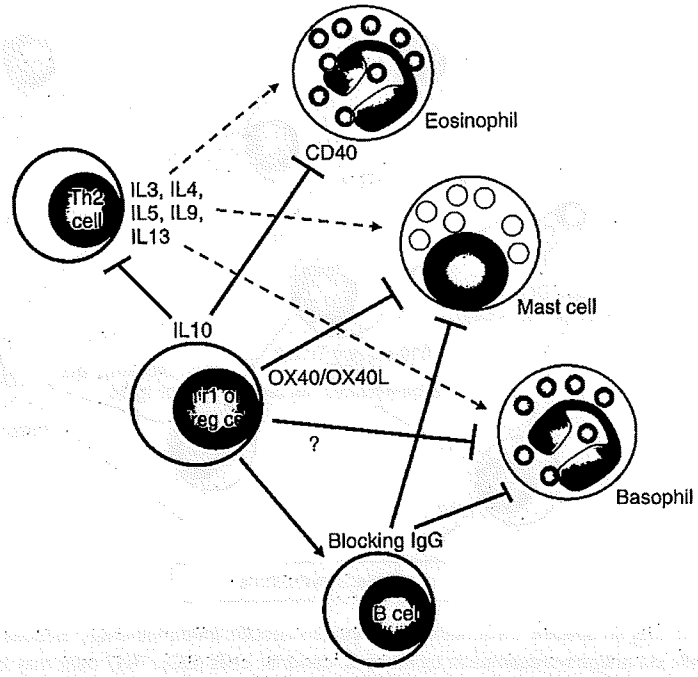

Fig. 3 Proposed roles of regulatory T cells on inflammatory cells in allergen-specific immunotherapy. Regulatory T cells, namely IL10-secreting Tr1 cells or adaptive/induced Treg cells, down-regulate inflammatory cells, directly or indirectly. Regulatory T cells down-regulate the activation of Th2 cells 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.

REFERENCES

1. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J* 2004;**24**:758-64.
2. Baba K, Nakae K. [Epidemiology of nasal allergy through Japan in 2008]. *Prog Med* 2008;**28**:2001-12(in Japanese).
3. Grote M, Vrtala S, Niederberger V, Wiermann R, Valenta R, Reichelt R. Release of allergen-bearing cytoplasm from hydrated pollen: a mechanism common to a variety of grass (*Poaceae*) species revealed by electron microscopy. *J Allergy Clin Immunol* 2001;**108**:109-15.
4. Maeda Y, Akiyama K, Shida T. A clinical study of Japanese cedar (*Cryptomeria japonica*) pollen-induced asthma. *Allergol Int* 2008;**57**:413-7.
5. Plotz SG, Traidl-Hoffmann C, Feussner I *et al.* Chemotaxis and activation of human peripheral blood eosinophils induced by pollen-associated lipid mediators. *J Allergy Clin Immunol* 2004;**113**:1152-60.
6. Traidl-Hoffmann C, Kasche A, Jakob T *et al.* Lipid mediators from pollen act as chemoattractants and activators of polymorphonuclear granulocytes. *J Allergy Clin Immunol* 2002;**109**:831-8.
7. Gutermuth J, Bewersdorff M, Traidl-Hoffmann C *et al.* Immunomodulatory effects of aqueous birch pollen extracts and phytoprostanes on primary immune responses in vivo. *J Allergy Clin Immunol* 2007;**120**:293-9.
8. Boldogh I, Bacsi A, Choudhury BK *et al.* ROS generated by pollen NADPH oxidase provide a signal that augments antigen-induced allergic airway inflammation. *J Clin Invest* 2005;**115**:2169-79.
9. Dharajiya N, Choudhury BK, Bacsi A, Boldogh I, Alam R, Sur S. Inhibiting pollen reduced nicotinamide adenine dinucleotide phosphate oxidase-induced signal by intrapulmonary administration of antioxidants blocks allergic airway inflammation. *J Allergy Clin Immunol* 2007; **119**:646-53.
10. Walker S, Khan-Wasti S, Fletcher M, Cullinan P, Harris J, Sheikh A. Seasonal allergic rhinitis is associated with a detrimental effect on examination performance in United Kingdom teenagers: case-control study. *J Allergy Clin Immunol* 2007;**120**:381-7.
11. Jauregui I, Mullol J, Davila I *et al.* Allergic rhinitis and school performance. *J Investig Allergol Clin Immunol* 2009;**19**:32-9.
12. Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911;**177**:1572-3.
13. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 2006;**6**:761-71.
14. American Academy of Allergy, Asthma and Immunology. The use of standardized allergen extracts. American Academy of Allergy, Asthma and Immunology (AAAAI). *J Allergy Clin Immunol* 1997;**99**:583-6.
15. Canonica GW, Baena-Cagnani CE, Bousquet J *et al.* Recommendations for standardization of clinical trials with Allergen Specific Immunotherapy for respiratory allergy. A statement of a World Allergy Organization (WAO) taskforce. *Allergy* 2007;**62**:317-24.
16. van Ree R, Chapman MD, Ferreira F *et al.* The CREATE project: development of certified reference materials for allergenic products and validation of methods for their quantification. *Allergy* 2008;**63**:310-26.
17. Salcedo G, Diaz-Perales A, Sanchez-Monge R. The role of plant panallergens in sensitization to natural rubber latex. *Curr Opin Allergy Clin Immunol* 2001;**1**:177-83.
18. Harwanegg C, Laffer S, Hiller R *et al.* Microarrayed recombinant allergens for diagnosis of allergy. *Clin Exp Allergy* 2003;**33**:7-13.
19. Deinhofer K, Sevcik H, Balic N *et al.* Microarrayed allergens for IgE profiling. *Methods* 2004;**32**:249-54.
20. Pittner G, Vrtala S, Thomas WR *et al.* Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy* 2004;**34**:597-603.
21. Bhalla PL, Singh MB. Biotechnology-based allergy diagnosis and vaccination. *Trends Biotechnol* 2008;**26**:153-61.
22. Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol* 2005;**116**: 608-13.
23. Pauli G, Larsen TH, Rak S *et al.* Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2008;**122**:951-60.
24. Akdis M, Akdis CA. Therapeutic manipulation of immune tolerance in allergic disease. *Nat Rev Drug Discov* 2009;**8**: 645-60.
25. Zoss A, Koch C, Hirose R. Alum-ragweed precipitate: preparation and clinical investigation; preliminary report. *J Allergy* 1937;**8**:329-35.
26. Muller U, Hari Y, Berchtold E. Premedication with antihistamines may enhance efficacy of specific-allergen immunotherapy. *J Allergy Clin Immunol* 2001;**107**:81-6.
27. Klunker S, Saggar LR, Seyfert-Margolis V *et al.* Combination treatment with omalizumab and rush immunotherapy for ragweed-induced allergic rhinitis: Inhibition of IgE-facilitated allergen binding. *J Allergy Clin Immunol* 2007; **120**:688-95.
28. Calderon M, Brandt T. Treatment of grass pollen allergy: focus on a standardized grass allergen extract - Grazax. *Ther Clin Risk Manag* 2008;**4**:1255-60.

