosorbent test (CAP-RAST score), (iii) subjects with a moderate total symptom score (comprising symptoms of sneezing, runny nose, nasal congestion, itchy and watery eyes, scored for JCP in the past 2 years) according to the "Practical Guidelines for the Management of Allergic Rhinitis in Japan," 5th edition.[19] Subjects who lived or worked in the suburbs of Tokyo were preferred as study subjects.

The following subjects were excluded from the study: (i) those whose symptoms had developed before the cedar pollen season, (ii) those with nasal disease that might have affected efficacy evaluation in this trial (perennial allergic rhinitis, acute and chronic rhinitis, *etc.*), (iii) those who planned to travel to Hokkaido, Okinawa, or abroad, and (iv) others whom the physician in charge judged unfit as study subjects.

Prior to participation, written consent was obtained from all the subjects after the physician in charge had explained the study to the group. In addition, the study received the approval of the Ethics Committee of the Division of Research and Development of Meiji Dairies Corporation, and it was performed in accordance with the Declaration of Helsinki.

Study design. The study was a randomized, double-blind, placebo-controlled clinical trial performed at a single institution in Tokyo between January 10, 2007 and April 6, 2007. The study protocol is summarized in Fig. 1. After obtaining informed consent from the subjects, they were screened to confirm compliance with the inclusion and exclusion criteria and to examine the physical condition of each individual. Subject background, clinical laboratory analysis (hematology, blood biochemistry, and serology), and physical examination were included in the screening.

Following a 1-week pre-observation period, the subjects were randomly divided into two groups: one group of subjects who were orally administered tablets containing 100 mg (approximately 1×10^{10} cells) of heat-killed (75 °C for 60 min) *L. gasseri* OLL2809 per d and the other, who received placebo tablets. The placebo tablets contained dextrin instead of heat-killed *L. gasseri* OLL2809, and were identical in color and taste to the OLL2809 tablets. Each subject received tablets for an 8-week course (from February 5 to April 6).

On dividing the groups, a controller who was not directly involved in the study was responsible for group allocation. The subjects were divided randomly into the active (OLL2809) and placebo groups according to the total symptom scores for JCP in the past 2 years, the Japanese-cedar-pollen specific IgE not to be different between the groups. A group allocation number was given to each subject. To prevent leakage of information, this number was closely guarded jointly by the controller and a member of the ethical committee who was not directly involved in the study, until accessed with the key after completion of the study.

The physician in charge examined each subject a total of 3 times: during the pre-observation period, and 4 and 8 weeks after treatment. The subjects were asked to fill out the Japanese Allergic Rhinitis QOL standard Questionnaire (JRQLQ) during the pre-observation period, and 4 and 8 weeks after treatment. They were asked to record pollinosis symptoms (sneezing, runny nose, nasal congestion, itchy eyes, watery eyes, and interference with daily life) and compliance with the administration schedule in the subject diary.

Evaluation items.

Nasal cavity findings. Nasal examinations were conducted by rhinoscopy during the pre-observation period and after 4 and 8 weeks of treatment. Mucosal swelling of the inferior turbinate and the amounts of watery rhinorrhea were scored on a 4-point scale of severity (0 = none to 3 = severe).[19]

Allergy diary. Allergy diaries were kept by the subjects to self-assess items from a list, including sneezing (number of attacks per d), runny nose (number of incidences of nose blowing per d), nasal congestion, and itchy and watery eyes. These symptoms were scored subjectively on a 5-point severity scale, where 0 indicated no symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms; and 4, very severe symptoms.[19] The total scores for sneezing, runny nose, and nasal congestion were counted as the nasal symptom score, while the total scores for itchy and watery eyes were counted as the ocular symptom score. The medication score was recorded as described elsewhere.[19] Severity during the season was scored daily as nasal or ocular symptom medication scores (SMS), and the mean SMS each week was compared between the placebo and OLL2809 groups.

Japanese allergic rhinitis QOL standard questionnaire (JRQLQ). JRQLQ was used for evaluation of the subjects' QOL during the pollen season. The questionnaire is composed of three parts: nasal and eye symptoms (JRQLQ-I), 17 questions regarding the QOL (JRQLQ-II), and a comprehensive evaluation (face scale).[20]

Nasal and ocular symptoms included the following six categories: runny nose, sneezing, nasal congestion, itchy nose, and itchy and watery eyes. The symptoms of each subject were evaluated on a 5-point scale, 0 denoting no symptoms; 1, mild; 2, moderate to severe; 3, severe; and 4, very severe.

