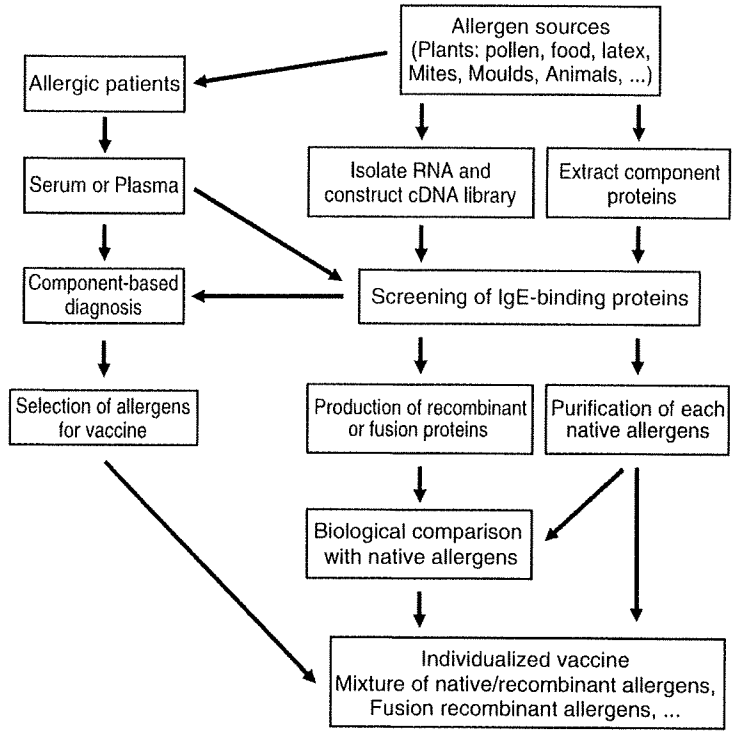

Fig. 1 Schematic procedure of the steps involved in the identification and development of an individualized vaccine using only sensitized antigens for immunotherapy. To identify component allergens which have the capacity to react with serum IgE from allergic patients, it is important to establish individualized vaccines to avoid secondary sensitization. Allergens with which an individual patient reacted can be elucidated by a component-based diagnosis, and an individualized vaccine can be established using a mixture of the purified native or the standardized recombinant allergens to which the patient is sensitized.

ROUTE OF VACCINE ADMINISTRATION FOR IMMUNOTHERAPY AND ITS SAFETY

Immunotherapy vaccines against allergies were originally injected subcutaneously without an adjuvant.[12] However, subcutaneous injection of allergens often induces severe adverse reactions like local allergic reactions, urticaria, asthma, and frequent anaphylaxis. To increase the safety and therapeutic efficacy of immunotherapy vaccines, aqueous allergen extracts absorbed into adjuvants such as aluminum hydroxide have been used in SCIT.[25] Pretreatment with antihistamine or anti-IgE antibody has been used to prevent the adverse events that can be induced after subcutaneous vaccine injection, and the pretreatments also enhance the therapeutic efficacy of SCIT.[26,27]

In this decade, SLIT has been developed as a safer method for immunotherapy and has been used with increasing frequency, especially in Europe and the US. SLIT is noted to be a very safe method without fetal adverse reactions. In most cases, adverse reactions to SLIT have been mild local reactions such as oral pruritus, edema of the mouth, throat irritation, and sneezing.[28] However, a few cases of anaphylaxis have been reported after SLIT using a crude or standardized vaccine.[29-33] These reports suggest that SLIT is not always safe for patients, especially those with severe asthma or who have experienced severe adverse reactions to SCIT. It has been recommended that the first dose of the vaccine is to be administered in a doctor's office under observation.[32]

The administration regimens for SLIT, including dosing, the build-up phase, duration of the treatment, and frequency of the maintenance dose, differ greatly among the clinical trials.[34] The sublingual and supralingual administration methods of oral drops were evaluated by a double-blind, placebo-controlled study using mixed standardized extract in patients allergic to grass pollen. In this report, sublingual administration significantly reduced the nasal, ocular, and bronchial symptoms, as well as the intake of symptom-reducing drugs compared to the placebo. Supralin-

Table 1 Comparison between SLIT and SCIT

	SLIT	SCIT
Administration	Sublingual spitting or sublingual swallowing	Subcutaneous injection with or without adjuvant
Pre-treatment	None	Medication or anti-IgE
Build-up phase	A few weeks, one day for rush protocol, or no up-dosing phase	A few weeks or a few days for rush protocol
Vaccination	Once daily or a few times weekly	A few times weekly or monthly
Adverse event	Local mild reaction in most cases, a few reports of fetal adverse reactions	Sometimes induces fetal adverse reactions

gual treatment also attenuated the symptoms and symptom-reducing drugs intake; however, only the nasal symptom score showed a significant reduction compared to the placebo-control group.[35] Thus, holding the vaccine under the tongue may be an important way to achieve better therapeutic effects with SLIT.

Vaccines for SLIT can also be delivered by two methods: sublingual spitting, in which the vaccine is spat out after being held under the tongue, and sublingual swallowing, in which the vaccine is swallowed after being kept under the tongue. In studies using radiolabeled allergens, most of the allergens remained in the mouth after the vaccine was spat out. However, plasma radioactivity began to increase only after swallowing.[36-38] The author concluded that contact between the allergens and the oral mucosa is a crucial step in the mechanisms of SLIT, and suggested that the more appropriate and advantageous way to administer the allergen sublingually is via the sublingual swallowing procedure.[38]

It has been recommended that the administration of SLIT vaccine be started at least 8 weeks before pollen season for better therapeutic effects.[39] However, an ultra-rush scheme of SLIT treatment for children allergic to grass pollen was reported to significantly improve the symptoms and the medication score compared to the placebo group. In this 2-year randomized, double-blind, placebo-control trial, the authors administered standardized extract of five grass pollen (*Dactylis glomerata*, *Anthoxanthum odoratum*, *Lolium perenne*, *Poa prantensis*, and *Phleum pretense*) beginning 2 weeks before the pollen season started with one day for ultra-rush induction, and followed by daily treatment (120 IR, 10 μg major allergen) for 6 months. It has been reported that SLIT significantly improved the asthma symptom score and reduced the nasal symptom score and the use of rescue medication score compared to the placebo group.[40] The starting point and duration of treatment varied among the clinical trials, and the best procedure for administration remains unclear.[41] (Table 1)

As a novel route to enhance the therapeutic efficacy of the vaccine, direct intralymphatic injection was proposed for the administration of peptide vaccine against viral infection and tumor in the mouse. This paper reported that the direct administration of peptide vaccine into a lymph node induced enhanced immunogenicity compared to subcutaneous and intradermal vaccination.[42] This novel technique was recently applied to patients with hay fever in an open-label, randomized control trial.[43] The authors injected 1,000 SQ-U of aluminum hydroxide-adsorbed grass pollen extract into a superficial inguinal lymph node under ultrasonic guidance. Three intralymphatic injections over 2 months resulted in long-lasting tolerance with the amelioration of hay favor symptoms, reduced skin prick test reactivity, and decreased serum allergen-specific IgE comparable with conventional SCIT. Furthermore, the author reported that there were fewer adverse events than in SCIT, even without premedication with antihistamines, and the injection was less painful than venous puncture.[43] Further clinical trials with a larger population are needed to evaluate the safety, therapeutic efficacy, and duration of tolerance of this treatment.

BIOMARKERS FOR SLIT

The therapeutic effects obtained by antigen-specific immunotherapy are commonly judged on the basis of clinical symptoms according to quality-of-life (QOL) score, symptom diary, and symptom-reducing drugs intake. The biomarkers correlated with the therapeutic effects are still controversial, especially for SLIT.

Antigen-specific IgG4 is considered to be a biomarker for antigen-specific immunotherapy; however, the correlation between the induction of IgG4 production and clinical symptoms is controversial.[44] In a report about the use of SLIT against timothy pollinosis, antigen-specific IgG4 was significantly upregulated in the SLIT group compared to the placebo group, and the authors concluded that the upregulation of IgG4 was correlated with the improvement of symptoms compared with the previous year. However, the clinical score and medication score were not significantly different between the SLIT group and the placebo group.[45] A recent study of dairy administration of grass allergen tablets showed dose-dependent efficacy of the SLIT and the induction of blocking IgG. This report showed that the administration of 75,000 SQ-T (15 μg Phl p 5) dose significantly reduced the symptom and medication

scores, and up-regulated specific IgG; however, a 2,500 SQ-T (0.5 μg Phl p 5) dose did not result in amelioration of the symptom and medication scores nor in the induction of IgG.[46] We previously reported that specific IgG4 was significantly increased in pollen season concomitant with improvement of the symptom medication score in the SLIT group compared to the placebo group.[47] The disagreement in results related to the induction of blocking IgG or IgG4 and the improvement of clinical symptoms may depend on the dose and/or the method of administration of the SLIT vaccine.

