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

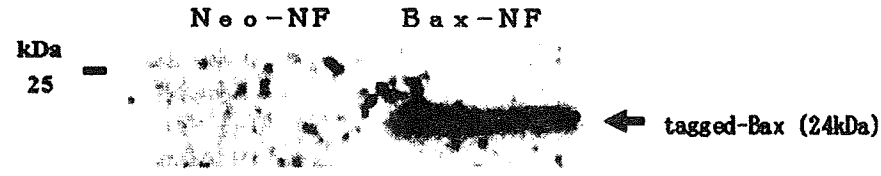

Fig. 1 Western blot analysis of Bax in nasal fibroblasts. Bax-NF was transiently transfected with hBaxpcDNA3. Neo-NF was transiently transfected with native pcDNA3. Equal amounts of cell extract (30 μg of protein) were loaded in each lane and blotted with anti-human Bax antibody. A band of Bax (24 kDa)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

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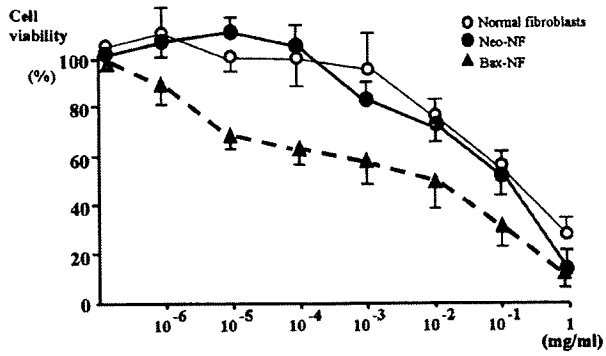

Fig. 2 Effect of Bax expression on the sensitivity of nasal fibroblasts to prednisolone in vitro. Bax-NF, Neo-NF, and wt-NF (wild-type) were incubated with various concentrations of prednisolone. Cell viability was assessed by the MTT assay. $*p < 0.05$, $**p < 0.01$, compared to cell viability in the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

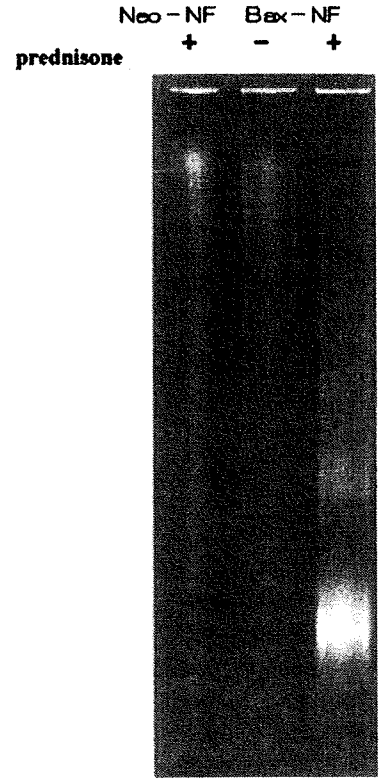

Fig. 3 Acrylamide gel electrophoresis of DNA. Bax-NF and Neo-NF were treated with prednisolone (1 μg/ml) for 72 h. A DNA ladder was seen in the prednisolone-treated Bax-NF

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 life-style-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 checkup 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

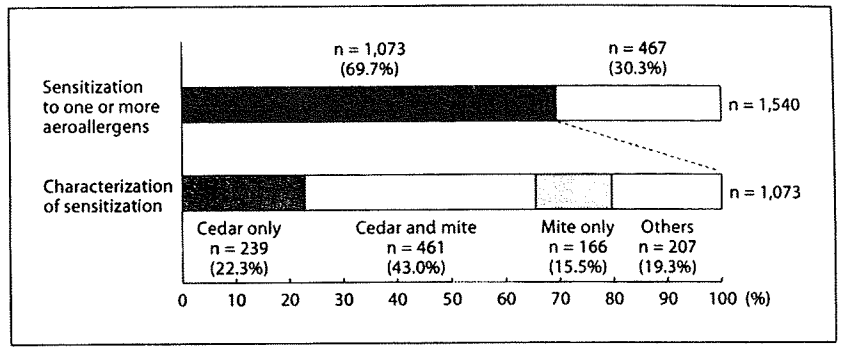

Fig. 1. The prevalence of sensitization to 7 test aeroallergens and characterization of sensitization.

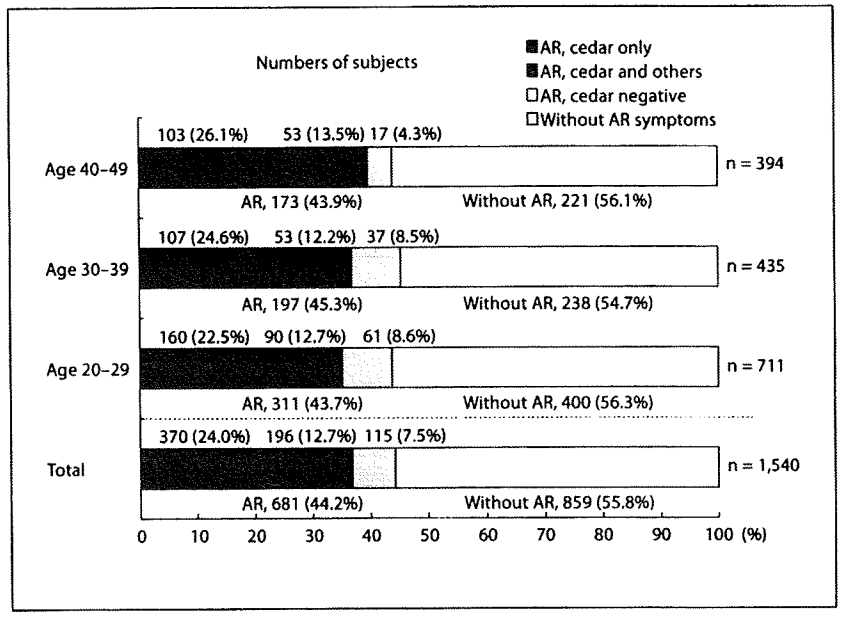

Fig. 2. Age effects on the prevalence of AR and sensitization to Japanese cedar pollen.