29. Antico A, Pagani M, Crema A. Anaphylaxis by latex sublingual immunotherapy. *Allergy* 2006;**61**:1236-7.
30. Dunsky EH, Goldstein MF, Dvorin DJ, Belecanech GA. Anaphylaxis to sublingual immunotherapy. *Allergy* 2006; **61**:1235.
31. Eifan AO, Keles S, Bahceciler NN, Barlan IB. Anaphylaxis to multiple pollen allergen sublingual immunotherapy. *Allergy* 2007;**62**:567-8.
32. de Groot H, Bijl A. Anaphylactic reaction after the first dose of sublingual immunotherapy with grass pollen tablet. *Allergy* 2009;**64**:963-4.
33. Cochard MM, Eigenmann PA. Sublingual immunotherapy is not always a safe alternative to subcutaneous immunotherapy. *J Allergy Clin Immunol* 2009;**124**:378-9.
34. Lombardi C, Incorvaia C, Braga M, Senna G, Canonica GW, Passalacqua G. Administration regimens for sublingual immunotherapy to pollen allergens: what do we know? *Allergy* 2009;**64**:849-54.
35. Panzner P, Petras M, Sykora T, Lesna I. Double-blind, placebo-controlled evaluation of grass pollen specific immunotherapy with oral drops administered sublingually or supralingually. *Respir Med* 2008;**102**:1296-304.
36. Bagnasco M, Mariani G, Passalacqua G *et al.* Absorption and distribution kinetics of the major *Parietaria judaica* allergen (Par j 1) administered by noninjectable routes in healthy human beings. *J Allergy Clin Immunol* 1997;**100**: 122-9.
37. Bagnasco M, Passalacqua G, Villa G *et al.* Pharmacokinetics of an allergen and a monomeric allergoid for oromucosal immunotherapy in allergic volunteers. *Clin Exp Allergy* 2001;**31**:54-60.
38. Passalacqua G, Villa G, Altrinetti V *et al.* Sublingual swallow or spit? *Allergy* 2001;**56**:578.
39. Calderon MA, Birk AO, Andersen JS, Durham SR. Prolonged preseasonal treatment phase with Grazax sublingual immunotherapy increases clinical efficacy. *Allergy* 2007;**62**:958-61.
40. Stelmach I, Kaczmarek-Wozniak J, Majak P, Olszowiec-Chlebna M, Jerzynska J. Efficacy and safety of high-doses sublingual immunotherapy in ultra-rush scheme in children allergic to grass pollen. *Clin Exp Allergy* 2009;**39**: 401-8.
41. Cox LS, Larenas Linnemann D, Nolte H, Weldon D, Finegold I, Nelson HS. Sublingual immunotherapy: a comprehensive review. *J Allergy Clin Immunol* 2006;**117**:1021-35.
42. Johansen P, Haffner AC, Koch F *et al.* Direct intralymphatic injection of peptide vaccines enhances immunogenicity. *Eur J Immunol* 2005;**35**:568-74.
43. Senti G, Prinz Vavricka BM, Erdmann I *et al.* Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. *Proc Natl Acad Sci U S A* 2008;**105**:17908-12.
44. Wachholz PA, Durham SR. Induction of 'blocking' IgG antibodies during immunotherapy. *Clin Exp Allergy* 2003; **33**:1171-4.
45. Lima MT, Wilson D, Pitkin L *et al.* Grass pollen sublingual immunotherapy for seasonal rhinoconjunctivitis: a randomized controlled trial. *Clin Exp Allergy* 2002;**32**:507-14.
46. Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006;**117**:802-9.
47. Horiguchi S, Okamoto Y, Yonekura S *et al.* A randomized controlled trial of sublingual immunotherapy for Japanese cedar pollinosis. *Int Arch Allergy Immunol* 2008;**146**:76-84.
48. Ciprandi G, Continia P, Fenoglio D *et al.* Relationship between soluble HLA-G and HLA-A, -B, -C serum levels, and interferon-gamma production after sublingual immunotherapy in patients with allergic rhinitis. *Hum Immunol* 2008;**69**:409-13.
49. Ciprandi G, Contini P, Pistorio A, Murdaca G, Puppo F. Sublingual immunotherapy reduces soluble HLA-G and HLA-A, -B, -C serum levels in patients with allergic rhinitis. *Int Immunopharmacol* 2009;**9**:253-7.
50. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005;**115**:911-9.
51. De Rosa V, Procaccini C, Cali G *et al.* A key role of leptin in the control of regulatory T cell proliferation. *Immunity* 2007;**26**:241-55.
52. Ciprandi G, De Amici M, Murdaca G, Filaci G, Fenoglio D, Marseglia GL. Adipokines and sublingual immunotherapy: preliminary report. *Hum Immunol* 2009;**70**:73-8.
53. Ciprandi G, De Amici M, Tosca M, Negrini S, Murdaca G, Marseglia GL. Two year sublingual immunotherapy affects serum leptin. *Int Immunopharmacol* 2009;**9**:1244-6.
54. Okano M, Otsuki N, Azuma M *et al.* Allergen-specific immunotherapy alters the expression of B and T lymphocyte attenuator, a co-inhibitory molecule, in allergic rhinitis. *Clin Exp Allergy* 2008;**38**:1891-900.
55. Rolinck-Werninghaus C, Kopp M, Liebke C, Lange J, Wahn U, Niggemann B. Lack of detectable alterations in immune responses during sublingual immunotherapy in children with seasonal allergic rhinoconjunctivitis to grass pollen. *Int Arch Allergy Immunol* 2005;**136**:134-41.
56. Savolainen J, Nieminen K, Laaksonen K *et al.* Allergen-induced in vitro expression of IL-18, SLAM and GATA-3 mRNA in PBMC during sublingual immunotherapy. *Allergy* 2007;**62**:949-53.
57. Nieminen K, Valovirta E, Savolainen J. Clinical outcome and IL-17, IL-23, IL-27 and FOXP3 expression in peripheral blood mononuclear cells of pollen-allergic children during sublingual immunotherapy. *Pediatr Allergy Immunol.* Epub 2009 Jun 29.
58. Akdis M, Verhagen J, Taylor A *et al.* Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004;**199**:1567-75.
59. Jutel M, Akdis M, Budak F *et al.* IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003;**33**:1205-14.
60. Ciprandi G, Fenoglio D, Cirillo I *et al.* Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol* 2005;**95**:38-44.
61. Ciprandi G, Cirillo I, Fenoglio D, Marseglia G, Tosca MA. Sublingual immunotherapy induces spirometric improvement associated with IL-10 production: preliminary reports. *Int Immunopharmacol* 2006;**6**:1370-3.
62. Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 2007;**120**:707-13.
63. Savolainen J, Jacobsen L, Valovirta E. Sublingual immunotherapy in children modulates allergen-induced in vitro expression of cytokine mRNA in PBMC. *Allergy* 2006;**61**: 1184-90.
64. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT.