The QOL-related questionnaire included 17 items concerning (i) reduced productivity at work/home; (ii) poor mental concentration; (iii) reduced thinking power; (iv) impaired reading book/newspaper; (v) reduced memory; (vi) limitation of outdoor life (*e.g.*, sports, picnic); (vii) limitation of going out; (viii) hesitation visiting friend or relatives; (ix) reduced contact with friends or others by telephone or conversation; (x) not an easy person to be around; (xi) impaired sleeping; (xii) tiredness; (xiii) fatigue; (xiv) frustration; (xv) irritability; (xvi) depression; and (xvii) unhappiness. Each item was evaluated on a 5-point scale, 0 denoting no significant problem; 1, a mild problem; 2, moderately severe; 3, severe; and 4, very severe.

Blood examination. Blood samples were collected 3 times: at the pre-observation period and after 4 and 8 weeks of treatment. The samples were used to determine the concentrations of total and Japanese cedar pollen-specific IgE levels and number of eosinophils. The ratio of Th1 to Th2 cells (Th1/Th2 ratio) was determined 2 times: in the pre-observation period and after 8 weeks of treatment. The blood examinations described above were performed at SRL (Tokyo).

Assessment of outcomes. The primary efficacy outcome was the difference in the nasal and the ocular symptom medication scores over the 8-week administration period between the OLL2809 and placebo groups. The secondary assessments were based on the QOL-related scores on JRQLQ at 4 and 8 weeks after treatment.

Statistical analysis. A blind data review was performed before decoding, and decisions concerning the handling of drop-outs were made on the basis of blinded results. The assessment of efficacy was based on all the subjects who completed the study. Subgroup analyses based on CAP-RAST scores were done additionally after decoding.

Data were expressed as mean ± SE. Statistical differences between the placebo and OLL2809 groups and subgroups were analyzed by Student's t test or Mann-Whitney's U test. The differences between pre-observation and 4 and 8 weeks after treatment were analyzed in each group and subgroup by Student's paired-t test with Bonferroni's correction. Differences were considered significant when the p-value was less than 0.05.

Results

Background characteristics of subjects and cedar pollen count

Among 107 subjects (placebo $n = 54$, OLL2809 $n = 53$) enrolled, seven subjects withdrew from the study citing personal reasons and 100 (placebo $n = 53$, OLL2809 $n = 47$) successfully completed it. The baseline characteristics of the subjects were similar in the placebo and OLL2809 groups in terms of age, sex, duration of JCP, nasal cavity findings, nasal and ocular symptom medication scores in the allergy diary, and a blood examination (data not shown).

The Japanese cedar pollen season started on January 31 and continued to the end of April in 2007. The total pollen count during the study period was 1,263 grains/cm^2 in Chiyoda-ku, central Tokyo, slightly higher than the pollen count for 2006 (874 grains/cm^2)

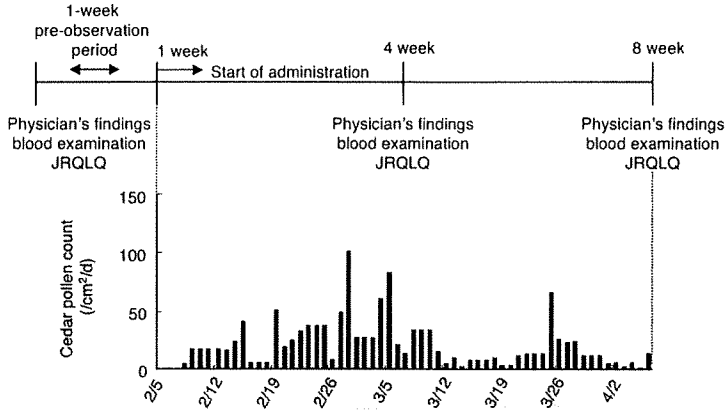

Fig. 1. Study Protocol and Diagrammatic Profile of Japanese Cedar Pollen Dispersion during the Clinical Trial in Tokyo, in 2007.
These data were obtained from the Bureau of Social Welfare and Public Health, Tokyo Metropolitan Government, by determining the pollen collected in a Durham pollen catcher on the roof of a building in Chiyoda Ward.

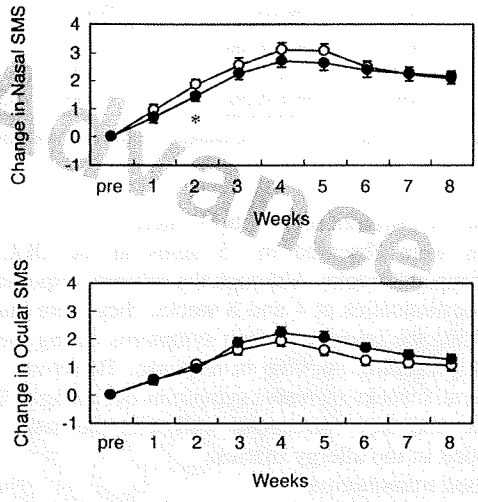

Fig. 2. Time-Course Change in Nasal and Ocular Symptom Medication Scores (SMS).
The scores are differences from those in the pre-observation period. Placebo group (○), $n = 53$; OLL2809 group (●), $n = 47$. $^*p < 0.05$ (Mann-Whitney's U test).