Other serological parameters have been recently reported to be useful as therapeutic biomarkers for SLIT. A 3-month course of pre-seasonal treatment of patients with grass pollen allergic rhinitis induced a reduction of the serum level of soluble human leukocyte antigen (sHLA)-G. The authors reported a significant relationship among the decrease of the sHLA-G serum level, the increase of interferon (IFN)-γ producing cells, and the decrease of sHLA-A, -B, and -C after SLIT.[48] Furthermore, the changes of serum sHLA levels were significantly correlated with the clinical symptom score measured using a visual analogue scale (VAS) after SLIT.[49] In this preliminary open-labeled study, the authors suggested that sHLA molecules might be considered as possible biomarkers of the response to SLIT.

Recently, two reports investigated the change of serum reptin levels after SLIT. Leptin is primarily produced by adipocytes and has been reported to protect T lymphocytes from apoptosis, regulate T cell activation, and up-regulate adhesion molecules in endothelial cells.[50] Furthermore, leptin was reported to modulate the hyporesponsiveness and proliferation of human naturally occurring $Foxp3^+CD25^+CD4^+$ regulatory T (nTreg) cells.[51] After a 3-month course of SLIT against pollinosis, serum leptin levels were reported to significantly correlate with symptom severity as assessed by VAS of nasal symptoms in women, the number of peripheral eosinophils in men, the allergen threshold dose for allergen-specific nasal challenge in both men and women, and the medication score in women. This 3-month course of SLIT showed a tendency to increase serum leptin levels compared to the levels before the SLIT, albeit the increase was not significant.[52] After a 2-year course of SLIT, the serum leptin level was significantly increased in men.[53] The relationship between the up-regulation of leptin by SLIT and clinical symptoms remains unclear; however, the difference of the clinical therapeutic efficacy may depend on gender and the presence or absence of obesity.

The reduction of antigen-specific Th2 responses is considered to be an important biomarker for antigen-specific immunotherapy. The increase in the size of the specific Th2 clone, which produces IL4 after being stimulated with Cry j 1 (a major allergen of the Japanese cedar pollen), after pollen season was reported to be significantly reduced in the SLIT group compared with the placebo group in a double-blind, placebo-controlled study of Japanese cedar pollinosis. The increase of specific IL5-producing cells after pollen season was also reduced in the SLIT group, but the reduction was not statistically significant.[47] It has also been reported that after a 2-year course of SCIT against Japanese cedar pollinosis, B and T lymphocyte attenuator (BTLA) expression on $CD4^+$ T cells was down-regulated in untreated patients after Cry j 1 stimulation and up-regulated in SCIT-treated patients. Furthermore, the change of BTLA expression was negatively correlated with IL5 production. The authors concluded that BTLA-mediated coinhibition of IL5 production may contribute to the regulation of allergen-specific T cell responses by antigen-specific immunotherapy.[54]

The therapeutic biomarkers of SLIT in children also remain unclear. In a study of the administration of the SLIT treatment to children with seasonal allergic rhinoconjunctivitis to grass pollen, the authors reported that a 2-year course of SLIT using a standardized 5-grass mixture (1.5 μg/week) did not alter the systemic immunologic reaction of IL4, IL5, and IFN-γ cytokine production, nor the proliferation of PBMC after stimulation with allergens in the SLIT group compared to the placebo group, although a positive effect on rescue medication use was achieved by SLIT treatment.[55] However, another study reported the up-regulation of mRNA expression in PBMC during SLIT in children using SQ-standardized tree pollen extracts. The authors reported that after the stimulation of PBMC with allergen *in vitro*, the mRNA expression of signaling lymphocytic activation molecule (SLAM) was significantly increased from baseline after 1 year in the SLIT group receiving a high-dose (weekly dose of 200,000 SQ-U) treatment. This up-regulation was reported to be correlated with IL10 and transforming growth factor-β (TGF-β) mRNA expression. The IL18 mRNA expression was also increased in the high-dose group over a 1-year treatment compared to the placebo group and was reported to be inversely correlated with the late-phase skin reaction after the second study year. The authors reported that this up-regulation of SLAM and IL18 mRNA expression suggested the down-regulation of Th2-type inflammatory responses by increased Th1-type responses.[56] Another study of SLIT in children using SQ-standardized tree pollen extract (weekly dose of 200,000 SQ-T, 30 μg major allergen containing Bet v 1, Aln g 1, and Cor a 1) reported that specific allergen-induced Foxp3 mRNA expression after a 2-year course of SLIT treatment was significantly increased in PBMCs compared to the placebo group and compared to the level before treatment. Changes in allergen-induced Foxp3 expression that significantly correlated with IL10 mRNA expression

were reported in the whole study group, including the low-dose (weekly dose of 24,000 SQ-T) group and the placebo group, after 1- and 2-year courses of treatment, and correlated with TGF-β1 mRNA after 1 year of treatment. Furthermore, IL17A mRNA expression was significantly correlated with symptom-medication score (SMS) in the whole study group and especially in the high-dose treated group. The authors concluded that IL17 expression may be associated with a poor therapeutic outcome of SLIT.[57]

MECHANISMS OF ANTIGEN-SPECIFIC IMMUNOTHERAPY

Numerous data showing that antigen-specific Th2-type responses are down-regulated and, in contrast, Th1-type and/or regulatory T cell (Treg) responses are up-regulated by immunotherapy have been accumulated. The imbalance of the population among the antigen-specific Th1, dominant Th2, and Treg is considered to induce sensitization and subsequent allergic inflammation in response to invading allergens, and immunotherapy may correct the imbalance of these cells. Actually, the high frequency of IL4-secreting Th2 cells was reported in allergic individuals, as was, in contrast, the dominance of IL10-secreting Tr1 cells in healthy subjects.[58] These authors suggested that the balance between allergen-specific Tr1 cells and Th2 cells causes the development of the allergy.

IL10-producing regulatory cells are considered to play a crucial role in clinical therapeutic mechanisms in immunotherapy. In a study of SCIT using house dust mite (HDM) extract in patients allergic to HDM, SCIT induced the suppression of PBMC proliferation and the suppression of IFN-γ, IL5, and IL13 production in PBMC stimulated with Der p 1 (a major allergen of HDM) at 70 days after treatment compared to the levels before treatment. In contrast to the suppression of Th1 and Th2 cytokines, the production of both IL10 and TGF-β was significantly increased. The report also showed that the suppression of proliferation was dependent on IL10 and TGF-β and that the source of IL10 is $CD25^+CD4^+$ T cells.[59] It has also been reported that IL10 production was induced by SLIT against HDM. The authors also reported the suppression of the proliferation of PBMC stimulated with extract of mite (*Dermatophagoids farinae*) and the increase of IL10 production compared to non-treated subjects.[60] The IL10 production after 3 years of SLIT treatment was significantly correlated with the improvement of clinical symptoms as assessed by forced expiratory flow between 25% and 75% (FEF_{25-75}).[61]

In a report about the use of SLIT to treat birch pollinosis, the authors investigated the antigen-specific proliferation and mRNA levels of cytokines and Foxp3. They reported that 4 weeks of SLIT induced a reduction in Bet v 1-specific proliferation and induced mRNA expression of IL10 and Foxp3 in $CD3^+$ cells compared to the levels before SLIT. These up-regulations of IL10 and Foxp3 mRNA expression were not seen after 52 weeks after SLIT; however, IFN-γ mRNA expression was significantly induced at 52 weeks after SLIT. The reduced Bet v 1-specific proliferation was significant after both 4 and 52 weeks, and this down-regulation was dependent on IL10 at 4 weeks. It has also been reported that neither TGF-β levels nor cell-cell contact-mediated suppression of $CD25^+CD4^+$ cells were changed during the course of SLIT.[62] Another report shows the significant reduction of IL5 mRNA expression and increased IL10 expression compared to the placebo group after 1 and 2 years of SLIT at a weekly dose of 200,000 SQ-U (30 μg major allergen) in children with tree pollinosis. It has been reported that TGF-β expression remained low after 1 and 2 years of SLIT; however, TGF-β expression was inversely correlated with IL5 and positively correlated with IL10 expression after 1 year of SLIT.[63]