Grass pollen immunotherapy induces Foxp3-expressing $CD4^+$ $CD25^+$ cells in the nasal mucosa. *J Allergy Clin Immunol* 2008;**121**:1467-72.
65. Pereira-Santos MC, Baptista AP, Melo A *et al.* Expansion of circulating $Foxp3^+CD25^{bright}$ $CD4^+$ T cells during specific venom immunotherapy. *Clin Exp Allergy* 2008;**38**: 291-7.
66. Gri G, Piconese S, Frossi B *et al.* $CD4^+CD25^+$ regulatory T cells suppress mast cell degranulation and allergic responses through OX40-OX40L interaction. *Immunity* 2008;**29**:771-81.
67. Ohkawara Y, Lim KG, Xing Z *et al.* CD40 expression by human peripheral blood eosinophils. *J Clin Invest* 1996; **97**:1761-6.
68. Fujisawa T, Nagao M, Hiraguchi Y *et al.* Biomarkers for allergen immunotherapy in cedar pollinosis. *Allergol Int* 2009;**58**:163-70.
69. Okubo K, Gotoh M, Fujieda S *et al.* A randomized double-blind comparative study of sublingual immunotherapy for cedar pollinosis. *Allergol Int* 2008;**57**:265-75.
70. Hirahara K, Tatsuta T, Takatori T *et al.* Preclinical evaluation of an immunotherapeutic peptide comprising 7 T-cell determinants of Cry j 1 and Cry j 2, the major Japanese cedar pollen allergens. *J Allergy Clin Immunol* 2001; **108**:94-100.
71. Yamanaka K, Yuta A, Kakeda M *et al.* Induction of IL-10-producing regulatory T cells with TCR diversity by epitope-specific immunotherapy in pollinosis. *J Allergy Clin Immunol* 2009;**124**:842-5.
72. Yang L, Suzuki K, Hirose S, Wakasa Y, Takaiwa F. Development of transgenic rice seed accumulating a major Japanese cedar pollen allergen (Cry j 1) structurally disrupted for oral immunotherapy. *Plant Biotechnol J* 2007;**5**: 815-26.
73. Takagi H, Hiroi T, Yang L *et al.* A rice-based edible vaccine expressing multiple T cell epitopes induces oral tolerance for inhibition of Th2-mediated IgE responses. *Proc Natl Acad Sci U S A* 2005;**102**:17525-30.
74. Ishii Y. [Allergen-specific immunotherapy utilizing mechanisms for immune regulation]. *Nihon Rinsho Meneki Gakkai Kaishi* 2008;**31**:392-8(in Japanese).
75. Linhart B, Jahn-Schmid B, Verdino P *et al.* Combination vaccines for the treatment of grass pollen allergy consisting of genetically engineered hybrid molecules with increased immunogenicity. *FASEB J* 2002;**16**:1301-3.

Allergology International. 2009;58:155-162
DOI: 10.2332/allergolint.08-RAI-0074

REVIEW ARTICLE

Present Situation of Cedar Pollinosis in Japan and its Immune Responses

Yoshitaka Okamoto[1], Shigetoshi Horiguchi[1], Heizaburo Yamamoto[1], Syuji Yonekura[1] and Toyoyuki Hanazawa[1]

ABSTRACT

Recent observations have suggested significant worldwide increase in the prevalence of allergic rhinitis and cedar pollinosis. In Japan, Japanese cedar (*Cryptometria japonica*) and Japanese cypress (*Chamaecyparis obtusa*) pollens are considered to be the major unique allergens and their extent of dispersal is quite large, travelling more than 100 km and thus causing serious pollinosis. Cedar pollinosis is a typical type 1 allergic disease by an adaptive immune response that occurs through the induction of allergen-specific effector T cells from naïve T cells. We examined the number of Japanese cedar pollen specific memory Th cells in the peripheral blood of the patients and found that the cedar pollen specific IL-4-producing Th2 memory cells increased during the pollen season and decreased during the off-season. However, more than 60% of the cedar-specific memory Th2 cells survived up to 8 months after the pollen season. Natural killer T(NKT) cells represent a unique lymphocyte subpopulation and their activity is not restricted to MHC antigens. NKT cells play an important role in innate immunity, however, the participation in development of allergic rhinitis could not be clarified.