(Fig. 1), according to a survey conducted by the Bureau of Social Welfare and Public Health of the Tokyo Metropolitan Government.

Analysis of subjects

The nasal cavity findings, blood examinations, and JRQLQ results revealed no significant differences between the placebo and OLL2809 groups that were administered *L. gasseri* OLL2809. Examination of the allergy diaries revealed that although ocular SMS did not differ between the groups, and nasal SMS at 2 weeks was significantly low ($p = 0.0409$) in the OLL2809 group as compared with the placebo group (Fig. 2). Overall, no efficacy of *L. gasseri* OLL2809 was observed in this study.

Subgroup analysis: analysis of high CAP-RAST score group

Type I allergic diseases are characterized by an elevation in serum antigen-specific IgE levels.[21] The CAP-RAST score of the subjects enrolled in this study was 3.22 ± 0.80 (placebo group, 3.28 ± 0.77, $n = 53$; OLL2809 group, 3.15 ± 0.83, $n = 47$; $p = 0.3371$). Hence, each group was divided into two subgroups. The first subgroup consisted of subjects with CAP-RAST scores of 2 or 3, and the second group of subjects with CAP-RAST scores of 4 or 5. Subsequently, these subgroups were analyzed again to investigate the efficacy of *L. gasseri* OLL2809 on them. Although there were no significant differences between the placebo and OLL2809 subgroups with CAP-RAST scores of 2 or 3 (data not shown), *L. gasseri* OLL2809 was found to be effective, particularly in subgroups of subjects with CAP-RAST scores of 4 or 5, as described below.

Background characteristics of subjects with CAP-RAST scores of 4 or 5

The baseline characteristics of the subjects were similar in the placebo and OLL2809 subgroups, with CAP-RAST scores of 4 or 5, as well as those of the all subjects, in terms of age, sex, duration of JCP, nasal cavity findings, nasal and ocular symptom medication scores taken in allergy diaries and blood exams (Table 1).

Nasal cavity findings

Both the nasal cavity scores for mucosal swelling of the inferior turbinate and the amount of watery rhinorrhea significantly ($p < 0.01$) increased at 4 weeks in both the placebo and OLL2809 subgroups. However, no differences were observed between these subgroups. When the scores were summed and total scores were compared, the results tended to be low ($p = 0.0991$) in the OLL2809 subgroup at 4 weeks (Table 2). The scores decreased at 8 weeks as compared to those at 4 weeks, and no difference was observed between the subgroups.

Nasal and ocular SMS

Both nasal and ocular SMS increased and symptoms were exacerbated, with a peak at 4 to 5 weeks during the experimental period. While no differences were observed in the ocular SMS between the subgroups, the nasal SMS exhibited significantly ($p < 0.05$) lower values in the OLL2809 subgroup than in the placebo subgroup at 1, 5, 6, 7 and 8 weeks (Fig. 3).

JRQLQ

The scores for all the QOL items in the JRQLQ-I increased at 4 weeks, and then slightly decreased at 8

Table 1. Background Factors of Subjects with CAP-RAST Scores of 4 or 5

	Placebo ($n = 19$)	OLL2809 ($n = 12$)	p-value
Age (year)	30.3 ± 1.6	30.7 ± 2.1	0.8766[a]
Sex (male:female)	8:11	6:6	0.9524[b]
Duration of JCP (year)	10.3 ± 1.6	9.9 ± 1.4	0.8503[a]
Mucosal swelling of the inferior turbinate	0.474 ± 0.177	0.750 ± 0.250	0.3273[c]
Amount of watery rhinorrhea	0.211 ± 0.096	0.167 ± 0.112	0.7671[c]
Total nasal finding scores	0.684 ± 0.242	0.971 ± 0.336	0.5015[c]
Ocular symptom medication score	0.233 ± 0.085	0.036 ± 0.020	0.1066[c]
Nasal symptom medication score	1.02 ± 0.27	1.17 ± 0.31	0.6245[c]
Total-IgE (IU/ml)	281 ± 60	296 ± 87	0.8845[a]
Japanese cedar pollen-specific IgE (UA/ml)	38.7 ± 4.3	42.6 ± 6.0	0.5887[a]
Eosinophils (%)	2.58 ± 0.44	2.58 ± 0.34	0.9937[a]
Th1/Th2 ratio	8.16 ± 0.60	8.77 ± 1.36	0.6486[a]

[a,b,c] Analyzed by Student's t-test, Chi-square test, and Mann-Whitney's U test respectively.
Th1/Th2 ratio represents the proportion of IFN-γ^+CD4$^+$ and IL-4$^+$CD4$^+$ peripheral mononuclear cells.