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

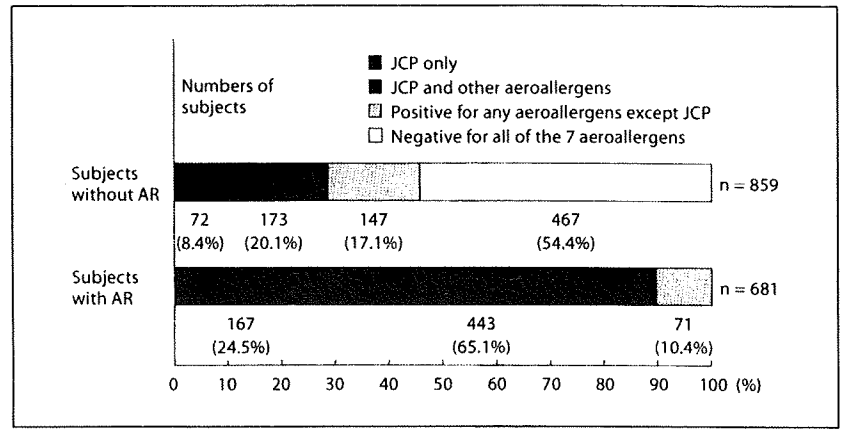

Fig. 3. Prevalence of AR and sensitization to Japanese cedar pollen.

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.

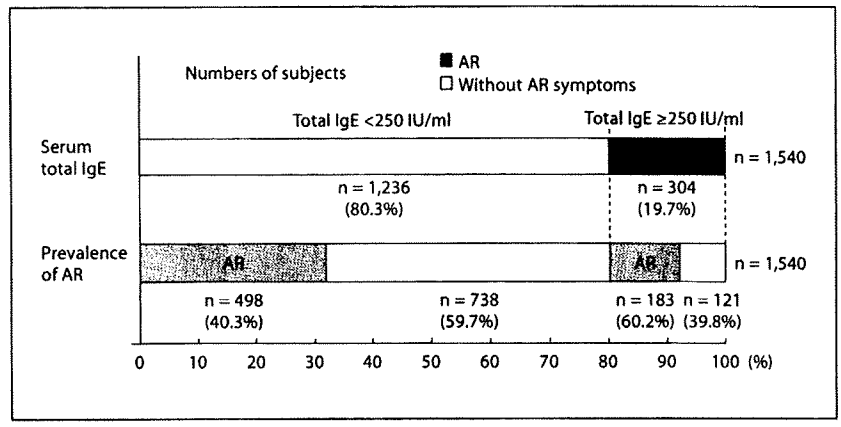

Fig. 4. Serum total IgE levels and prevalence of AR.

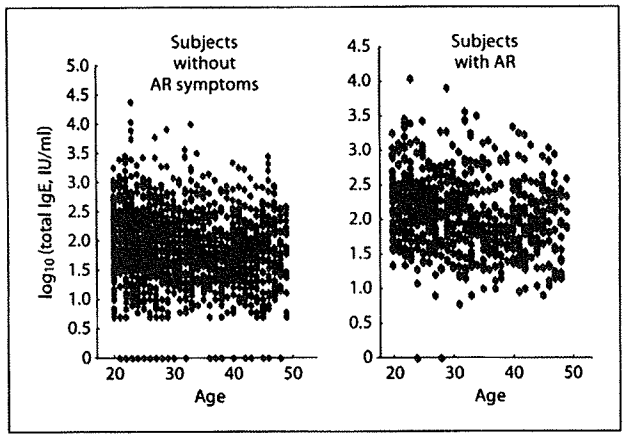

Fig. 5. Age effects on serum total IgE levels in subjects with AR and non-AR.

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 ($r_s = -0.21$, $p < 0.01$) (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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Mechanisms and clinical implications of glucocorticosteroids in the treatment of allergic rhinitis

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Summary

Allergic rhinitis is a common airway disease characterized by hypersensitivity, exudation, hypersecretion, inflammatory cell infiltration and remodelling. Intranasal glucocorticosteroids are the most effective drugs for controlling the inflammation caused by allergic rhinitis. Glucocorticosteroids exert anti-inflammatory effects through at least two pathways: the transactivation pathway and the transrepression pathway. Glucocorticosteroids also exert regulatory functions by inducing regulatory cytokines and forkhead box P3 (FoxP3$^+$) regulatory T cells. Evidence suggests that intranasal glucocorticosteroids control not only nasal symptoms but also ocular symptoms. In contrast to sedating H1 receptor antagonists, intranasal glucocorticosteroids can improve impaired performance symptoms, such as daytime sleepiness, associated with allergic rhinitis. Recent studies suggest that intranasal glucocorticosteroids might also be useful for the prophylactic treatment of pollinosis; this possibility is supported by the molecular mechanism of the anti-inflammatory action of glucocorticosteroids. These findings suggest that intranasal glucocorticosteroids might be positioned as first-line drugs for the treatment of both perennial and seasonal allergic rhinitis.