KEY WORDS

cedar pollinosis, cedar specific Th memory cell, epidemiology, natural killer T cell

CEDAR POLLEN

In recent years, many countries have experienced an increase in the prevalence of allergic rhinitis.[1,2] Dust mite allergen is responsible for at least 90% of cases of perennial allergic rhinitis, while arboreal pollen, including that of cedar and Japanese cypress, is important in Japan.[3,4] Cedar forest covers nearly 18% of the total land area of Japan, while Japanese cypress is concentrated in the Kanto region and the western part of the country. Both cedar and Japanese cypress produce enormous amounts of pollen. In Japan, pollen counts are typically measured using the gravimetric method with a Durham sampler, in contrast to Western countries in which a Burkard sampler is typically used. In a study in Chiba Prefecture in 2005, the amount of air-borne pollen counted with a Burkard sampler was about 12 times greater than that counted with a Durham sampler.[5] In addition, distinct from grass pollen, which only spreads less than 100 meters, cedar and cypress pollen travel a long distance and reach major cities, including Tokyo and Osaka, causing wide-spread pollinosis, although no actual data describing the distance traveled was available. A detailed simulation study considering the results of real-time pollen distributing information was conducted using large computers and Figure 1 shows the source and areas from which the cedar pollen detected at Chiba University Hospital had spread. These dark spots indicate the areas where the cedar pollen originated. Pollens blow to Chiba city from the cedar planting areas of Boso Peninsula, as well as from the north Kanto area, Nikko, Izu Peninsula and Shizuoka Prefecture. This study suggests that cedar pollen actually can travel more than 100 km and cause pollinosis in a large area.

Cedar pollen dispersal precedes Japanese cypress pollen dispersal, and approximately 70% of patients with cedar pollinosis are also allergic to Japanese cypress pollen because of a common antigen.[6] Dispersal of cedar and Japanese cypress pollen generally exhibits an arch-shaped pattern with time: cedar pollen dispersal starts in early February and reaches a peak between late February and early March, and is fol-

[1]Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan.
Correspondence: Yoshitaka Okamoto, MD, PhD, Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan.
Email: yokamoto@faculty.chiba-u.jp
Received 9 December 2008.

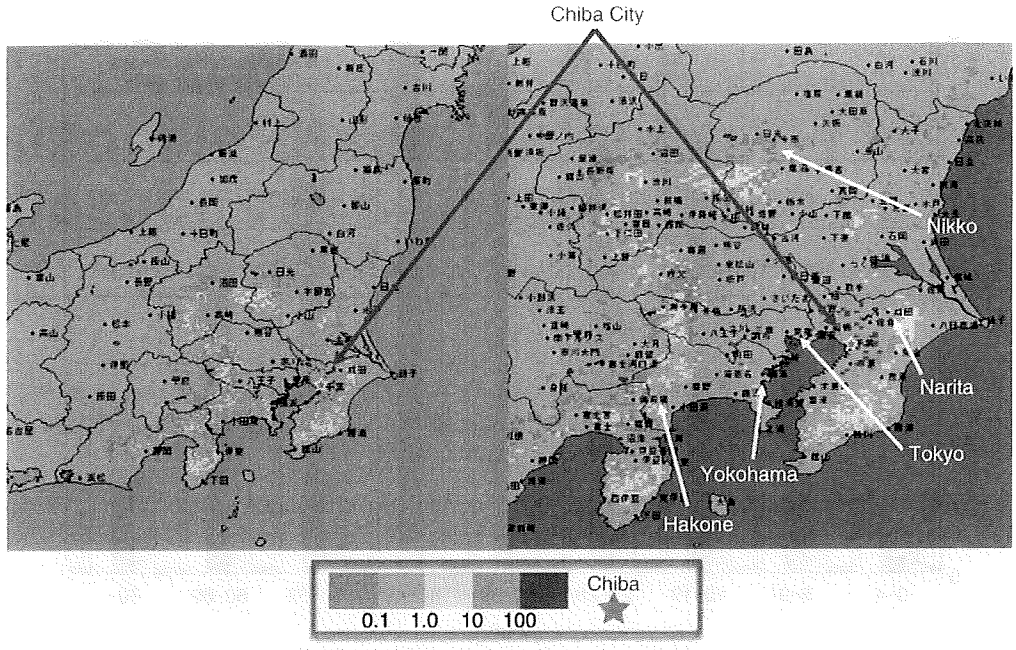

Fig. 1 The source areas from which the cedar pollen detected at Chiba University Hospital spread. This is the computer simulation study done by Mr. Kunihiko Yokota *et al.*, at Weather Service Co.,Ltd..

lowed by dispersal of Japanese cypress pollen, which reaches a peak from late March to early April, with some variation due to changes in the climate each year.[7,8] The pollen dispersal season lasts for more than 10 weeks in and around the Tokyo area.

PREVALENCE OF CEDAR POLLINOSIS IN JAPAN

A survey based only on a questionnaire has the risk of inclusion of a high rate of false-positive cases, because allergic rhinitis is sometimes difficult to distinguish from acute upper respiratory infection and even normal healthy individuals may exhibit mild, nonspecific nasal symptoms, such as sneezing and nasal secretion. In particular, cedar pollen dispersal season is also high flu season. An allergen-specific IgE test is necessary to avoid a high incidence of false positives, but it has been difficult to conduct an epidemiological study in Japan because of laws preventing use of personal information. In 2008, a questionnaire was posed to the Otorhinolaryngologists nationwide to determine whether their families suffered from allergic rhinitis. Although the rate of return of the questionnaire was low, i.e., 40% and the bias of the population could not be ignored, an accurate diagnosis was expected.

According to the analysis of this questionnaire,[9] the prevalence of perennial allergic rhinitis and of cedar pollinosis was 23.4% and 26.5%, respectively. In particular, the prevalence of cedar pollinosis increased more than 10% compared with that observed in a similar questionnaire conducted in 1998. Although the peak of cedar pollinosis is in those in their thirties to forties, the age onset of pollinosis has been decreasing (Fig. 2).

Figure 3 shows the annual amount of cedar pollen dispersal in Japan, which we examined in 2005. The darker brown parts indicate areas where cedar pollen counts were high. We studied the influence of various amounts of pollen exposure on the development of pollinosis and mite allergic rhinitis in elementary school students from schools in rural areas where the movement of students out of or into the school was uncommon. The annual amount of cedar and cypress pollen differed among these five regions. The pollen level was very high in southern Yamanashi: about 7,000/cm^2 on average for the last five years, as determined using Durham pollen samplers. In contrast, the pollen level was low in northern Yamanashi and inland Akita, at about 2,000/cm^2, and very low in coastal Akita, at about 500/cm^2. The pollen level in Chiba was about 4,000/cm^2.