Table 2. Mean Change in Scores for Nasal Cavity Findings for Subjects with CAP-RAST Scores of 4 or 5 after 4 and 8 Weeks of Treatment

	4 weeks			8 weeks		
	Placebo	OLL2809	p-value	Placebo	OLL2809	p-value
Mucosal swelling of the inferior turbinate	0.842 ± 0.206	0.500 ± 0.230	0.3837	0.474 ± 0.160	0.417 ± 0.149	0.8018
Amount of watery rhinorrhea	0.895 ± 0.130	0.667 ± 0.142	0.2766	0.211 ± 0.123	0.500 ± 0.195	0.1411
Total nasal finding scores	1.737 ± 0.295	1.167 ± 0.271	0.0991	0.684 ± 0.242	0.917 ± 0.260	0.2478

The scores are differences from those in the pre-observation period. Total nasal finding scores represent sums of the scores for swelling of the inferior turbinate and amount of watery rhinorrhea. Placebo subgroup, $n = 19$; OLL2809 subgroup, $n = 12$.

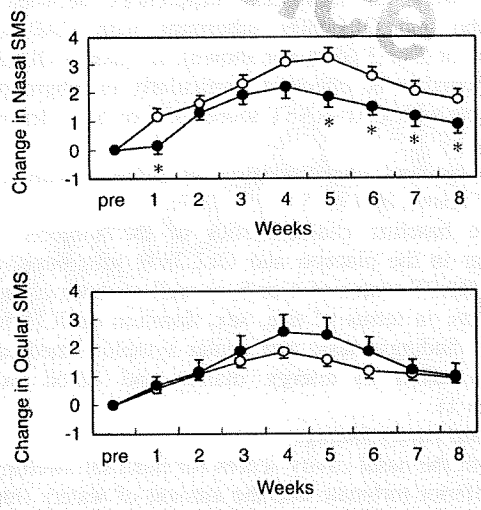

Fig. 3. Time-Course Change in Nasal and Ocular Symptom Medication Scores (SMS) of Subgroups with CAP-RAST Scores of 4 or 5.

The scores are differences from those in the pre-observation period. Placebo subgroup (○), $n = 19$; OLL2809 subgroup (●), $n = 12$. *$p < 0.05$ (Mann-Whitney's U test).

weeks. Although there was no significant difference in the scores between the subgroups at 4 weeks (data not shown), the scores for nasal congestion and itchy nose were significantly lower ($p = 0.009$ and 0.0156 respectively), and the frequency of the subjects who exhibited no symptoms was higher in the OLL2809 subgroup at 8 weeks (Fig. 4).

Of the 17 items in JRQLQ-II, the scores for reduced memory, reduced contact with friends or others by telephone or conversation at 4 weeks and for tiredness at 8 weeks tended to be low ($p = 0.0666$, 0.0934, and 0.0848 respectively) in the OLL2809 subgroup (Table 3). Furthermore, of the 17 items, scores at lower values were observed in 15 items in the OLL2809 subgroup at 8 weeks. Although the subjects responded to the questionnaires at 4 and 8 weeks, they were queried regarding the severity of their symptoms during the 1–2 weeks preceding the time of response. Therefore, these scores at 8 weeks represent symptoms occurring at 6 to 7 weeks, and they correspond well with the nasal SMS recorded in the allergy diaries.

Blood examination

At 4 weeks, all the blood examination items, including the total IgE and Japanese cedar pollen-specific IgE levels, numbers of eosinophils, and the Th1/Th2 ratio, increased, with a peak at 4 weeks, but no differences were observed between the placebo and OLL2809 subgroups. When we analyzed the relative values where the mean values in each subgroup at the pre-observation period were expressed as 1.0, the Japanese cedar pollen-specific IgE levels in the OLL2809 subgroup tended to be lower than in the placebo subgroup at 4 weeks ($p = 0.0525$, Fig. 5). While there were no intra-subgroup differences even in the relative values of eosinophils, intra-period analysis in each subgroup revealed that although this value significantly ($p < 0.05$) increased in the placebo subgroup at 8 weeks as compared with the pre-observation period, this increase was not significant in the OLL2809 subgroup ($p = 0.2104$). Further, the Th1/Th2 ratio tended to increase in the OLL2809 subgroup but not in the placebo subgroup as compared with the respective values for the pre-observation period ($p = 0.0687$). Total IgE did not significantly change between the subgroups during the study period.