Keywords: impaired performance, intranasal glucocorticosteroids, ocular symptoms, regulatory T cells

Introduction

Allergic rhinitis (AR) is a common manifestation of allergic diseases, affecting approximately 500 million people worldwide [1]. AR is increasing in prevalence. For example, the prevalence of AR in Japan increased from 29·8% in 1998 to 39·4% in 2008. The prevalence of pollinosis, the typical seasonal AR, has been increased from 19·6% in 1998 to 29·8% in 2008 [2].

AR is a major chronic inflammatory condition in the upper airway characterized by hypersensitivity, exudation, hypersecretion, inflammatory cell infiltration and remodelling [3]. Although glucocorticosteroids (GC) are highly effective in mitigating inflammation, their potent action often causes severe adverse effects [4,5]. To decrease the potential for adverse effects, intranasal glucocorticosteroid (INS) formulations with low systemic availability have been developed for the treatment of allergic rhinitis [6].

In this review, we discuss the pathophysiology of allergic rhinitis and the mechanism of action of GC, including the induction of regulatory T cells (T_{regs}), in the pathogenesis of AR. We also discuss the usefulness and pitfalls of INS in the clinical setting and assess the current status of INS for the treatment of AR.

Pathophysiology of AR

Pathogenesis of AR

Most causal antigens for AR are inhalant allergens. House dust mite, animal dander and pollens are the principal allergens. Many allergens, including the major house dust-mite allergen, Der p 1, have protease activity that impairs epithelial barrier function and facilitates the penetration of allergens into nasal mucosa [7]. Following nasal exposure to the inhalant allergens, professional antigen-presenting cells in the nasal mucosa, such as dendritic cells (DC), capture the allergens and provide two distinct signals, the allergen-derived peptide/MHC complex and co-stimulatory molecules such as CD80 and CD86, to naive T cells [8–10]. Allergen-specific T helper type 2 (Th2) cells are generated in patients with AR, whereas allergen-specific Th1 cells are

generated in healthy individuals [11,12]. Early interleukin (IL)-4 and thymic stromal lymphopoietin (TSLP) produced by basophils in response to allergens with protease activity may contribute to Th2 differentiation [12]. Th2 cells produce IL-4/IL-13 and express CD40L, which promote the class-switching of B cells to immunoglobulin (Ig)E [13,14]. When sensitized subjects inhale antigens, the antigens pass through the epithelial tight junctions in the nasal mucosa to bind IgE on the surface of mast cells in the epithelial layer of the nasal mucosa, inducing the release of chemical mediators including histamine, prostaglandins and cysLTs by aggregation of FcεRI. Histamine regulates tight junctions via the coupling of H1 receptors and increases paracellular permeability [15]. This increased permeability allows DC to penetrate epithelial tight junctions easily and enhance antigen presentation to T cells [16]. The early-phase response, which consists of sneezing, rhinorrhoea and nasal congestion, is caused by interactions between chemical mediators and the sensory nerve terminals and blood vessels in the nasal mucosa [17].

After the nasal exposure to allergen, infiltration of inflammatory cells, such as activated eosinophils and Th2 cells, into the nasal mucosa is induced by cytokines, chemical mediators, chemokines and growth factors [18,19]. Cytokines such as IL-5, IL-4, IL-13 and granulocyte–macrophage colony-stimulating factor (GM-CSF) are produced mainly in Th2 cells and mast cells; however, eosinophils also have the potential to produce these cytokines [18,20,21]. Chemical mediators such as platelet-activating factor (PAF), leukotriene B4 (LTB_4), cysteinyl leukotrienes (cysLTs) and thromboxane A_2 (TXA_2) are also released mainly from mast cells and eosinophils [17,20]. Chemokines such as eotaxin, regulated upon activation normal T cell expressed and secreted (RANTES) and thymus and activation regulated chemokine (TARC) are produced mainly in fibroblasts, epithelial cells and vascular endothelial cells [22]. Proinflammatory cytokines such as tumour necrosis factor (TNF)-α from mast cells and eosinophil-derived granules such as eosinophil cationic proteins are also produced and participate in allergic inflammation [23,24]. The sensitivity of the nasal mucosa to different stimulants increases along with the progress of allergic inflammation in the nasal mucosa; this increased sensitivity is referred to as the priming effect [25]. The secondary reaction with inflammatory cells and their mediators, especially the cysLTs produced by eosinophils, causes oedema of the nasal mucosa [26]. This inflammation, which develops 6–10 h after the allergen challenge, is referred to as the late-phase response [17]. Management of allergic rhinitis should be determined based on its mechanism (Fig. 1).

Onset of three major AR symptoms

Sneezing. Sensory nerves containing substance P (SP) and calcitonin gene-related peptide (CGRP) are distributed throughout the epithelial and subepithelial layers of the nasal mucosa [27]. Sensory nerve terminals are located in the epithelial junctions and subepithelial layers. In the guinea pig model of allergic rhinitis, the sneezing reflex following allergen challenge is inhibited significantly by pretreatment with capsaicin, which depletes SP and CGRP from the nasal mucosa [28]. When various chemical mediators are applied to the nasal mucosa, histamine is the only mediator that induces a significant sneezing reflex [28,29]. Therefore, the sneezing reflex following allergen challenge is a respiratory reflex induced by the interaction between histamine and the H1 receptor at the sensory nerve terminals containing SP and CGRP and might be a sensory stimulation response amplified by hyperreactivity in the nasal mucosa [25].