In addition to IL10-secreting Tr1 cells, $Foxp3^+$ Treg cells are also considered to play a crucial role in the therapeutic effects achieved by immunotherapy (Fig. 2). It has been reported that 2 years of SCIT against hay fever significantly induced an increase in the number of $Foxp3^+CD25^+$ and $Foxp3^+CD4^+$ cells in the nasal mucosa compared to the number before SCIT and the number in untreated patients out of season. Twenty per cent of $CD3^+CD25^+$ cells were reported to also be Foxp3-positive, and 18% of $CD3^+$IL10-expressing cells were Foxp3-positive in the nasal mucosa after immunotherapy. This report suggested that the increase of $Foxp3^+CD25^+CD3^+$ cells in the nasal mucosa was associated with the clinical efficacy and suppression of seasonal allergic inflammation. This report also suggested the involvement of different types of regulatory T cells, namely IL10-secreting Tr1 cells and adaptive or induced Foxp3-positive Treg, in the therapeutic mechanisms of immunotherapy.[64] The involvement of Treg cells in immunotherapy was also reported in SCIT against hymenoptera venom allergy. In this report, the authors showed that the numbers of peripheral Treg cells defined as $Foxp3^+CD25^{bright}CD4^+$ T cells were significantly increased by venom immunotherapy, and the increase of circulating Treg cells was significantly correlated with the venom specific IgG4/IgE ratio.[65]

Antigen-specific Tr1 and Treg cells are considered to be involved not only in the suppression of Th2 cells but also, directly or indirectly, in the suppression of peripheral allergic inflammation[24] (Fig. 3). It has been reported that $CD25^+CD4^+$ Treg cells, more than 90% of which are $Foxp3^+$, directly inhibited the FcεR1-dependent mast cell degranulation after crosslinking of IgE, and this inhibition was dependent on cell-cell contact involving OX40-OX40L interactions between Treg and mast cells in the mouse.[66] Furthermore, al-

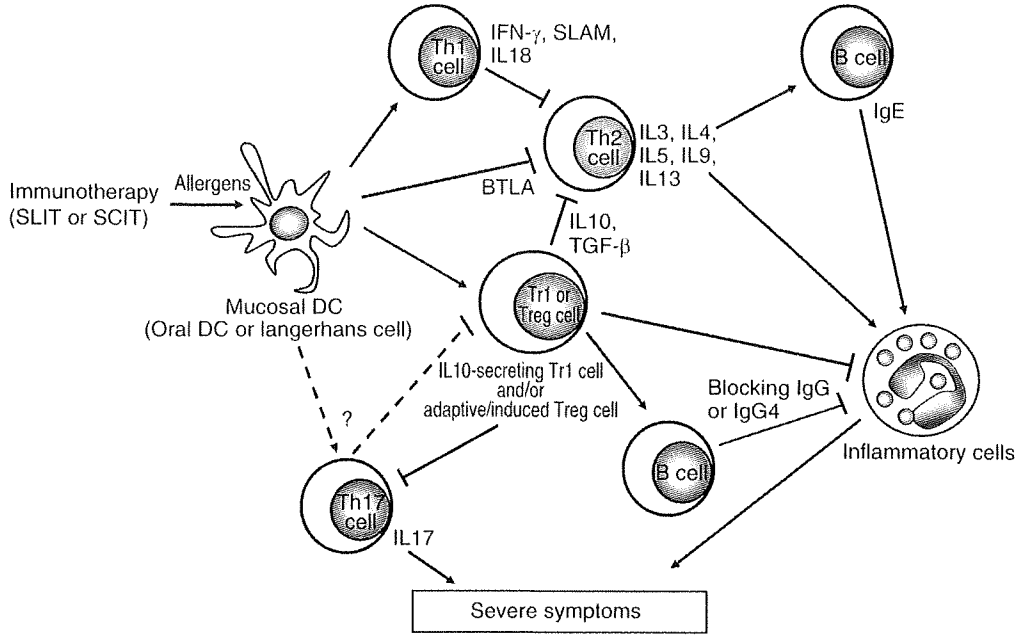

Fig. 2 T cells in antigen-specific immunotherapy. Antigen-specific immunotherapy induces regulatory T cells and Th1 cells via antigen-presentation by mucosal dendritic cells (DC). Th17 cells may be induced in a non-responder population by immunotherapy. The induced Th1 cells and/or regulatory T cells down-regulate the activation of Th2 cells and subsequently the activation of inflammatory cells such as eosinophils and mast cells. The regulatory T cells also activate B cells to produce blocking IgG or IgG4, and the blocking antibody inhibits binding between allergen and surface IgE on inflammatory cells to prevent the secretion of inflammatory chemical mediators.

lergic human eosinophils in peripheral blood and chronically inflamed nasal tissues were reported to express CD40, and the cross-linking of CD40 and CD40L enhanced the survival of eosinophils and induced the release of granulocyte/macrophage colony-stimulating factor (GM-CSF). In this report, IL10 down-regulated the constitutive expression of CD40 mRNA expression in eosinophils.[67] The induction of IL10-producing Tr1 or Treg cells in the nasal mucosa may play an important role in the reduction of nasal symptoms via cross-talk down-regulation of mast cells and eosinophils.

In a reports on the rush protocol of SCIT against Japanese cedar pollinosis using standardized pollen extract, the percentage of $CD203c^{high}$ cells in $CD3^-$ $CRTH2^+$ basophils after allergen stimulation was reported to be down-regulated after rush immunotherapy without a decrease of the serum specific IgE titer. Furthermore, the percentage of $CD203c^{high}$ on basophils after *in vitro* stimulation was reported to be significantly correlated with symptom score.[68] The mechanisms which attenuate the sensitivity of peripheral basophils without a change in serum specific IgE remain unclear; however, this attenuation may be partially due to the up-regulation of inhibitory blocking antibody on the surface of basophils.

ANTIGEN-SPECIFIC IMMUNOTHERAPY AGAINST JAPANESE CEDAR POLLINOSIS

In Japan, Japanese cedar pollinosis is one of the most prevalent types of seasonal allergic rhinitis, with a prevalence estimated to be 26.5%.[2] Two clinical trials described the therapeutic effects of SLIT against Japanese cedar pollinosis.[47,69] In both trials, standardized Japanese cedar pollen extract was used at a monthly cumulative dose of 8,000 JAU, which contains approximately 10 μg of Cry j 1. This dosage is less than that reported in Europe, where a dose of 75,000 SQ-T (15 μg of a major grass allergen Phl p 5) was administered once daily for 18 weeks.[46] Unless the monthly cumulative dose is approximately $1/40^{th}$ of the amount required to be considered a major allergen (10/450 μg as a major allergen) in Japan, SLIT with an active treatment group against Japanese cedar pollinosis is still effective for improving quality of life and significantly ameliorates patients' SMS and symptom score during the pollen season. The up-regulation of the IL4-producing clone size specific to epitopes from Cry j 1 and Cry j 2[70] was reported to be significantly attenuated, and Cry j 1-specific IgG4 production was also significantly induced by active SLIT.[47] Furthermore, IL10-producing Tr1 cells were