Safety

No adverse effects were observed throughout the study. No significant changes in blood or biochemistry

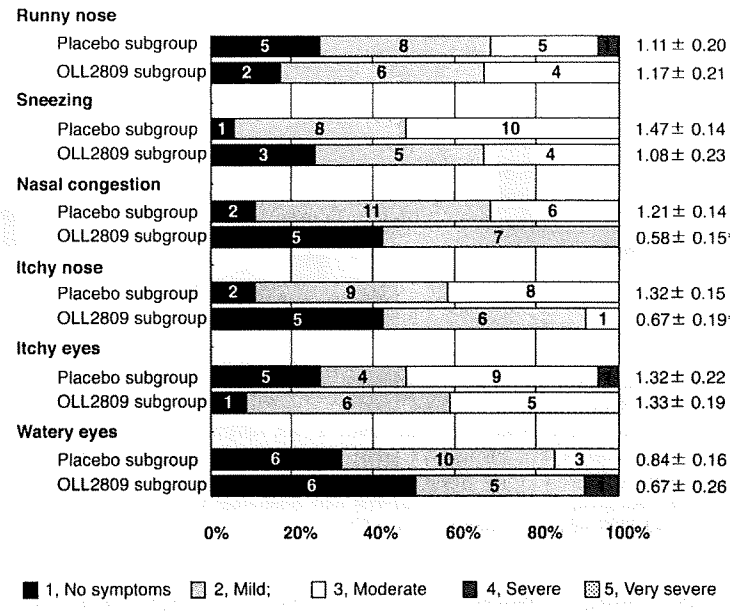

Fig. 4. Japanese Allergic Rhinitis Standard Quality of Life Questionnaire for Subgroups with CAP-RAST Scores of 4 or 5 after 8 Weeks of Treatment.

The numbers in the histogram represent the numbers of subjects, and the mean scores ± SE are presented on the right. Placebo subgroup, $n = 19$; OLL2809 subgroup, $n = 12$. $^*p < 0.05$ (Mann-Whitney's U test).

Table 3. Mean Change in JRQLQ-II Scores of Subgroups with CAP-RAST Scores of 4 or 5 after 4 and 8 Weeks of Treatment

	4 weeks			8 weeks		
	Placebo	OLL2809	*p*-value	Placebo	OLL2809	*p*-value
1. Reduced productivity at work/home	1.158 ± 0.220	0.667 ± 0.225	0.2142	0.632 ± 0.191	0.333 ± 0.188	0.3555
2. Poor mental concentration	1.158 ± 0.206	0.833 ± 0.271	0.4170	0.632 ± 0.157	0.583 ± 0.229	>0.999
3. Reduced thinking power	1.053 ± 0.223	0.833 ± 0.271	0.6399	0.632 ± 0.175	0.417 ± 0.229	0.5180
4. Impaired reading book/newspaper	0.842 ± 0.206	0.417 ± 0.193	0.5083	0.526 ± 0.160	0.333 ± 0.142	0.5083
5. Reduced memory	0.789 ± 0.224	0.167 ± 0.167	0.0666	0.316 ± 0.134	0.083 ± 0.149	0.3137
6. Limitation of outdoor life	0.842 ± 0.175	0.417 ± 0.228	0.1014	0.579 ± 0.221	0.333 ± 0.225	0.6341
7. Limitation of going out	1.053 ± 0.235	0.750 ± 0.279	0.4114	0.579 ± 0.176	0.417 ± 0.193	0.5745
8. Hesitation visiting friend or relatives	0.789 ± 0.196	0.417 ± 0.260	0.4558	0.421 ± 0.139	0.250 ± 0.131	0.4558
9. Reduced contact with friends or others by telephone or conversation	0.632 ± 0.175	0.250 ± 0.131	0.0934	0.316 ± 0.134	0.250 ± 0.131	0.8731
10. Not an easy person to be around	0.632 ± 0.191	0.250 ± 0.131	0.8672	0.263 ± 0.129	0.250 ± 0.131	0.8672
11. Impaired sleeping	0.421 ± 0.221	0.750 ± 0.372	0.4932	0.316 ± 0.134	0.333 ± 0.142	0.7584
12. Tiredness	1.000 ± 0.229	1.000 ± 0.348	0.8301	0.737 ± 0.185	0.250 ± 0.131	0.0848
13. Fatigue	0.789 ± 0.211	1.083 ± 0.358	0.5735	0.579 ± 0.221	0.333 ± 0.142	0.5817
14. Frustration	0.789 ± 0.224	0.917 ± 0.358	0.9828	0.316 ± 0.154	0.417 ± 0.149	0.5548
15. Irritability	0.842 ± 0.191	1.000 ± 0.326	0.8251	0.368 ± 0.114	0.250 ± 0.131	0.4991
16. Depression	0.737 ± 0.200	0.833 ± 0.366	0.8419	0.263 ± 0.129	0.250 ± 0.131	0.8814
17. Unhappiness	0.759 ± 0.192	0.833 ± 0.366	0.8423	0.211 ± 0.164	0.333 ± 0.142	0.7151

The scores are differences from those in the pre-observation period.
Placebo subgroup, $n = 19$; OLL2809 subgroup, $n = 12$.

results were observed during the study period in any of the subjects.