Rhinorrhoea. Synchronously with the sneezing reflex, sensory stimulation on the nasal mucosa induces excitation reflexively in the parasympathetic centre. After allergen challenge on the hemilateral nasal mucosa of patients with allergic rhinitis, the weight of rhinorrhoea induced in both sides of nasal cavities is correlated with the number of sneezes. In addition, the weight of rhinorrhoea in the nasal cavity with allergen challenge is correlated with that on the opposite side. Therefore, rhinorrhoea can be regarded as the secretion from the mucous glands by parasympathetic stimulation [30]. Furthermore, allergic inflammation induced by nasal allergen exposure augments this 'naso-nasal' reflex [31]. Possible mechanisms for sensory nerve hyperresponsiveness include the increased release of nerve growth factor during allergic inflammation [32].

Chemical mediators including histamine, cysLTs, and PAF induce plasma exudation directly from the blood vessels in the nasal mucosa, which constitutes a part of rhinorrhoea. However, only 4–15% of total rhinorrhoea is attributed to plasma exudation, according to calculations based on the albumin concentration in the rhinorrhoea induced by allergen challenge [33].

Nasal congestion. The underlying causes of nasal congestion in the early phase of allergic rhinitis are the relaxation of the smooth muscle layer of capacitance vessels in the nasal mucosa and the interstitial oedema induced by plasma exudation. Swelling of the nasal turbinate is induced by the parasympathetic reflex and the axon reflex through the nerve centre and the direct effects of the chemical mediators on the vascular system. Dilation of the capacitance vessels and plasma exudation after excitation of the parasympathetic centre are caused by the nitric oxide (NO) released from parasympathetic terminals and vascular endothelial cells [34]. However, the participation of the nerve reflex in nasal turbinate swelling after allergen challenge is minor compared with the direct effects of chemical mediators, such as histamine, cysLTs, PAF and prostaglandin D_2 (PGD_2) and kinin, on the vascular system in the nasal mucosa [35,36]. Nasal congestion in the late phase is induced by the allergic inflammation, as described above.

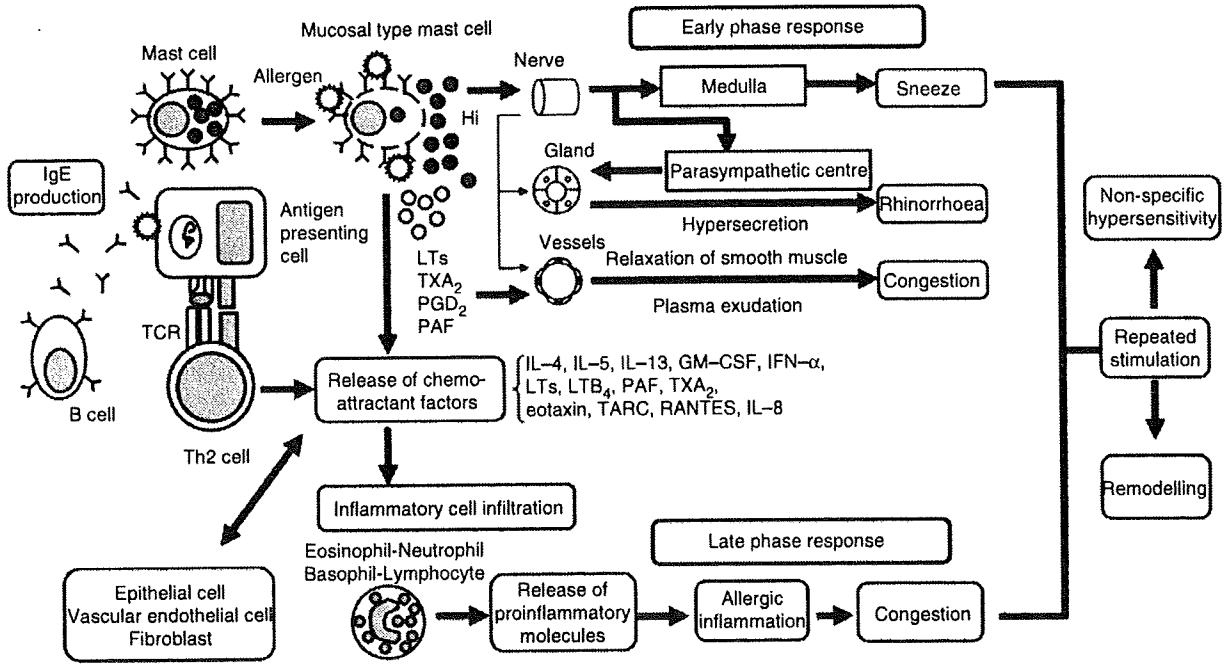

Fig. 1. Pathophysiology of allergic rhinitis as described in Practical Guideline for Management of Allergic Rhinitis in Japan (PG-MARJ). After allergens are inhaled into the nasal mucosa of sensitized subjects, they bind to immunoglobulin (Ig)E on the surface of mast cells, inducing the release of chemical mediators including histamine, prostaglandins and cysteinyl leukotrienes (cysLTs) by aggregation of FcεRI. Histamine regulates tight junctions by coupling the H1 receptor, which increases paracellular permeability. The early-phase response, which is characterized by sneezing, rhinorrhoea and nasal congestion, is the response of the sensory nerve terminals and blood vessels on the nasal mucosa to these chemical mediators. After the nasal exposure to allergen, infiltration of inflammatory cells, such as activated eosinophils and T helper type 2 (Th2) cells, into the nasal mucosa is induced by chemoattractant factors such as cytokines including interleukin (IL)-5, chemical mediators including cysLTs and chemokines including eotaxin. Oedema of the nasal mucosa develops as a secondary reaction with inflammatory cells. This inflammation, referred to as the late-phase response, develops 6–10 h after allergen challenge and causes prolonged nasal congestion.