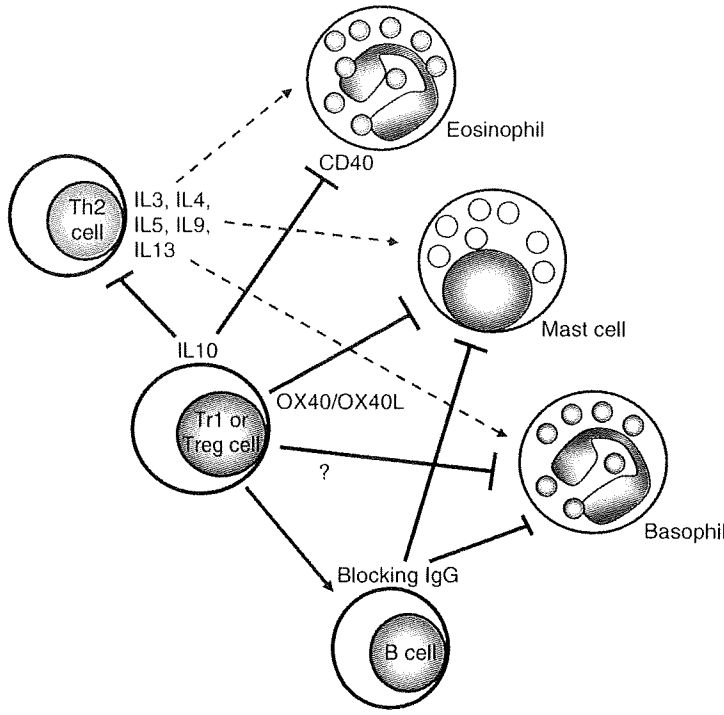

Fig. 3 Proposed roles of regulatory T cells on inflammatory cells in allergen-specific immunotherapy. Regulatory T cells, namely IL10-secreting Tr1 cells or adaptive/induced Treg cells, down-regulate inflammatory cells, directly or indirectly. Regulatory T cells down-regulate the activation of Th2 cells and subsequently Th2-type cytokine secretion. Regulatory T cells suppress the activation of inflammatory cells directly via their surface molecules and by secreting cytokines, and indirectly via the down-regulation of cytokine production in Th2 cells and by the activation of B cells to produce blocking IgG.

reported to be significantly increased in patients treated with SLIT compared with the levels in untreated patients and healthy subjects, and the proliferation of $CD4^+$ leukocytes stimulated with Cry j 1 and Cry j 2 was significantly suppressed by SLIT treatment in an IL10-dependent manner.[71] Supplementation with recombinant or native Cry j-allergens and/or updosing of the extract by bio-engineering may lead to more effective SLIT for treating pollinosis.

Another approach to safer immunotherapy is the use of oral immunotherapy using transgenic rice seed accumulating Cry j 1.[72] The generated transgenic rice plants expressed recombinant, structurally disrupted Cry j 1 peptides but spanned the entire Cry j 1 region as fusion proteins with the major rice storage protein glutenin. These fusion proteins aggregated with cysteine-rich prolamin and were deposited in endoplasmic reticulum-derived protein body I in rice seed. Transgenic rice expressing T cell epitopes from Cry j 1 and Cry j 2 successfully suppressed antigen-specific Th2-mediated IgE responses in a mouse model of allergic rhinitis.[73] Further clinical trials are needed to develop a rice-based edible vaccine as a tool for oral immunotherapy to control allergies.

An immunoregulatory liposome encapsulating the recombinant fusion protein of Cry j 1-Cry j 2 was manufactured as a novel vaccine for Japanese cedar pollinosis without risk of anaphylaxis.[74] The hybrid fusion allergen is expected to provide safer and more effective vaccines for immunotherapy. Vaccines using only T cell epitopes are also safer than native allergens, but there is wide variation among individual T cell epitopes. The fusion protein of major allergens covers all sequential T cell epitopes but is expected to have less IgE-binding capacity because its three-dimensional structure is disrupted in some B cell epitopes. Recombinant hybrid molecules using major allergens of timothy grass pollen induced stronger proliferation of PBMC in timothy-allergic patients than did mixtures of corresponding allergens, but still possess IgE-binding capacity and induce IgG production in sensitized mice.[75] In a mouse model sensitized with native Cry j 1 and Cry j 2, the vaccine that con-

tained Cry j 1-Cry j 2 fusion protein in the immunoregulatory liposome showed suppression of IgE and IgG antibody responses after being challenged with the allergens. Furthermore, oral administration of the vaccine showed efficient suppression of IgE antibody production.[74]

CONCLUSIONS

The standardization of a vaccine enables us to compare the results from varied clinical trials with respect to dose, clinical effects, and changes in biological parameters. Many reports have shown positive clinical therapeutic effects and suppressed effector/inflammatory responses. It is considered that IL10-producing Tr1 and/or adaptive or induced Treg cells may be involved in the suppression of the antigen-specific Th2-responses and local inflammation. However, how immunotherapy induces suppressor cells like Tr1 and Treg cells remains unclear, although the involvement of mucosal dendritic cells has been proposed. High-quality clinical studies are indispensable to clarify the therapeutic biomarkers and the mechanisms of induction of suppressor cells, and the resultant data from the studies may enable us to develop safer and more effective immunotherapy through the modification of the allergens, optimum dose, or administration regimen of a vaccine.

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CD14 and *IL4R* gene polymorphisms modify the effect of day care attendance on serum IgE levels

To the Editor:

The cause of atopy is generally traced to the interplay of genetic and environmental factors.[1] Day care appears to be one of the most frequently investigated environmental factors. Although the results of studies investigating the association between day care attendance and atopy, as assessed by skin prick test responses, were inconsistent, all studies[2-4] measuring serum IgE levels have thus far shown a constant decreasing effect on serum IgE levels.

Among the genes that show a gene-environment interaction for the development of atopy or allergic diseases, the most frequently investigated is the *CD14* gene.[1] However, there is no report that investigates interaction of this gene and day care attendance. CD14 is a pattern-recognition receptor involved in the clearance of bacterial endotoxin and is also known as a receptor of respiratory syncytial virus. We investigated *CD14*−159C/T (rs2569190) and *CD14*−550C/T (rs5744455) polymorphisms in Japanese patients with severe respiratory syncytial virus–induced bronchiolitis and found that *CD14*−550C/T but not *CD14*−159C/T was significantly associated with the condition.[5]

The IL-4 receptor α gene *(IL4R)* is also one of the most frequently investigated genes and has been shown to be associated with atopy and atopic diseases.[6] The Ile50Val polymorphism (rs1805010) of the *IL4R* gene is a functional polymorphism and has been reported to be strongly associated with atopy and atopic asthma in the Japanese population. To date, only one study has reported the interaction of the *IL4R* Ile50Val polymorphism and day care attendance in the first year of life.[7] The result showed a

TABLE I. Characteristics of the subjects

Total no. of participants	473
Age (mo)	
Mean ± SD	111.1 ± 19.9
Range	76-147
Sex ratio (male:female)	1.00:1.01
Day care attendance before age 2 y (%)	14.5
Total IgE (IU/mL), mean ± SD	
Male	254 ± 340
Female	241 ± 469
Prevalence of atopy (%)	
Male	76.9
Female	68.0
Prevalence of allergic disorders (%)	
Asthma	
Male	14.1
Female	6.6
Atopic dermatitis	
Male	11.5
Female	9.7
Allergic rhinitis	
Male	42.1
Female	31.2
Food allergy	
Male	3.0
Female	3.4

significant gene-environment interaction for IFN-γ production at 1 year of age. However, it is not known whether this modified cytokine response affects the chance of having atopy or allergic diseases in the later period of life.

Here we report a relationship between serum total and specific IgE levels in Japanese elementary school children and day care attendance during earlier life. Our results suggest that day care attendance is associated with serum IgE levels, and this effect is modified by *CD14*−550C/T and *IL4R* Ile50Val polymorphisms. This is the first report that suggests an interaction between early-life day care attendance and genetic variations on IgE levels in later life.

Children attending an elementary school located in the central area of Chiba city (population of approximately 930,000) were recruited for this study. We first asked all (n = 843) children for participate in the survey. We then sent a detailed questionnaire to those who had a positive response (n = 582). Children with congenital heart diseases and lung diseases caused by immature birth were excluded. A total of 473 school children aged 6 to 12 years were enrolled. Blood samples were collected from 411 children on 2 separate days (July 3 and 12, 2006) for serum and DNA preparation. A complete set of information on total and 8 specific IgE levels, genotypes, and environmental factors was obtained from 375 children. All parents provided written informed consent. The study protocol was approved by the Ethics Committee of Chiba University Graduate School of Medicine.

The status of allergic diseases was evaluated by using questions based on the International Study of Asthma and Allergies in Childhood. We asked whether the child regularly attends a day care center where time is spent with other children at or before 2 years of age. For parents who responded yes to this question, the age of entry of their child to the day care center was obtained. The questionnaire also included the following items to assess possible confounding factors: number of siblings; number of older siblings; allergic diseases of parents and siblings (family history: scored as positive if parents, siblings, or both had any of 4 allergic diseases [asthma, allergic rhinitis, atopic eczema, and food allergy]); residential area (6 categories), type of house structure (5 categories), and floor type of bedroom (5 categories); yogurt/fermented food consumption; pet ownership; and smoking among family members.