Figure 4 shows the detection rate of cedar- and mite-specific IgE in students in these regions. The positive rate for Japanese cedar was about 60%, except for students in coastal Akita, who had a rate of only 23%. The positive rate for mite IgE was about 50% in each region. These results suggest that the sensitization rate for mite allergen is almost the same nationwide, whereas that for cedar pollen is depend-

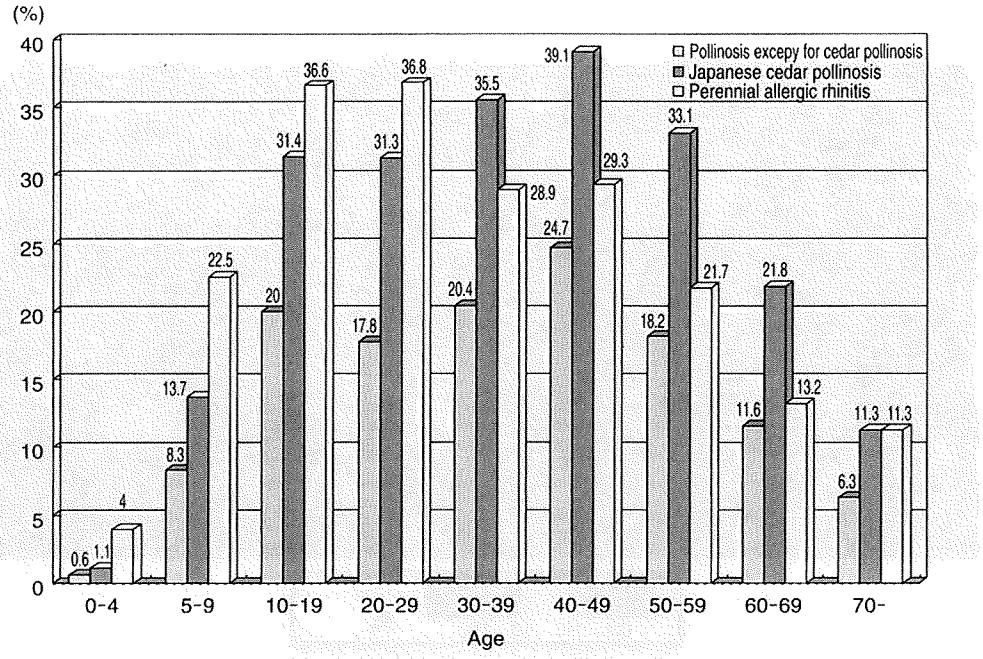

Fig. 2 The prevalence rate of allergic rhinitis in Japan in 2008 (from reference 9).

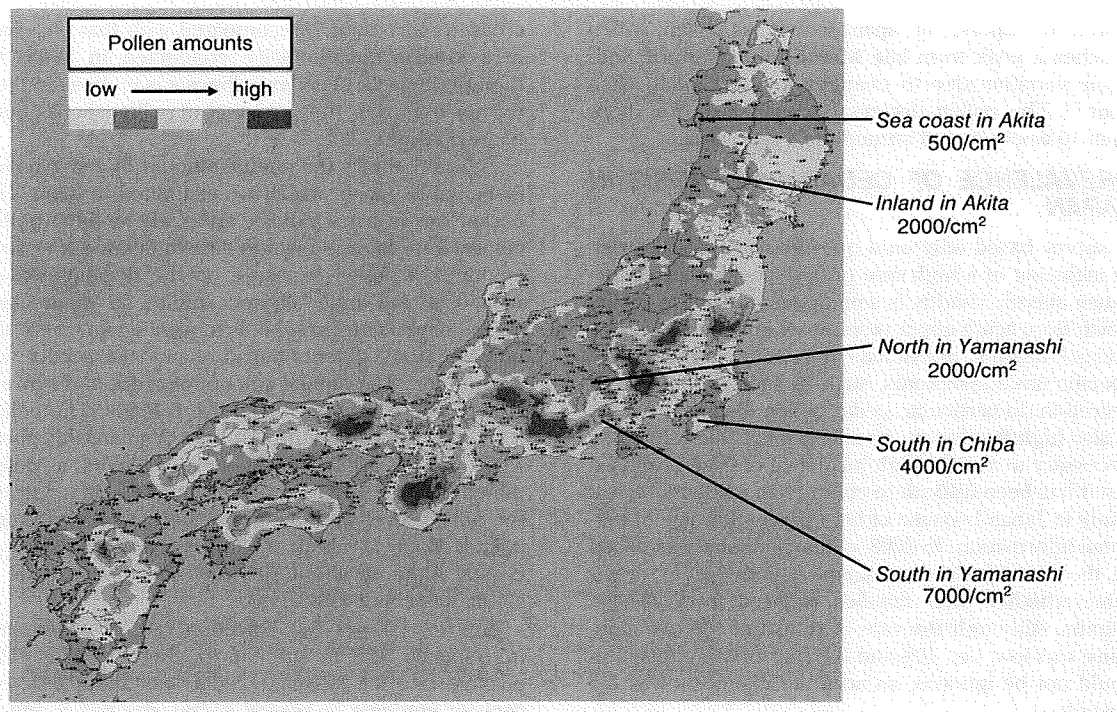

Fig. 3 Annual amount of cedar and cypress pollen dispersal in Japan in 2005.