Mechanisms of glucocorticosteroid

Molecular level

At the molecular level, the effects of GC begin when GC crosses the cell membrane and binds to the intracellular glucocorticosteroid receptor (GR) [37]. Cytoplasmic GR is maintained in an inactive form by heat shock protein (hsp)90 and hsp70 [38,39]. Binding of GC dissociates the hsps, allowing the GR complex to translocate into the nucleus or interact with cytoplasmic transcriptional factors. An alternative splicing variant, GRβ, lacks the ability to bind GC [40]. GRβ forms heterodimers with the wild-type GR (GRα) and may act as an inhibitor of GRα. In atopic nasal tissue, staphylococcal enterotoxin induces GRβ expression and steroid resistance [41].

GC exerts its anti-inflammatory effects through at least two pathways, transactivation and transrepression [42]. Transactivation occurs when the receptor complex binds to the glucocorticosteroid-response elements (GRE) in the promoter regions of glucocorticosteroid-responsive genes, which encode anti-inflammatory genes such as annexin 1, IκB and CD163 [43]. Alternatively, the GR complex represses the transcription of proinflammatory genes by protein–protein interactions such as GR–nuclear factor kappa B (NFκB) and GR–activator protein 1 (AP-1) [44]. Evidence for a co-activator competition model of transrepression involving CBP/p300 was first provided for GR transrepression of AP-1 target genes [45].

Cellular level (Fig. 2)

GC inhibits the functions of infiltrating inflammatory cells and their recruitment into the nasal mucosa. GC inhibits the maturation, cytokine production, FcεRI expression and mediator release of mast cells [46,47]. GC inhibits histamine release from basophils [48,49], induces apoptosis of eosinophils [50] and reduces the recruitment of antigen-presenting cells such as Langerhans cells [51]. GC decreases the numbers of GATA-3$^+$ Th2 cells and the production of Th2 cytokines, such as IL-4, IL-5, IL-6 and IL-13, while having little effect on T-bet$^+$ Th1 cells and the production of Th1 cytokines such as IL-2, IL-12 and interferon (IFN)-γ [52,53]. Although the inhibitory effect of GC on B cell recruitment is limited, GC inhibits class-switching to IgE in the nasal mucosa [51,54].

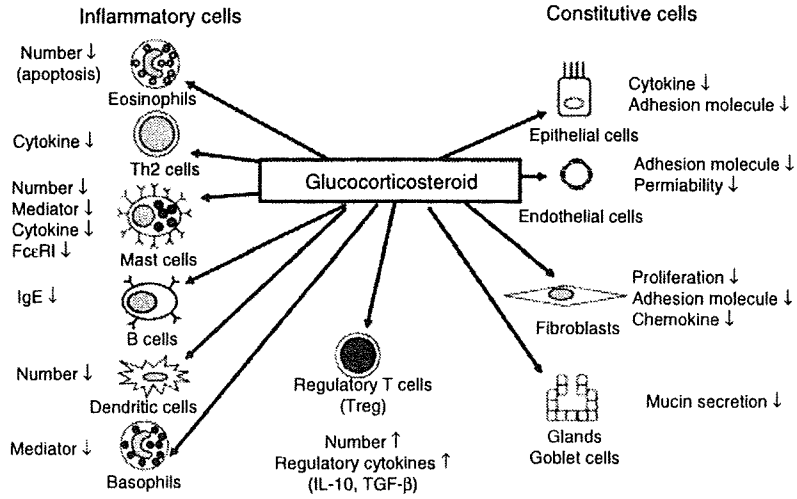

Fig. 2. Effect of glucocorticosteroids (GC) on nasal mucosa. The anti-inflammatory effects of GC on allergic rhinitis are mediated not only by inflammatory cells such as eosinophils, T helper type 2 (Th2) cells, mast cells, B cells, dendritic cells and basophils, but also by nasal constitutive cells such as epithelial cells, endothelial cells, fibroblasts and glands/goblet cells. In addition, treatment with GC can induce regulatory T cells.

GC also has anti-inflammatory effects on nasal constitutive cells, such as epithelial cells, fibroblasts, vascular endothelial cells and glands. GC inhibits intercellular adhesion molecule 1 (ICAM-1) expression [49] and GM-CSF production [55] by nasal epithelial cells. GC down-regulates nasal fibroblast functions, including basic fibroblast growth factor (bFGF)-induced proliferation, TNF-α-induced ICAM-1 expression, TNF-α- or IL-4-stimulated eotaxin release [56], TNF-α-induced matrix metalloproteinase production [57] and TNF-α-induced vascular endothelial growth factor (VEGF) and bFGF production [58]. GC inhibits TNF-α- or IL-1β-stimulated E-selectin expression on nasal vascular endothelial cells [59]. The effect of GC on vascular cel adhesion molecule 1 (VCAM-1) expression on nasal vascular endothelial cells is controversial [60,61]. The effect of GC on vascular permeability reflects the inhibition of cellular inflammatory processes indirectly rather than the direct effect on nasal vascular endothelial cells [62].