Genotyping of the *CD14*−550C/T polymorphism was performed as described previously,[5] whereas that of the *IL4R* Ile50Val (rs1805010) polymorphism was carried out with the TaqMan allele-specific PCR method.[8] Primer sequences were as shown in this article's Online Repository at www.jacionline.org.

Table I shows the characteristics of the investigated population. The percentage of children who had regularly attended day care before 2 years of age was 14.5%. Atopy was defined as the presence of positive (≥0.35 IU/mL) specific IgE level against at least 1 of the 8 allergens. Although the prevalences of asthma, atopic dermatitis, and food allergy were compatible with those in a recent large study,[9] prevalences of allergic rhinitis and atopy were about 10 to 20 points higher, suggesting that children who had allergic rhinitis were more likely to attend this study.

Table II shows the association between day care attendance and serum IgE levels or atopy after being stratified with the *CD14*−550C/T genotype. Day care significantly decreased total IgE levels ($P = 9.7 \times 10^{-5}$), mite-specific IgE levels ($P = .0016$), and rate of atopy ($P = .00041$) in individuals with the C/T or T/T genotype, whereas the effect of day care was not observed in those with the C/C genotype. Numbers of children with the C/T+T/T genotype and those with the C/C genotype were similar, suggesting that the difference is not likely due to the statistical power for detecting association. Multivariate analyses with confounding factors were performed to evaluate the significance of this gene-environment interaction. The interaction between the *CD14*−550C/T polymorphism and day care was significant for $\log_{10}$(total IgE) ($P = .0046$), mite-specific IgE classes ($P = .00047$), and atopy ($P = .0097$) after adjusting for age, sex, family history, and number of siblings.

Table III shows the association between day care attendance and serum IgE levels or atopy after being stratified with the *IL4R* Val50Ile genotype. The effects of day care on total and some specific IgE levels were significant in Val/Ile heterozygotes but not in Val/Val or Ile/Ile homozygotes. In Val/Ile individuals day care significantly decreased total IgE levels ($P = .0012$), mite-specific ($P = .011$) and cedar pollen–specific ($P = .034$) IgE levels, and rate of atopy ($P = .018$). No such trend was observed in Val/Val or Ile/Ile individuals. The numbers of Val/Val and Val/Ile individuals were similar. It is therefore unlikely that the lack of significant association in Val/Val individuals was due to smaller statistical power for detecting association. When the significance of gene-environment interaction was assessed with the confounding factors, the interaction term between *IL4R* and day care attendance was significant for $\log_{10}$(total IgE) ($P = .019$) and mite-specific ($P = .0025$) and cedar pollen–specific ($P = .040$) IgE classes but not for atopy.

Total IgE levels in 4 genotype groups (group 1: *CD14* C/C, *IL4R* Ile/Ile+Val/Val; group 2: *CD14* C/C, *IL4R* Val/Ile; group 3: *CD14* C/T+T/T, *IL4R* Ile/Ile+Val/Val; and group 4: *CD14* C/T+T/T, *IL4R* Val/Ile) were compared to evaluate the combined effect of 2 polymorphisms on total IgE levels. Fig 1 shows the box

TABLE II. Effects of day care attendance on IgE levels when stratified by *CD14*–550C/T genotype

	C/C				C/T + T/T				
	Day care attendance		Effect size or odds ratio (95% CI)	*P* value	Day care attendance		Effect size or odds ratio (95% CI)	*P* value	Gene-environment interaction *P* value*
	No	Yes			No	Yes			
No. of subjects	169	22			157	28			
Log_{10}(total IgE)									
Mean	1.88	1.98	0.094 (−0.21 to 0.39)¶	0.54†	2.09	1.58	−0.50 (−0.26 to −0.76)¶	**9.7×10^{-5}†**	**.0046****
SD	0.77	0.76			0.63	0.51			
Specific IgE (positive‡ rate)									
Mite	0.49	0.59	1.50 (0.61 to 3.69)#	.51§	0.61	0.32	0.30 (0.13 to 0.71)#	**.0016§**	**.00047††**
Cedar pollen	0.45	0.46	1.02 (0.42 to 2.45)#	.92§	0.57	0.32	0.35 (0.15 to 0.83)#	**.032§**	.116††
Atopy (rate)	0.77	0.68	1.60 (0.56 to 4.55)#	.38‖	0.81	0.50	0.24 (0.10 to 0.55)#	**.00041‖**	**.0097‡‡**

Boldface indicates statistically significant values.
*Adjusted for age, sex, number of siblings, and family history.
†Analysis of variance for log_{10}(total IgE [in international units per milliliter]).
‡Class ≥1 (≥0.35 IU/mL).
§Kruskal-Wallis test for IgE value (in international units per milliliter).
‖χ^2 Test of independence.
¶Effect size.
#Odds ratio.
**General liner model.
††Generalized linear model (Poisson distribution, log link function).
‡‡Logistic regression.

TABLE III. Effects of day care attendance on IgE levels when stratified by *IL4R* Val50Ile genotype

	Val/Val				Val/Ile				Ile/Ile				
	Day care attendance		Effect size or odds ratio (95% CI)	*P* value	Day care attendance		Effect size or odds ratio (95% CI)	*P* value	Day care attendance		Effect size of odds ratio (95% CI)	*P* value	Gene-environment interaction *P* value*
	No	Yes			No	Yes			No	Yes			
No. of subjects	125	18			152	27			49	5			
Log_{10}(total IgE)													
Mean	1.94	1.91	−0.058 (−0.38 to 0.27)¶	.72†	1.88	1.55	−0.44 (−0.71 to −0.18)¶	**.0012†**	1.99	2.32	0.33 (−0.31 to 0.97)¶	12†	**.019****
SD	0.64	0.72			0.57	0.56			0.69	0.52			
Specific IgE (positive‡ rate)													
Mite	0.57	0.56	0.95 (0.35 to 2.57)#	.51§	0.52	0.30	0.39 (0.16 to 0.94)#	**.011§**	0.59	0.80	2.76 (0.29 to 26.5)#	.36§	**.0025††**
Cedar pollen	0.50	0.50	1.01 (0.38 to 2.73)#	.93§	0.51	0.30	0.41 (0.17 to 0.99)#	**.034§**	0.55	0.40	0.54 (0.083 to 3.54)#	.91§	**.040††**
Atopy (rate)	0.74	0.72	0.93 (0.31 to 2.82)#	.90‖	0.74	0.52	0.37 (0.16 to 0.86)#	**.018‖**	0.76	0.80	1.30 (0.13 to 12.8)#	.82‖	.118‡‡

Boldface indicates statistically significant values.
*Adjusted for age, sex, number of siblings, and family history.
†Analysis of variance for log_{10}(total IgE [in international units per milliliter]).
‡Class ≥1 (≥0.35 IU/mL).
§Kruskal-Wallis test for IgE value (in international units per milliliter).
‖χ^2 Test of independence.
¶Effect size.
#Odds ratio.
**General liner model.
††Generalized linear model (Poisson distribution, log link function).
‡‡Logistic regression.

plot of log_{10}(total IgE) in 4 genotype groups. Among children who attended day care compared with group 1, the mean log_{10} (total IgE) values of groups 2, 3, and 4 decreased by 0.41, 0.35, and 0.69, respectively. This magnitude of change suggests that the effects of *CD14* and *IL4R* were additive. The children in group 4 showed significantly (P = .0046) lower total IgE levels than those in group 1. On the other hand, among children who did not attend day care, the log_{10}(total IgE) levels of children in groups 3 (P = .031) and 4 (P = .036) were significantly higher than those of children in group 1. The *CD14* C/T and T/T genotypes appeared to show the opposite effect on the serum total IgE level in children who did not attend day care compared

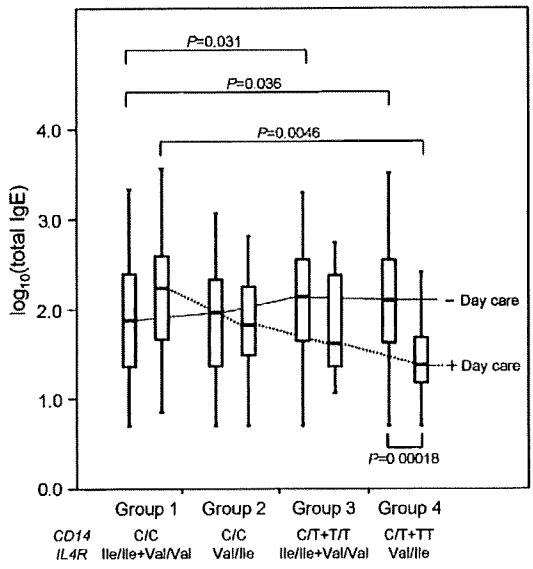

FIG 1. Total IgE levels in 4 groups of children classified based on a combination of *IL4R* and *CD14* genotypes. Box plot of $\log_{10}$(total IgE) values is shown for children who attended day care *(+Day care)* and for those who did not *(−Day care)*. Results are presented as medians and interquartile ranges. Only significant *P* values (<.05) are shown.

with those who did attend day care. When we examined the effect of day care in each genotype group, the effect was not sufficiently large to show a significant change in IgE level in groups 2 and 3, in which individuals had only 1 IgE level–decreasing genotype. However, in group 4, in which individuals had 2 IgE level–decreasing genotypes, the effect was sufficiently large to show a significant difference ($P = .00018$). Significance of interaction between the *CD14* and *IL4R* genotypes was also evaluated by using general linear models in which age, sex, family history, number of siblings, and day care were included as variables. The interaction term of the 2 genes was not significant, suggesting an independent effect of the *CD14* and *IL4R* genes.