Discussion

In recent years, a number of clinical trials have evaluated the efficacy of probiotics and of heat-killed lactobacilli in allergic diseases. The results imply that some clinical effects on pollinosis,[22–25] atopic dermatitis,[26,27] perennial allergic rhinitis induced by house-dust mites,[28] and food allergy[29] occurred. On the other hand, Brouwer *et al.* and Grüber *et al.* reported that there were no clinical or immunological effects of probiotic *L. rhamnosus* GG, which was used in infants with atopic dermatitis.[30,31] Such diverse results were due to differences in study design, and perhaps were due to heterogeneity of exposure to the allergens, the allergic backgrounds of the subjects, and the efficacy of the microorganisms used. Hence, further studies are required to determine the efficacy of probiotics and of lactic acid bacteria with immunoregulatory activity.

Although our clinical study had a substantial sample size, no obvious efficacy of *L. gasseri* OLL2809 was observed. Hence, we performed subgroup analyses based on the CAP-RAST scores. Because serum antigen-specific IgE levels play a crucial role in the

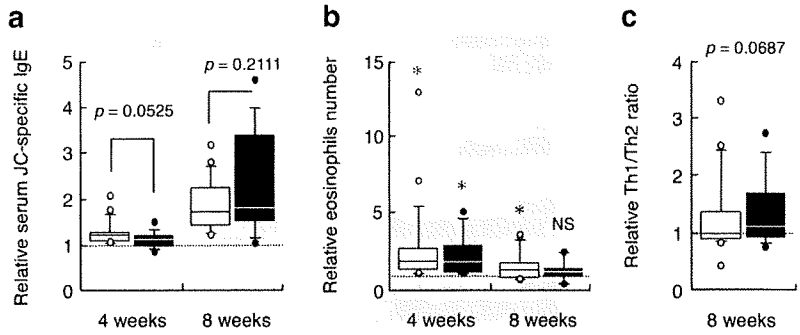

Fig. 5. Relative Changes in Serum Japanese Cedar Pollen-Specific IgE (a), Number of Peripheral Blood Eosinophils (b), and the Th1/Th2 Ratio (c) in Subgroups of Subjects with CAP-RAST Scores of 4 or 5.

Data are represented by their relative values where the mean values in each subgroup at the pre-observation period are expressed as 1.0 (broken line). The differences in the relative serum Japanese cedar pollen-specific IgE and relative Th1/Th2 ratio between the placebo and OLL2809 subgroups were analyzed by Student's *t*-test. Those in the relative eosinophil numbers between pre-observation and 4 and 8 weeks after treatment were analyzed in each group by Student's paired *t*-test with Bonferroni's correction (*$p < 0.05$). NS, not significant. Placebo subgroup (open bars), $n = 19$; OLL2809 subgroup (solid bars), $n = 12$.

onset of symptoms in type I allergic diseases, it is possible that subjects with high CAP-RAST scores have a higher predisposition to allergic diseases. The results of the subgroup analyses revealed that subjects with CAP-RAST scores of 4 or 5 exhibited the efficacy of *L. gasseri* OLL2809: it caused a significant reduction in the nasal SMS throughout the administration period, and the nasal symptoms in JRQLQ at 8 weeks. In addition, the relevant evaluation items such as clinical scores for the nasal cavity at 4 weeks and the serum allergy-related items, including the Japanese cedar pollen-specific IgE levels, eosinophils, and the Th1/Th2 ratio improved, though the differences were not statistically significant. There was a difference in that efficacies were observed for each item, *e.g.*, the nasal cavity findings tended to be low in the OLL2809 subgroup at 4 weeks, but other parameters such as nasal SMS, nasal congestion, and itchy nose in JRQLQ-I were lower in the OLL2809 subgroup in the later period (5–8 weeks). This might have been caused by a difference in objective and subjective evaluation. For instance, even if an objective examination item is improved, subjective symptoms are not necessarily improved, and *vice versa*. This sometimes occurs in clinical examination. Yet, considering these data comprehensively, it was assumed that *L. gasseri* OLL2809 mainly ameliorated the nasal symptoms of the subjects with CAP-RAST scores of 4 or 5, *via* affecting the immune systems.