Induction of regulatory cytokines and T_{regs}

Among the cells with regulatory functions such as $CD8^+$, $CD4^-CD8^-$ and γδ T cells, $CD4^+CD25^+$forkhead box P3 ($FoxP3^+$) T_{reg} cells play a central role in immune tolerance and immune homeostasis [63]. T_{regs} are derived from the thymus and the periphery [64]. The suppressive effect of T_{regs} is associated with expression of the transcription factor FoxP3, which is used as a T_{reg} marker [65]. In addition, T_{regs} express high-affinity IL-2 receptor (CD25), and IL-2 is vital for the development and survival of T_{regs} [64]. T_{regs} regulate effector cells by cell-to-cell contact, the production of inhibitory cytokines such as IL-10 and transforming growth factor (TGF)-β, cytotoxicity mediated by perforins and granzymes, and competition for T cell growth factors, especially IL-2 [66].

The impaired expression or function of T_{regs} is involved in the pathogenesis of allergic rhinitis. For example, regulatory $CD4^+CD25^+$ T cells from patients with birch pollinosis but not healthy controls were defective in down-regulating birch pollen-induced IL-13 and IL-5 production by $CD4^+CD25^-$ T cells during the pollen season, while their capacity to suppress IFN-γ production and proliferation was retained [67]. The ratio of $FoxP3^+$/GATA binding protein 3 ($GATA\text{-}3^+$) cells in nasal mucosa was decreased significantly in patients with pollinosis as compared with healthy controls outside the pollen season, and the ratio was decreased further during the pollen season in allergic patients [53]. In addition, T_{regs} are induced in both peripheral blood and nasal mucosa following allergen-specific immunotherapy [68,69].

Treatment with GC induces T_{regs}. FoxP3 mRNA expression in $CD4^+$ cells was increased significantly in adult asthmatic patients receiving GC, and systemic GC treatment led to an early increase in FoxP3 mRNA and T_{reg} expression in patients with asthma [70]. Paediatric asthma patients treated with GC also had an increased frequency of T_{regs} in $CD4^+$ cells from peripheral blood and bronchoalveolar lavage fluid (BALF). In addition, T_{regs} in the BALF of asthmatic patients failed to suppress proliferation and production of Th2-associated cytokines by responder T cells, which was restored after inhalation of GC [71]. FoxP3 and IL-10 were down-regulated in nasal polyps compared with control mucosa, and their expression was increased after intranasal GC treatment [72]. We have demonstrated that GC induced $CD4^+$ $CD25^+$ $FoxP3^+$ T_{regs} in dispersed nasal polyp cells in the presence of IL-2. In fact, combined treatment with GC and IL-2 expands T_{regs} *in vivo*, and the induced T_{regs} suppress the proliferation of responder T cells in mice [73]. GC leads to the production of glucocorticosteroid-induced leucine zipper (GILZ) by dendritic cells; GILZ is critical for commitment of DCs to differentiate into regulatory DCs and for the generation of antigen-specific T_{regs} [74]. The detailed mechanism by which GC induces T_{regs} has not been elucidated.

Practical Guideline for Management of Allergic Rhinitis in Japan (PG-MARJ)

To address the classification, epidemiology, pathophysiology and management of allergic rhinitis in Japan, a practical guideline for the management of this condition, PG-MARJ, was first released in 1993. Based on the latest basic and clinical evidence, the sixth edition of PG-MARJ was published in 2008 [2]. The following discussion summarizes the PG-MARJ guidelines regarding the positioning of INS and systemic GC for the management of allergic rhinitis.

INS are potent agents indicated for the treatment of allergic rhinitis. In the treatment of type I allergy, INS are used as anti-inflammatory drugs. INS exert anti-inflammatory effects by the following mechanisms: inhibiting the local infiltration of effector cells of allergic inflammation such as mucosal-type mast cells, eosinophils and lymphocytes; inhibiting the production and release of cytokines; inhibiting vascular permeability and mucus gland secretion; and down-regulating the production of leukotrienes and prostaglandins by inhibiting arachidonic acid cascades. INS are not effective in controlling acute-phase allergic reactions but are effective for late-phase allergic reactions. However, INS are effective in controlling acute-phase allergic reactions when administered continuously.

Beclomethasone propionate, fluticasone propionate, mometasone furoate and fluticasone furoate are currently available as nasal sprays in Japan. These INS have potent local effects at small doses; they are not absorbed easily into the systemic circulation and are metabolized rapidly when absorbed [75]. Thus, the incidence of systemic adverse effects is low, even in patients receiving these drugs for ≥ 1 year, and reliable clinical effects can be expected with their use [76,77]. In addition, INS with lower bioavailability are believed to show fewer systemic adverse effects [78]. Because these drugs are administered locally, mild nasal irritation, dry nose and nasal bleeding may develop in winter when the air is dry.

The onset of the effects of INS is rapid, with efficacy observed in as little as 1 day [79]. Efficacy increases as the treatment period is prolonged. These drugs are effective even in patients with severe allergic rhinitis; their effects are clearly observable, and many patients obtain excellent results. INS are effective for the treatment of nasal obstruction that is unresponsive to H_1-receptor antagonists, for aiding withdrawal from vasoconstrictive nose drops (α-sympathetic stimulants) and for the treatment of vasomotor rhinitis [80].