The interaction of the *CD14* gene with day care attendance suggests that the mechanism of the effect of day care involves at least in part a response to infection, environmental endotoxin exposure, or both. The interaction of the *IL4R* gene with day care attendance suggests that the mechanism also involves those related to T_H2 cell proliferation and IgE production. These results suggest that the complex nature of mechanisms underlies the effect of day care attendance on serum IgE levels.

Environmental factors investigated in the present study were determined based on a questionnaire on past day care attendance, and therefore recall bias can be a potential problem. The number of subjects investigated in this study was not so large and might be the acceptable minimum for investigating gene-environment interactions. The subjects evaluated were children who attended a single school and lived in a medium-populated city, thus representing those living in rather small regional environments in Japan. Nevertheless, these characteristics of the present sample might have contributed to minimizing the variances of background and outcome parameters and might have resulted in the positive findings obtained from a relatively small number of subjects. It is necessary to perform a cohort study to follow children with or without day care attendance until they reach school age to validate the current observations.

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3. 抗原特異的免疫療法の現状とその作用機序

岡本美孝

患者の著しい増加がみられるアレルギー性鼻炎であるが，自然改善は少なく，仕事，学業や睡眠の障害など患者の日常生活への影響も大きい．現在，唯一アレルギー性鼻炎の自然経過を改善し，根本治療になり得る治療として抗原特異的免疫療法が用いられているが，従来の皮下注射による抗原エキスの投与は頻回な通院，稀とはいえ重い副作用発現の危惧から患者負担が大きい．抗原エキスの舌下投与は患者負担を大きく軽減する方法として期待され，欧州では多くのランダム化試験から有効性が確認され用いられている．日本特有のスギ花粉症に対しても舌下投与による抗原特異的免疫療法が期待されるが，本稿ではその有効性に対する検討の現状，さらにその作用機序に対する検討の現状について解説する．

はじめに

抗原特異的免疫療法（減感作療法）は，1911年に花粉エキスを用いての報告以来，長い歴史を有する．欧米ではいくつものランダム化比較試験が実施され，その有効性は確立されている．現在，唯一根本治療となることが期待される治療法であり，完全な寛解に至らなくても高い割合で症状の改善効果が期待され，また少なくとも治療後数年はその効果が持続することがエビデンスとして確立している[1]．

抗原特異的免疫療法は，国内ではアレルギー性鼻炎を中心に治療に用いられてきた．アレルギー性鼻炎は現在も患者数の増加が目立っている．昨年の全国調査の報告では罹患率が全国民の40％近くに達し，特にスギ花粉症の増加が目立っており，26.5％の有病率と10年前の調査に比較して10％以上の増加が報告されている[2]．アレルギー性鼻炎は直接死に至る疾患ではないが，このような患者数の増加と，睡眠，就業，学業への影響から患者のQOL障害が強いこと，さらに青壮年では自然改善が非常に少ないことから根本的な対応が望まれており，免疫治療に対する期待は大きい．ただ，最近は喘息，さらに食物アレルギーに対しても抗原特異的免疫療法の意義が注目され見直されている．

[キーワード&略語]
スギ花粉症，抗原特異的免疫療法，舌下投与，抗原特異的Th2細胞，制御性T細胞

ITIMs：intracellular immunoreceptor tyrosine-based inhibition motifs
PHA：phytohemagglutinin

1 抗原特異的免疫療法（減感作療法）の現状

これまで抗原特異的免疫療法は皮下投与法で行われてきている．確かに欧米でのプラセボを対照とした比較試験での症状の改善効果は認められ[1)3)]，かつ臨床

Concurrent situation of antigen specific immunotherapy and its mechanisms
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効果は投与終了後も少なくとも数年は持続することが確認されていて，小児期のアレルギー性鼻炎に対する免疫治療はその後の喘息発症の抑制，ほかの抗原感作の獲得も抑制することが報告されている[1]．このように従来の皮下注射による免疫治療の意義は評価されているものの，一方で2年以上の治療期間が必要で注射での投与のためその間50回以上の通院をする必要がある．また，頻度は少ないとはいえ副作用がみられ，喘息発作は1,000～2,000回に1回，重篤な致命傷にもなる全身アナフィラキシーは200万回の注射で1回生ずるとされている[5]．実際の投与にあたっては，注射後30分は医師の監視下に置き，ショックなどの反応出現に備えておく必要がある．この患者負担の大きさから，免疫療法は有効性が示され，かつ国内外のガイドラインで推奨されているのにもかかわらず，実際には実施する医療機関や受ける患者は減り続けている．

2 舌下粘膜投与による抗原特異的免疫療法の現状

従来の抗原の皮下注射による抗原特異的免疫療法に替わる方法として，抗原の粘膜投与が期待されている．経口，経鼻免疫といった方法も検討されてきたが，最も注目されているのは舌下免疫療法である．舌裏面に抗原の保存をはかり，口腔底粘膜を利用した粘膜投与であり，抗原をパンに染み込ませたり，専用の保持材料も開発されている．医師の指導下ではあるが，自宅での投与が可能であり，重篤な副作用の減少から患者の負担が著しく軽減されるものとして注目されている．舌下免疫療法に関する研究は，これまでヨーロッパで主に行われ，100を超える臨床試験の報告がみられ，特に南ヨーロッパではすでに治療として広く認められている．英国ではチモシーに対して認可され，米国でもFDAに登録した36の臨床試験が進行中とされ，関心は非常に高い．

投与量は従来の皮下投与に用いられた抗原量の0.017～500倍の濃度で検討が行われている[6]～[8]．また，投与回数についても連日から週1回，週3回とさまざまな方法で行われている．投与量を比較したランダム化試験は少ないが，いずれも高い濃度での有効性を示している．投与回数，投与期間の比較を行った報告も少ないが，ダニ抗原（Dep 1）1日1回1滴（6 μg）連日投与と1回5滴週3回とを比較した報告では連日投与の有効性を認めている．最近は連日投与の安全性，有効性を示したものが多い[9][10]．

一方，投与期間についても2カ月から5年とさまざまであり，投与期間の比較を行った検討では小児ダニアレルギー性鼻炎および喘息合併例を対象としたオープン試験で，6カ月投与で改善なく，12カ月投与でいずれの疾患にも有効性を示した，という報告や，やはりオープン試験で3年間の投与で確認されたというもの[11]，ランダム化試験では2年以上の投与が必要といったものがある．投与期間については特に花粉症では花粉飛散前投与，飛散中投与，飛散前ならびに飛散中投与，通年投与とさまざまな投与法での検討が報告されている．

さらに，実際の舌下免疫療法では2～3分間舌裏面に含んだ後に吐き出すspit法と，飲み込んでしまうswallow法がある．抗原の有効利用といった面からはswallow法が望ましいといった指摘もあるが[12]，有効性の違いの比較検討は行われていない．

このように舌下免疫療法についてはさまざまな抗原濃度，投与回数，投与期間，投与形態で検討が行われているが，試験内容の違いからこれらの試験のメタ解析が容易ではない．ただ，最近の大規模な臨床試験の結果から[13][14]，投与期間は長く，花粉症については飛散開始の少なくとも8週間前から，抗原投与量は高濃度での連日投与が推奨されている．