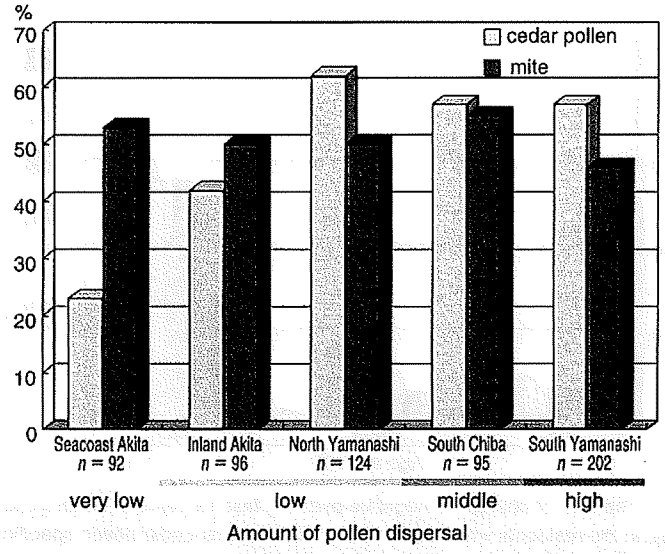

Fig. 4 The detection rate of cedar and cypress pollen-specific IgE in all 4th and 5th grade students in the elementary schools.

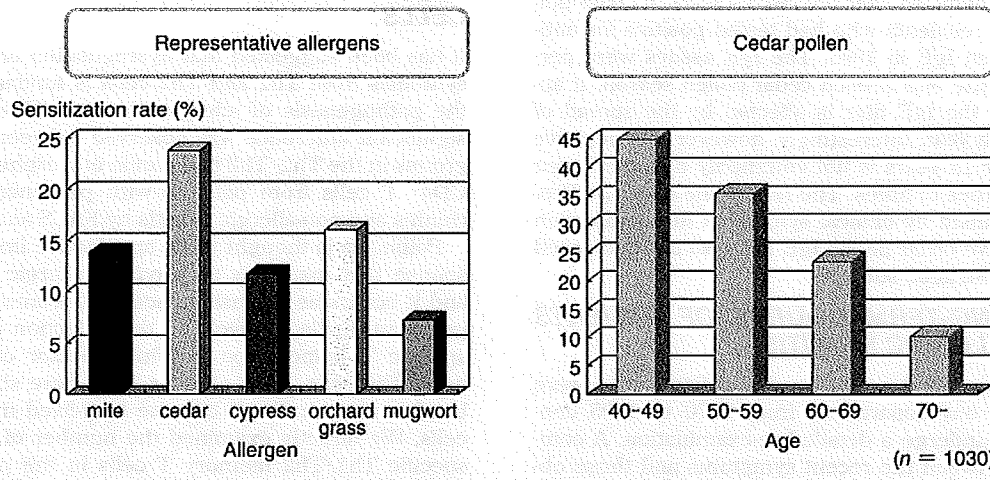

Fig. 5 The sensitization rate to the representative allergen and age distribution of cedar pollen-specific IgE in the adult residents in the forties to seventies in the rural small town in South Chiba.

ent on pollen counts. A very low level of pollen results in a low rate of detection and allergen avoidance is undoubtedly important for prevention. However, a high rate of allergic sensitization can be induced by a relatively small amount of pollen, and it is likely to be very difficult to reduce the amount of pollen exposure to a level that will prevent sensitization. Furthermore, tolerance was not easily induced in students in southern Yamanashi who had been receiving high pollen exposure every year since birth. Interestingly, the incidence of mite allergic rhinitis and pollinosis in these sensitized students was almost the same; about 30 to 35% in each region, respectively.

We have also undertaken medical examination of middle-aged adult residents in their forties to seventies in a rural small town (Maruyama-cho) in South Chiba every year since 1995.[10] The examination includes responses to a questionnaire and testing for specific IgE in serum using a CAP-RAST system. Figure 5 shows the sensitization rate to the representative allergens and the age distribution of cedar pollen-specific IgE. Deterioration of cedar-specific IgE is ob-

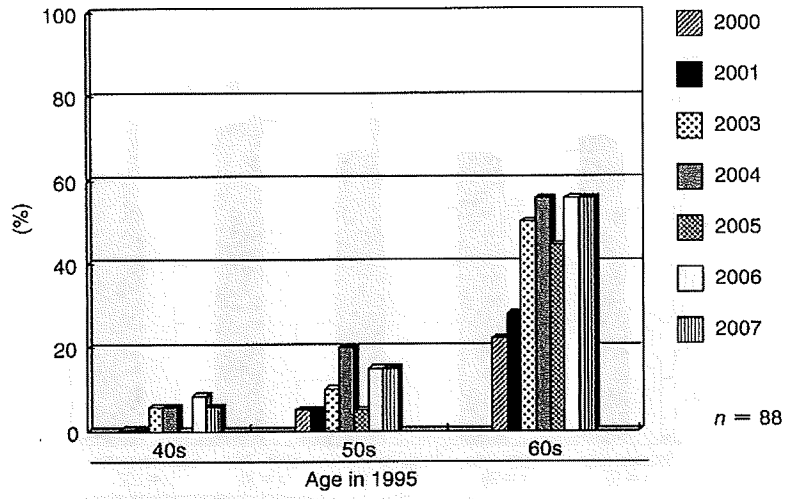

Fig. 6 The rate of change to negative over the last 13 years in cedar pollen-specific IgE in the residents who had tested positive for anti-cedar pollen specific IgE in 1995 and then had received examination every year.

served in elderly subjects. Figure 6 shows the rate of change to negative over the last 13 years in cedar pollen IgE in residents who had tested positive for anti-cedar pollen IgE in 1995. The IgE assays were performed at the end of each cedar pollen season. It appears that the IgE titer is affected by the spread of pollen each year. Interestingly, however, the negative change for 13 years is not commonly observed even in their forties to fifties. The rate of the cedar pollinosis determined by clinical symptoms in combination with positive cedar pollen IgE has also not decreased among these aged subjects.

THE LONG-TERM COURSE OF PATIENTS WITH ALLERGIC RHINITIS

One hundred and seventy-seven patients who were treated in our department from 1970 to 1995 consented to undergo a detailed re-examination. A comparison between the recent symptoms and those observed 10 to 30 years ago showed that 30% of adult patients exhibited some improvements and 10% had resolution. However, only 20% of the pediatric patients exhibited mild improvement of symptoms, whereas the remaining had the same or even worse symptoms as those in childhood (data not shown: in preparation for submitting). Regarding the allergen-specific IgE, a change to negative was not observed in any patients with cedar pollinosis and was seen in only a few of the mite-allergic patients. Thus, natural resolution is not commonly observed in allergic rhinitis and most pediatric patients grow to adulthood without natural improvement of symptoms.