Oral GC may be used in patients who do not respond to INS (such as those with severe, very severe and intractable allergic rhinitis). Celestamine® (a mixture of H_1-receptor antagonist *d*-chlorpheniramine maleate and betamethasone) is used relatively widely in Japan; however, no placebo-controlled trials have been reported. In addition, evidence regarding a suitable dosage of this drug is lacking. Among oral GC, only methylprednisolone tablets are confirmed as an effective treatment for allergic rhinitis by a placebo-controlled trial; this trial showed that a daily dosage of 24 mg of methylprednisolone was necessary to obtain a significant improvement in all nasal symptoms [81]. Thus, the use of oral GC corresponding to 20–30 mg of prednisolone should be limited to a brief period of time (within 1 week) when treating patients with allergic rhinitis. Caution is needed to avoid adverse effects including adrenal cortical suppression and difficulty in withdrawing GC following prolonged administration (longer than 2 weeks) [82].

Although some physicians use intramuscular injection with depot glucocorticosteroids for the treatment of pollinosis [83], these injections may induce systemic adverse effects. Therefore, a careful examination including the serum cortisol level and blood glucose level should be performed both before and after treatment. Because adverse effects such as moon face, skin/skin appendage disorders, menstrual disorder, application site disorders, including atrophy, and adrenal cortical hypofunction may develop, depot glucocorticosteroids are not recommended for patients with pollinosis [84].

Based on the above observations, glucocorticosteroids are recommended for patients with moderate-to-severe perennial allergic rhinitis (Table 1) and mild-to-severe pollinosis, except for prophylactic treatment (Table 2).

Effect of INS on ocular symptoms in patients with allergic rhinitis

Regarding statements on the mechanisms and efficacy of intranasal glucocorticosteroids, the Japanese guideline (PG-MARJ) has many similarities with Allergic Rhinitis and its Impact on Asthma (ARIA), the evidence-based international guideline for allergic rhinitis [1]. However, there are differences between these two guidelines, such as different conclusions regarding the efficacy of INS for ocular symptoms. According to the PG-MARJ, INS are effective only against nasal symptoms [2]. However, the updated ARIA documented that INS are effective not only for nasal but also ocular symptoms in patients with pollinosis.

Bernstein *et al.* performed a double-blind, double-dummy, randomized study comparing fluticasone propionate aqueous nasal spray 200 μg once daily, oral loratadine 10 mg once daily or placebo for the treatment of seasonal allergic rhinitis and found that fluticasone propionate reduced ocular symptoms, especially ocular itching, tearing and redness, compared with not only placebo but also oral loratadine [85]. More recently, Fokkens *et al.* performed a multi-centre, randomized, double-blind, placebo-controlled, parallel group study of fluticasone furoate 110 μg once daily nasal spray *versus* placebo for the treatment of seasonal allergic rhinitis caused by grass pollen, and they found that fluticasone furoate is significantly effective for not only nasal symptoms and quality of life but also

Table 1. Management for perennial allergic rhinitis in PG–MARJ.

		Moderate		Severe	
Grade type	Mild	Sneeze/discharge type	Congestion type	Sneeze/discharge type	Congestion type
Management	① H1 RA ② CMRI ③ Th2 CS Either ①, ② or ③	① H1 RA ② CMRI ③ Th2 CS ④ INS Either ①, ②, ③ or ④ Combination of ④ with ①, ② or ③	① LT RA ② PGD_2/TXA_2 RA ③ INS Either ①, ②, or ③ Combination of ③ with ① or ②	HIS + H1 RA	INS + LT RA or PGD_2/TXA_2 RA Topical decongestant for 5–7 days at initial treatment if necessary
				Corrective surgery of nasal cavity	
		Allergen-specific immunotherapy			
		Allergen avoidance/elimination			

H1 RA, second generation H1 receptor antagonists; CMRI, chemical mediator release inhibitors; LT RA, leukotriene receptor antagonists; PGD_2/TXA_2 RA, PGD_2/TXA_2 receptor antagonist (ramatroban); T helper type 2 (Th2) C, Th2 cytokine suppressor (suplatast); INS, intranasal glucocorticosteroids.

ocular symptoms including eye itching/burning, eye tearing/watering and eye redness [86]. The efficacy of fluticasone furoate nasal spray against ocular symptoms was also confirmed in patients with ragweed allergy [87].

Although the precise mechanism remains unclear, several explanations regarding the effectiveness of INS drugs for the treatment of ocular symptoms have been proposed. Because of low bioavailability, systemic absorption and circulation is negligible among second-generation INS drugs [75]. The symptoms of itchy and watery eyes and bilateral ocular secretion weights increase after ipsilateral nasal challenge with allergen, suggesting that the ocular symptoms associated with allergic rhinitis arise, in part, from a naso-ocular reflex [88]. The reduced nasal inflammation caused by INS may lead to a normalization or modification of the naso-ocular reflex. In addition, the reduced inflammation in the nose may lessen the release of inflammatory mediators that can cause inflammation in neighbouring tissues including the conjunctiva. Reduction of oedema and inflammation surrounding the opening of the nasolacrimal duct might also reduce the retention of allergen in the conjunctiva.

Effect of INS on impaired performance

Allergic rhinitis itself impairs performance by causing daytime sleepiness and disrupting cognitive functions such as learning ability [89,90]. Nasal congestion due to allergic reaction and inflammation seems to be the major causative

Table 2. Management for pollinosis in PG–MARJ.