Similar results have been reported for *L. casei* strain Shirota. Tamura *et al.* reported that supplementation with *L. casei* strain Shirota did not affected the nasal or ocular SMS, but it tended to reduce the nasal SMS in the subgroup of subjects with moderate to severe nasal symptom scores prior to start of ingestion of the test samples.[25] Type I allergic diseases such as JCP are associated with elevated serum antigen-specific IgE. Binding of inhaled allergens to IgE on the surfaces of basophils and mast cells, with subsequent cross-linkage of IgE and aggregation of high-affinity receptors for IgE (FcεRI), triggers the release of histamine, leukotrienes, and other inflammatory mediators, followed by the onset of allergic symptoms.[32] Consequently, there must be a correlation between the symptoms and the Japanese cedar pollen-specific IgE levels.

Here, we hypothesize the mechanism by which *L. gasseri* OLL2809 exhibited efficacy in the subjects with CAP-RAST scores of 4 or 5. It has been widely reported that some *Lactobacillus* strains, such as *L. gasseri* OLL2809, stimulate IL-12 (p70) production by immune competent cells, and that this promotes a shift in the Th1/Th2 balance from Th2 toward Th1.[16–18] This immunoregulatory effect in the Th1/Th2 balance is observed as certain lactobacilli induce IFN-γ production and reduce IL-4 production by $CD4^+$ T cells.[16,33] However, this effect occurs when antigen-sensitized $CD4^+$ T cells are stimulated by antigens. For instance, when $CD4^+$ transgenic T cells expressing ovalbumin-specific T-cell receptors were cultured with *Mycobacterium tuberculosis* in the absence of ovalbumin, they did not produce substantial levels of IFN-γ or IL-4 as compared with those cultured in the presence of both *M. tuberculosis* and ovalbumin.[34] Likewise, stimulation of IFN-γ production and suppression of IL-4 production by *L. gasseri* OLL2809 are observed specifically in antigen-sensitized $CD4^+$ T cells when the antigen is present in the cell culture, whereas it does not occur in non-sensitized $CD4^+$ T cells even in the presence of the antigen (Sashihara *et al.*, unpublished observation). This suggests that the immunostimulatory effect of microbes in shifting the Th1/Th2 balance from Th2 to Th1 can be effective when the host immune cells are highly sensitized and the antigen level is sufficient to stimulate antigen-sensitized $CD4^+$ T cells. In the present clinical trial, it is hard to assume that there was a difference in the amount of cedar pollen exposed in the subjects. Therefore, the observation that *L. gasseri* OLL2809 was effective in the subjects with CAP-RAST scores of 4 or 5 suggests that their $CD4^+$ T cells were highly sensitized to the antigen, and that consequently the reactivity of the immune cells to *L. gasseri* OLL2809 was higher than those from subjects with CAP-RAST scores of 2 or 3.

In conclusion, although no obvious clinical efficacy of heat-killed *L. gasseri* OLL2809 was observed in subjects with JCP, this strain possesses efficacy to ameliorate symptoms by modulating the systemic immune responses in subjects with a high predisposition to allergic disease.

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ガイドラインのワンポイント解説

アレルギー性鼻炎—第6版改訂のポイント—

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Key words: allergic rhinitis —— guideline —— histamine H_1 receptor antagonists —— leukotriene receptor antagonists —— intranasal corticosteroids

はじめに

2008年10月に「鼻アレルギー診療ガイドライン」改訂第6版が発刊された[1]. その第2章に馬場らが1998年と2008年に行った疫学調査の結果が対比して記されており，我国におけるアレルギー性鼻炎の有病率はこの10年間で約10%増加し39.4%に達したとある[1]. (**図1**)この調査は全国の耳鼻咽喉科医とその家族を対象としてアンケート法によって行われたものであり，様々なバイアスがあることを否めないが，アレルギー性鼻炎そしてスギ花粉症患者の増加は日常臨床の中で確かに実感されるところである. したがって，耳鼻咽喉科医だけですべてのアレルギー性鼻炎患者に対応するのは不可能であり，とくに短期間に患者数が激増するスギ花粉症においては，他の診療科の多くの医師もその治療にあたることになる. そのような社会的背景を踏まえて，アレルギーを専門とする耳鼻咽喉科医はもとより，アレルギーを専門としない耳鼻咽喉科医そして耳鼻咽喉科以外の医師をも対象として，アレルギー性鼻炎に関する理解を深め診療レベルを向上させることを目的として1993年に本ガイドラインの初版が作成され，その後改訂を重ねて2008年にこの第6版が発刊された.

本ガイドラインには別途にその内容をまとめた医師向けのダイジェスト版も同時に出版されており，これに目を通せば大まかな診断と治療の流れは理解できると思われる. そこで，本稿では今回の改訂での変更箇所を整理し，本ガイドラインを使用するうえでのいくつかの留意点と問題点について私見を述べてみたい.