CEDAR POLLEN SPECIFIC MEMORY T CELLS

It has been suggested that dysregulation of cytokine synthesis from Th1 and Th2 cells is fundamental to the pathogenesis of allergic diseases. However, no significant difference was observed between the two groups in the Th1/Th2 cell profile in peripheral blood $CD4^+$ T cells from patients with perennial allergic rhinitis and non-allergic rhinitis by FACS analysis.[11]

Pollinosis is thought to be an adaptive immune response that manifests as a type 1 allergic reaction, and it occurs as a consequence of fundamental allergenic mechanisms involving the induction of pollen-specific T helper type 2 (Th2) effector cells from naïve Th0 cells. Most effector T cells are short-lived, but few effector T cell become long-lived memory T cells. We directly examined the number of allergen-specific Th1/Th2 memory T cells in the peripheral blood of patients of allergic rhinitis by an ELISPOT assay using specific peptides.[12] The Japanese cedar-specific IL-4 producing Th2 cells were detected in all patients examined and increased during the pollen season and decreased during the off-season. However, more than 60% of the cedar-specific memory Th2 cells survived up to 8 months after the pollen season (Fig. 7) .

Allergen-specific immunotherapy is the only current treatment that can change the natural course of allergic rhinitis with long-term effects. However, the conventional immunotherapy with subcutaneous administration is inconvenient because it requires frequent visits to the doctor and also carries the risk of anaphylactic shock.[13] A recent review of randomized

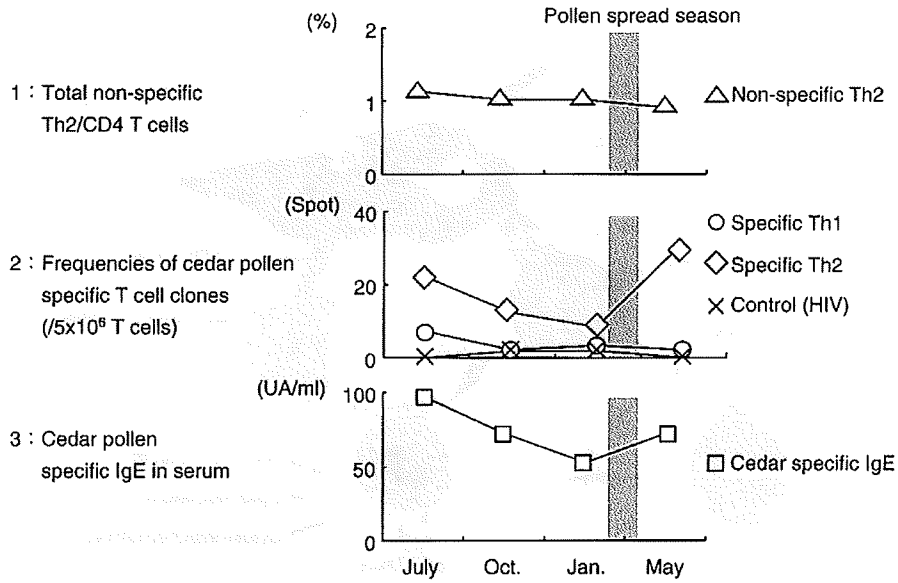

Fig. 7 The seasonal changes of total Th2 cells, frequency of cedar pollen specific T cell clones (spots number) and cedar pollen specific IgE.

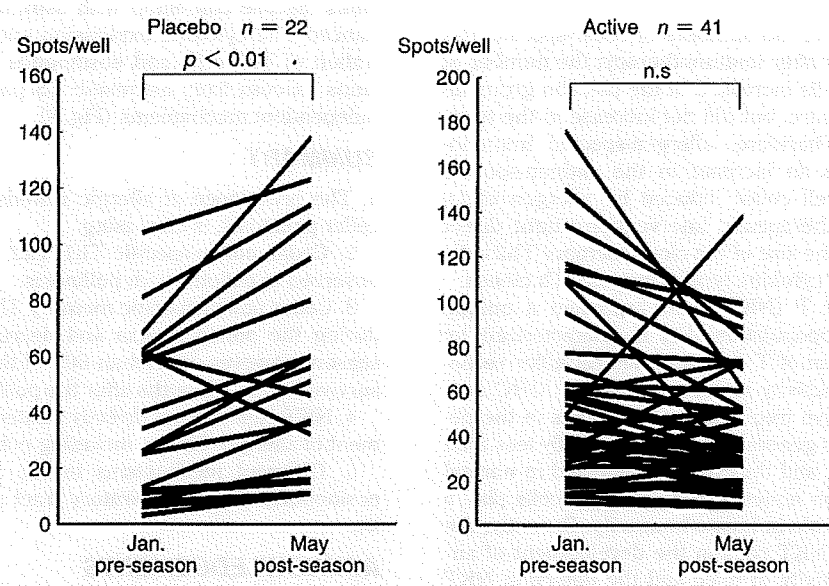

Fig. 8 The number of cedar-specific Th2 cells before and after sublingual immunotherapy.

controlled studies of sublingual immunotherapy suggested that this might be effective as an alternative method of administration.[14-16] To determine the efficacy of sublingual immunotherapy for Japanese cedar pollinosis, we conducted a blinded, randomized, placebo-controlled trial over a period of 6 months (from October 2005 to May 2006).[17] Sixty-seven subjects were enrolled and the nasal symptom scores during the cedar pollen season were evaluated using a symptom diary.