			Moderate		Severe	
Grade type	Prophylactic	Mild	Sneeze/discharge type	Congestion type	Sneeze/discharge type	Congestion type
Management	① CMRI ② H1 RA ③ LT RA ④ Th2 CS ⑤ PGD_2/TXA_2 RA Either ①, ②, ③, ④ or ⑤	① H1 RA ② INS Start with ① with eye drops Add ② if necessary	H1 RA + INS	LT RA + INS + H1 RA	INS + H1 RA	INS + LT RA + H1 RA Topical decongestant for 7–10 days at initial treatment if necessary Short-term administration (4–7 days) of oral glucocorticoids may be chosen for patients with extremely severe congestion
		Eye drops of either H1 RA or CMRI			Eye drops of either H1 RA, CMRI or glucocorticoids Corrective surgery of nasal cavity	
		Allergen-specific immunotherapy				
		Allergen avoidance/elimination				

H1 RA, second generation H1 receptor antagonists; CMRI, chemical mediator release inhibitors; LT RA, leukotriene receptor antagonists; PGD_2/TXA_2 RA, PGD_2/TXA_2 receptor antagonist (ramatroban); T helper type 2 (Th2) CS, Th2 cytokine suppressor (suplatast); INS, intranasal glucocorticosteroids.

factor of daytime sleepiness, as this symptom can cause obstructive sleep apnoea and microarousals during sleep [91]. Symptomatic seasonal allergic rhinitis has been associated with significant detrimental effects on examination performance in young people [90].

Treatment with sedating H_1-receptor antagonists exacerbates impaired performance [90,92]; students taking these medications on examination days exhibited a significant tendency to unexpectedly drop a grade [90].

On the other hand, INS can improve impaired performance in allergic rhinitis patients [93,94]. Craig *et al.* [93] showed that intranasal budesonide 128 μg/day, flunisolide 200 μg/day and fluticasone 200 μg/day were each effective in improving sleep and daytime fatigue and somnolence, although significant changes in polysomnography did not always occur. Moreover, treatment with intranasal fluticasone propionate 200 μg once daily significantly improved not only nasal symptoms and daytime sleepiness but also cognitive performance, as measured by the test of variables of attention (TOVA) in patients with seasonal allergic rhinitis [94].

Efficacy of INS for prophylactic (initial) treatment of pollinosis

The PG-MARJ recommends that patients who experience severe symptoms of pollinosis every year should receive prophylactic treatment immediately after the start of pollen release or the onset of symptoms [2,95]. Considering the amount of pollen release expected during the season and the type and severity of symptoms usually experienced by patients during the peak pollen season, physicians should determine the drug regimen for each individual patient by selecting from among chemical mediator–release inhibitors, second-generation H_1-receptor antagonists, leukotriene receptor antagonists, Th2 cytokine inhibitor (suplatast) and PGD_2/TXA_2 receptor antagonist (ramatroban) [2]. Patients with sneezing/rhinorrhoea-type rhinitis should receive chemical mediator–release inhibitors or second-generation anti-histamines, whereas patients with congestion-type disease should be treated with leukotriene receptor antagonist, Th2 cytokine inhibitor or PGD_2/TXA_2 receptor antagonist.

Several reports suggest that INS drugs are effective for the prophylactic treatment of pollinosis. One study of prophylactic treatment with mometasone furoate 200 μg once daily aqueous nasal spray, beclomethasone dipropionate 168 μg b.i.d. aqueous nasal spray or placebo was initiated in patients with ragweed pollinosis 4 weeks before the estimated start of pollen season. Both the proportion of minimal symptom days from start of ragweed season and the number of days from start of ragweed season to first non-minimal symptom day were significantly higher in patients treated with either mometasone furoate or beclomethasone dipropionate compared with placebo [96]. Yokoo [97] compared the efficacy of prophylactic treatment with intranasal fluticasone propionate 200 μg twice daily *versus* the second-generation oral H_1-antagonist olopatadine 10 mg twice daily in patients with Japanese cedar pollinosis and found that fluticasone propionate delayed the onset of nasal symptoms significantly compared with olopatadine. In addition, treatment with fluticasone suppressed symptoms significantly during peak pollen season. Okubo *et al.* [98] reported that initial treatment with fluticasone propionate 100 μg b.i.d. prevented exacerbation of nasal symptoms in paediatric patients with seasonal allergic rhinitis. Indeed, nasal symptoms disappeared in 44·0% of patients who had mild symptoms at initiation of treatment.

As described above, one of the pathways of the anti-inflammatory effect of GC is the down-regulation of proinflammatory genes by several mechanisms such as protein–protein interactions that sequester protein kinase A and cAMP enhancer binding protein (CREB)-binding protein from NF-κB [44,45]. The interaction between NF-κB, CREB and CREB-binding protein leads to the acetylation of chromatin and the subsequent transcription of proinflammatory genes, such as genes encoding cytokines, inflammatory enzymes, adhesion molecules and inflammatory receptors [99]. Thus, GC may be more effective for prophylactic treatment compared with post-onset treatment because increased levels of NF-κB in the nose after the onset of pollinosis can attenuate protein–protein interaction by glucocorticosteroids.

Conclusions

In addition to the novel information that appeared in the sixth edition of the PG-MARJ in 2008, considerable evidence supports the use of GC against allergic rhinitis. GC can induce regulatory cytokines and $FoxP3^+$ T_{regs} in the nose. The appropriate use of INS may improve nasal symptoms, ocular symptoms and impaired performance. Moreover, INS can be used for the first-line prophylactic treatment of pollinosis. These recent findings may provide additional information for incorporation into future editions of guidelines for allergic rhinitis treatment, including the PG-MARJ. On the other hand, several issues remain unsolved. For example, although inhaled GC have not been incriminated as teratogens in humans and are used commonly by pregnant women who have asthma, there are no placebo-controlled, randomized, double-blind studies of INS during the first trimester of pregnancy.

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