1. 鼻アレルギー診療ガイドライン第6版の改訂箇所

第6版ではエビデンスとなる新たな文献を追加し，その内容を見直すとともに，実地診療での様々な場面を想定した解説が新たに加えられた. 以下に，その変更点を列挙する.

まず，冒頭でも述べたように，第2章「疫学」に馬場らが10年振りに行った全国疫学調査の結果が示されている. アレルギー性鼻炎全体の増加は花粉症とくにスギ花粉症の増加によるもので，成人のみならず，小児から高齢者まですべての年齢層で増加していることが分かる.

花粉症の診断と治療には，その原因となる植物

利益相反（conflict of interest）に関する開示：著者は本論文の研究内容について他者との利害関係を有しません.

ONE POINT MESSAGE OF PG-MARJ 2009 (ALLERGIC RHINITIS)—THE CLINICAL POINTS AND ISSUES IN THE REVISION—

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Abbreviations: PGD2 "prostaglandin D2", TXA2 "thromboxane A2", LT "leukotriene"

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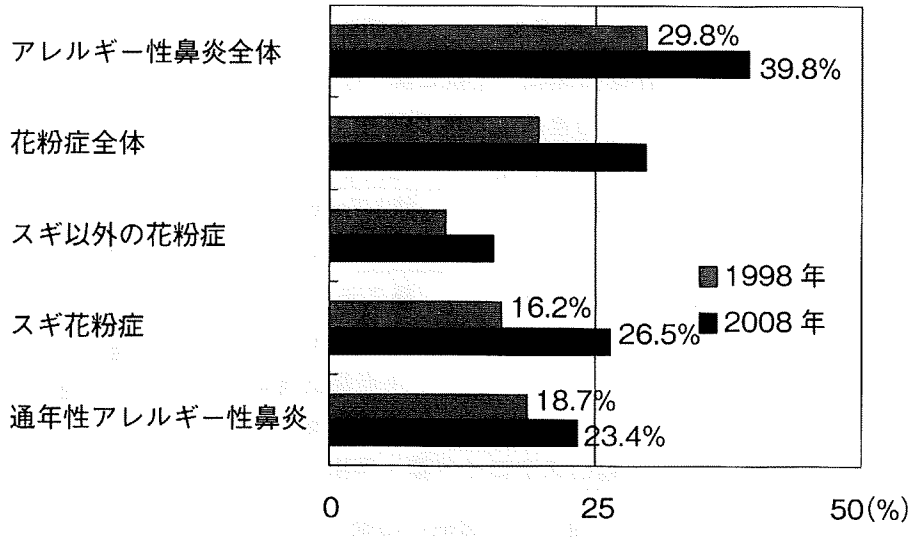

図1.　アレルギー性鼻炎有病率の1998年と2008年との比較.
（文献1より引用）

	くしゃみ・鼻漏型	鼻閉型	
軽　症	第2世代抗ヒスタミン薬		特異的免疫療法
	遊離抑制薬，Th2サイトカイン阻害薬		
中等症	第2世代抗ヒスタミン薬	抗LTs薬	
	遊離抑制薬，Th2阻害薬	抗PGD_2/TXA_2薬	
	鼻噴霧用ステロイド薬	鼻噴霧用ステ薬	
重　症	鼻噴霧用ステロイド薬	鼻噴霧用ステ薬	
	＋	＋抗LTs or	
	第2世代抗ヒスタミン薬	抗PGD_2/TXA_2	
		点鼻血管収縮薬，手術	抗原除去・回避

図2.　通年性アレルギー性鼻炎の治療.
（文献1より引用改変）

の分布やその開花期を知ることが重要である．そこで，第4章「検査・診断法」の主な花粉症原因植物開花期の図が変更され，花粉の飛散数により3段階表記にされている．

アレルギー性鼻炎は長期間の治療が必要であり，そこで最も大切なことは患者とのコミュニケーションの取り方である．しかし，どのようにしてこれを構築するかは，これまでのガイドラインには明記されていなかった．これを明文化するのはなかなか難しい作業であるが，今回の改訂では第5章「治療」に患者との十分なコミュニケーションを図るための具体的な方法，そしてその際に使用するアレルギー性鼻炎の問診票や鼻アレルギー日記の1例が掲載されている．

アレルギー性鼻炎の治療でもっとも大きな比重を占めるのは薬物治療であり，今回，通年性アレルギー性鼻炎の軽症そして中等症のくしゃみ・鼻漏型の治療薬としてTh2サイトカイン阻害薬が追加された．(**図2**)また，花粉症の初期療法に使用する薬剤としてもTh2サイトカイン阻害薬，そして抗プロスタグランジンD2・トロンボキサンA2(PGD2・TXA2)薬が新たに加えられた．(**図3**)