舌下免疫療法の副作用の多くは口内の軽度の違和感や腫脹で治療も必要とせず，治療継続可能であるが，なかには舌下免疫療法との関連が示唆される重篤な副作用が報告され[15]，喘息発作，腹痛，嘔吐，口内腫脹，全身の蕁麻疹などで，喘息の1例は入院治療を必要としたとされる．また，最近アナフィラキシー発症の報告もある[16]．正確な副作用の頻度は不明であるが，全身的な副作用は1万回の投与で6回程度の頻度とされる．

ただ，重症喘息患者を対象にした検討は十分行われておらず，適応は現在のところ難しい．

3 抗原特異的免疫療法の作用機序

皮下注射による抗原特異的免疫療法での検討では，血清中抗原特異的IgE抗体は抗原曝露を受けても徐々に増加しなくなり，長期的には減少するとした報告が多い．皮膚テストは免疫療法の実施中は反応が低下し

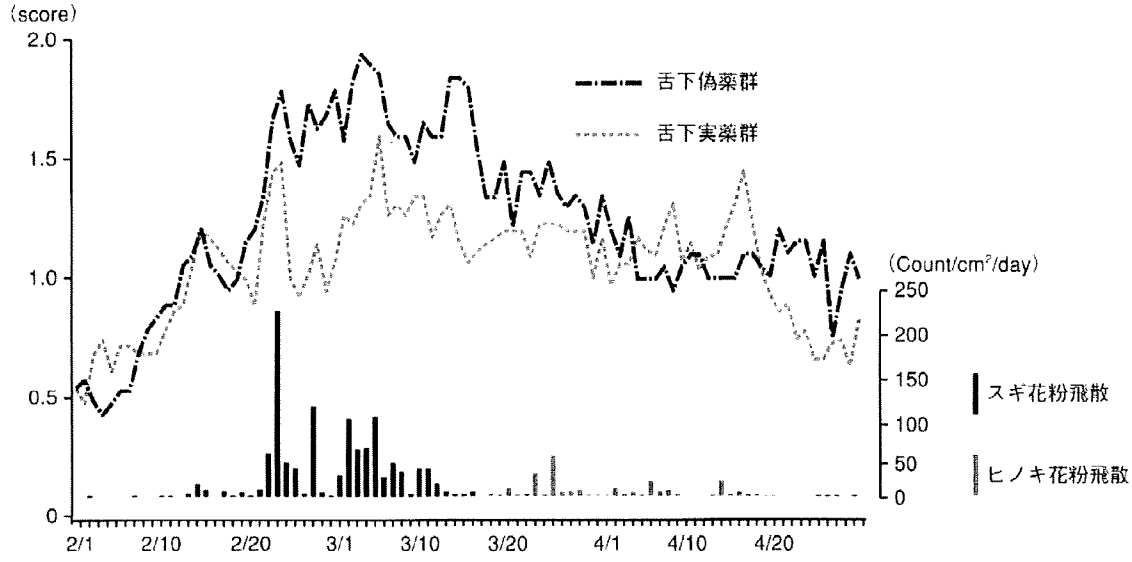

図1　舌下免疫治療による症状-薬物スコアの改善（平均値）
スギ花粉症患者を対象に2005年秋から半年間行われた二重盲検試験の症状-薬物スコアの結果．花粉飛散ピーク時には，実薬舌下投与群は偽薬投与群に比較して有意に症状改善がみられた．ただ，後半のヒノキ花粉飛散期には効果は明らかではなかった（文献26より改変）

ていくが，その後は再び陽性になるものがみられる．また，抗原エキスの皮下注射により誘導された抗原特異的IgG抗体による遮断抗体としての関与が指摘されている．代表はIgG4抗体で，IgE抗体との割合の重要性が指摘されたが，IgG4抗体自体は抗原エキスの量に依存するもので，臨床効果と抗体価とは関連を認めないとする報告が多い．その他遮断抗体として，抗原とIgE抗体のcomplexとB細胞表面上のCD23との結合を介したT細胞への抗原提示能を抑制すること，肥満細胞・好塩基球表面上のFcg2受容体のintracellular immunoreceptor tyrosine-based inhibition motifs（ITIMs）を介したIgE抗体による脱顆粒の抑制といった作用，さらにIgA抗体の誘導によるIL-10産生亢進，鼻粘膜局所での炎症細胞浸潤の抑制などが報告されている[17) 18)]．さらに最近は花粉症患者鼻生検組織の免疫染色の検討から，抗原特異的免疫療法により鼻粘膜局所にFoxP3陽性CD4$^+$Cd25$^+$の制御性T細胞が誘導されるといった報告が注目されている[19)]．現在，抗原特異的免疫治療によりTh1細胞誘導から遮断抗体としてのIgG抗体の誘導，制御性T細胞誘導からIL-10を介したIgG4抗体，TGF-βを介したIgA抗体の誘導とTh2細胞の抑制が機序として考えられている．

一方，舌下免疫療法の作用機序についても種々の検討が進んでいる．鼻粘膜や結膜中のICAM-1発現低下，鼻汁中tryptaseの増加抑制，炎症細胞浸潤低下が指摘されている[20) 21)]．IL-10については血清中の変化は報告がないが，3年以上ハウスダスト・ダニによる舌下免疫療法を受けた患者の末梢血単核球のPHA（phytohemagglutinin），カンジタ刺激によるIL-10産生が免疫療法例と比較して増加したといった指摘もある[22)]．

抗原特異的IgG4抗体価は舌下免疫療法開始後12カ月程度まで増加し，以後プラトーに達するとされ，投与抗原濃度との関連が示されているが，投与期間との関連は明らかではない．その意義と臨床効果との関連は不明である．血清抗原特異的IgE抗体価については，高濃度抗原による舌下免疫療法では6カ月ぐらいまで上昇し，以下再び開始前値に戻る．低濃度抗原を用いた場合には変化がないといったものが多い[23) 24)]．

プリック法による皮膚テストへの影響が検討されているが，一般に高濃度抗原を長期間使用した舌下免疫療法では変化を認めても，低濃度抗原使用やシーズン中のみの投与のものでは変化は認められていない．鼻粘膜抗原誘発に関しては，閾値の上昇を認めたとする

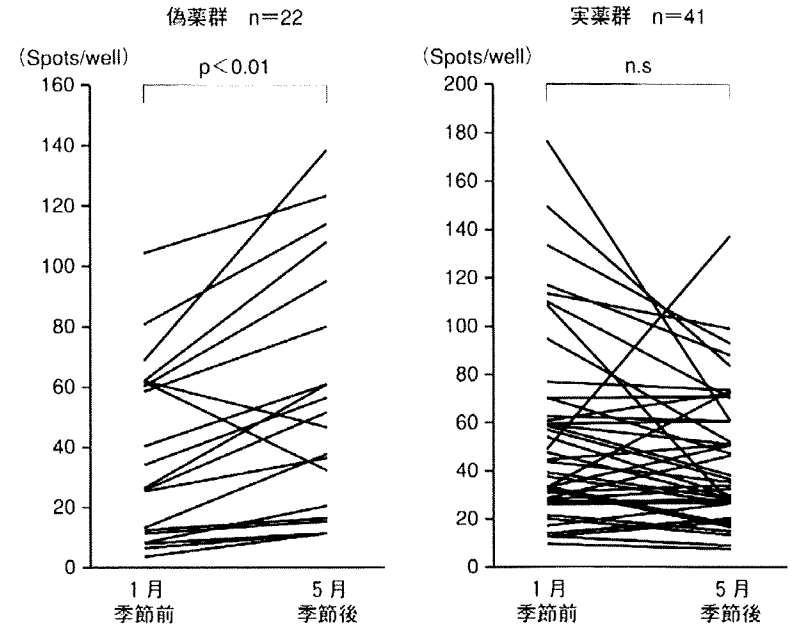

図2　スギ花粉特異的Th2クローンの変化
スギ花粉主要抗原であるCry jに対するTh2メモリー細胞数の花粉飛散による増加は，実薬舌下免疫群では有意な抑制がみられた（文献26より改変）

報告が多いが，変化がなかったとする報告もある[25]．

4 スギ花粉症に対する舌下免疫療法の臨床試験の現状

わが国特有といえるスギ花粉症に対する皮下注射法による抗原特異的免疫治療の有効性について，ランダム化試験による科学的検討は残念ながら全く行われていない．しかし，舌下免疫療法については厚生労働省の科学研究補助金にてわれわれも精力的に進めている．現在，スギ花粉症に対しては，皮下注射で用いられているスギ花粉エキス（トリイ[R]）しかないが，本エキスを用いて，スギ花粉症に対する舌下免疫療法の有効性を検討するため，プラセボ対照にランダム化比較試験を2005～'06年に行った[26]．花粉曝露量をほぼ一定にする必要があるため，対象は千葉市近郊に在住する成人スギ花粉症ボランティア67名で，'05年10月よりスギ花粉エキス（実薬群）あるいはinactive placebo（偽薬群）を投与した．実薬，偽薬を2：1で振り分けた盲検法により，投与は2分間舌下に保持して吐き出すspit法を用いた．spit法を用いたのは，これまでスギ花粉症に対する舌下免疫療法の安全性が確認されていなかったためであり，1 JAU/回の投与より開始して1カ月で維持量1,000 JAU/回として以後週1回で4月末まで投与を行い，アレルギー日記よりスギ花粉飛散時期の症状，および薬物スコアを調査した．従来の皮下注射法では200 JAU 0.2 mLを維持量としていたが，維持期の月あたりのエキスの投与量として300倍に設定した．また，試験開始直前，花粉飛散直前（'06年1月），投与終了後（'06年5月）に採血を行い，スギ，ヒノキ花粉特異的IgE抗体，特異的IgG4抗体，総Th1/Th2細胞，Cry j特異的Th2（IL-4）メモリー細胞を測定した．

4名が個人的理由で脱落したが，重篤な有害事象は認められなかった．'06年の千葉市でのスギ，ヒノキ花粉飛散数を図1に示すが，スギ花粉飛散は2月中旬に始まり，3月中旬に終息，以後はヒノキ花粉飛散が始まり4月中旬まで続いた．症状・薬物スコアの検討から，スギ花粉飛散ピーク時には有意に実薬群でスコア値が低値を示した．副作用として口の中のにがみ，かゆみ，蕁麻疹様の湿疹などが実薬群の13名に出現したが，いずれも一過性で投与の継続は可能であった．これらの結果はスギ花粉症に対しても，舌下免

疫療法の安全性と有効性を示唆するものであった．一方，血中IgE値，総Th1/Th2細胞に2群間に差はみられなかったが，血中のCry j特異的IgG4抗体は実薬群のみで上昇がみられた．Cry j特異的Th2（IL-4産生）細胞は，季節性変動を示すことを以前確認しており[27]，偽薬群では確かにスギ花粉飛散後に飛散前と比較して増加がみられた．しかし，実薬群では増加は認められず（図2），免疫療法の作用機序と考えられた．

この結果をもとに，120名の成人スギ花粉症患者を対象に'06年秋から'08年4月まで1年半に及ぶ同様の投与法によるプラセボ対照比較試験を行った．一定の期待した効果が得られている（投稿中）．ただ，スギ花粉症に対する免疫治療の問題点は，抗原エキスの濃度がWHOやこれまでの欧米での臨床試験から推奨されている濃度に比べ100分の1以下と低い点である．この背景には欧米で花粉症の中心である草本花粉に比較してスギ花粉の抗原抽出は非常に難しいこと，皮下注射では現在の標準化されたスギ花粉エキス低濃度でも強い痛みの誘発と免疫応答の誘導は確認されていることから，花粉の違いにより至適投与抗原濃度，投与プロトコールの設定は独自に必要である．'08年からはスギ花粉エキスの連日投与による比較試験を行っており，現在この試験についてもキーオープンに向けて解析を進めているところである．また，作用機序の解明から，有効性を示すバイオマーカーの検討，抗原特異的免疫療法の効果を有する症例の予測因子の検討はこの治療法の普及に欠かせない．前述した最初のランダム化試験結果から抗原特異的Th2細胞の増加抑制がみられていることから，'07～'08年にまず少数例ではあるがスギ花粉症患者対照にオープン試験を行い，バイオマーカーの検討を行った．その結果，末梢血液中のCry j 1特異的FoxP3$^+$，IL-10$^+$，CD4$^+$，CD25$^+$細胞が臨床効果と高い関連を示すことが見出され（図3），現在二重盲検試験での多数症例でその結果を確認しており，改めて詳細を報告したい．

おわりに

患者数の増加が著しいスギ花粉症に対する舌下免疫療法の検討が進んでおり，早期の臨床治験の開始が期待されている．同時に有効性を示すバイオマーカーの確立も急がれており，さまざまな検討が進んでいる．

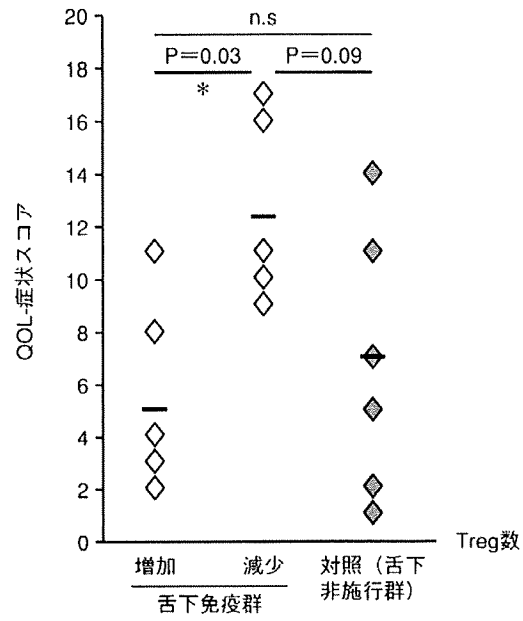

図3　舌下免疫療法によるスギ抗原特異的制御性T細胞の増減と臨床症状との関連
Cry j 1特異的FoxP3$^+$IL-10$^+$CD4$^+$CD25$^+$T細胞の末梢血中の増加は舌下免疫療法の臨床効果と関連がみられた（文献28より改変）

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特 集 『免疫療法はどこまで解明されたか』

スギ花粉症に対する舌下免疫療法の有効性の検討と治療バイオマーカーの探索

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Analysis of clinical therapeutic effects by sublingual immunotherapy against Japanese cedar pollinosis and elucidation of its therapeutic biomarkers

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ABSTRACT

Background: Japanese cedar (*Cryptomeria japonica*) pollinosis is one of the most prevalent allergies in Japan. We performed two clinical trials to clarify the therapeutic effects and mechanisms of sublingual immunotherapy (SLIT) against Japanese cedar pollinosis.

Methods: We performed the key-opened study over one pollinosis season in 2007, enrolling 19 patients from in-house volunteers suffering from Japanese cedar pollinosis prior to a double-blind, placebo-controlled (DBPC) clinical trial. The DBPC trial was performed over two pollen seasons in 2007 and 2008, including 120 patients from Kanto area around Tokyo and Chiba prefecture. Peripheral blood was obtained from all participants before SLIT treatment, and at pre- and post-pollen season. We analyzed the induction of regulatory T cells (iTreg), namely, $IL10^{+}Foxp3^{+}$ cells in $CD25^{+}CD4^{+}$ leukocytes, by flow cytometry. The Th2-type responses were analyzed by the cytokine production from peripheral blood mononuclear cells after stimulation with Cry j 1. Clinical symptoms were evaluated using a QOL questionnaire and symptom diary in the pollen season.

Results: The numbers of iTreg were significantly decreased in the No-SLIT group, but maintained in the SLIT group after pollen season. Upregulation of Th2-type cytokine production stimulated with Cry j 1 was attenuated by SLIT. The iTreg-increased subgroup from the SLIT group showed more suppressed Th2-type cytokine profiles and symptom scores compared to those from the iTreg-decreased subgroup from the SLIT group and the No-SLIT group.

Conclusion: Antigen-specific iTreg has a potential as a therapeutic biomarker correlated with clinical pollinosis symptoms and may be involved in the therapeutic mechanisms of SLIT.

Key words: allergic rhinitis, biomarker, Japanese cedar polinosis, regulatory T cell, sublingual immunotherapy

Abbreviations: CBA, cytometric bead assay; iTreg, induced regulatory T cell; PBMC, peripheral blood mononuclear cell; SLIT, sublingual immunotherapy

1. 緒言

スギ花粉症の有症率は，2008年のBaba等の報告によれば全国平均26.5%であり[1]，2003年にOkuda等により行われた全国調査の有症率13.1%と比較すると[2]，この5年間でその有症率は約2倍に増加している。特に若い世代である10代から50代でのスギ花粉症有症率が高いことから[3]，スギ花粉飛散時期における集中力の低下，作業効率の低下

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