The patients in the active treatment group exhibited significantly lower symptom scores compared to the placebo group. This result suggests that sublingual immunotherapy may offer a safe approach to the management of allergic rhinitis, although the *in vivo* mechanisms of allergen-specific immunotherapy are

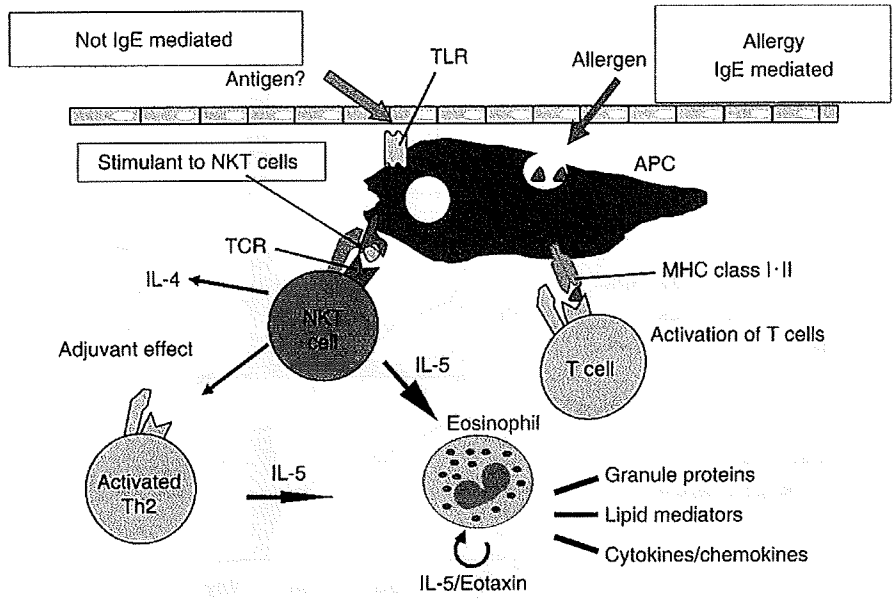

Fig. 9 Mechanism of eosinophil accumulation in respiratory mucosa. Eosinophil accumulation could be observed in MHC class-2 independent.

unknown.

Figure 8 shows the numbers of cedar-specific Th2 cells before and after immunotherapy: the number of Th2 memory cells increased in the placebo group after pollen exposure, but did not increase in the treatment group. Therefore, allergen-specific immunotherapy inhibits an increase in the antigen-specific Th2 memory cell count induced by allergen exposure. Immune-therapeutic intervention might direct at diminishing the size of the clone memory Th2 cells and shifting the cytokine type of memory Th clones.

Natural killer T (NKT) cells represent a unique lymphocyte subpopulation that is characterized by the co-expression of T cells and natural killer receptors.[18,19] Their activity is not restricted to MHC antigens. The relative frequency of NKT cells in the peripheral blood is generally quite low, usually less than 0.1% of PBMCs, and they are not detected in normal peripheral lymph nodes. However, NKT cells play a very important role in innate immunity. Recently, the involvement of NKT cells in the development of airway hypersensitivity in mice and the detection NKT cells in bronchoalveolar-lavage fluid samples from patients with moderate to severe asthma were reported. However, we could not detect the NKT cells in the nasal mucosa of the patients with allergic rhinitis by a polymerase chain reaction. However, NKT cells were detected to varying degrees in the sinus mucosa from asthmatic choronic sinusitis (CS) patients.

These results suggest that NKT cells are not directly related to the development of allergy, but that they may play important roles in the development of sinus disease combined with asthma and in the enhanced Th2 cytokine expression and increased infiltration of Th2 cells and eosinophils observed in the sinus mucosa from asthmatic CS patients via MHC-independent mechanisms (Fig. 9).

SUMMARY

1. The prevalence of allergic rhinitis, in particularly cedar pollinosis, is increasing.

2. Cedar pollen-specific Th1/Th2 dysregulation is observed in patients with pollinosis.

3. Cedar pollen specific memory Th cells increased during the pollen season and decreased during off season, however, morethan 60% of the memory cells survived up to 8 months after the pollen season.

4. NKT cells are not directly related to the development of allergic rhinitis, including pollinosis.

5. Different mechanisms in the accumulation of eosinophilia in the respiratory tract mucosa may exist.

ACKNOWLEDGEMENTS

The studies described in this manuscript were supported in part by a grant from the Ministry of Health, Labour and Welfare in Japan and by Global COE Program (Global Center for Education and Reseach in Immune System Regulation and Treatment), MEXT, Japan. The studies were conducted with the staff of Department of Otorhinolaryngology and Head & Neck Surgery, Graduate School of Medicine, Chiba University and Dr. Ozawa, Ozawa ENT Clinic, and Mr. Kunihiko Yokota, Weather Service Co. Ltd. We

appreciate their efforts and collaboration. We thank very much Dr. Akiyoshi Konno and Dr. Peary L. Ogra, under whom I have studied for a long time, for helpful comments and suggestions.

REFERENCES

1. Bousquet J, Van Cauwenberge P, Khaltaev N; Aria Workshop Group; World Health Organization. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001; **108**:S147-334.
2. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjuctivitis, and atopic eczema: ISAAC. *Lancet* 1998;**351**: 1225-32.
3. Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. *Ann Allergy Asthma Immunol* 2003;**91**: 288-96.
4. Kaneko Y, Motohashi Y, Nakamura H, Endo T, Eboshida A. Increasing prevalence of Japanese cedar pollinosis: a meta-regression analysis. *Int Arch Allergy Immunol* 2005; **136**:365-71.
5. Delaunay JJ, Sasajima H, Okamoto Y, Yokota M. Side-by-side comparison of automatic pollen counters for use in pollen information systems. *Ann Allergy Asthma Immunol* 2007;**98**:553-8.
6. Ito Y, Takahashi Y, Fujita T, Fukuyama S. Clinical effects of immunotherapy on Japanese cedar pollinosis in the season of cedar and cypress pollination. *Auris Nasus Larynx* 1997;**24**:163-70.
7. Ito H, Nishimura J, Suzuki M *et al.* Specific IgE to Japanese cypress (*Chamaecyparis obtusa*) in patients with nasal allergy. *Ann Allergy Asthma Immunol* 1995;**74**:299-303.
8. Sasaki K, Okamoto Y, Yonekura S *et al.* Cedar and cypress pollinosis and allergic rhinitis: Quality of life effects of early intervention with Leukotriene receptor antagonists. *Int Arch Allergy Immunol*. In press.
9. [*Practical Guideline for the Management of Allergic Rhinitis in Japan—Perennial Rhinitis and Pollinosis—2009 Edition*], 6th edn. Tokyo: Life Science, 2008(in Japanese).
10. Okawa T, Konno A, Yamakoshi T, Numata T, Terada N, Shima M. Analysis of natural history of Japanese cedar pollinosis. *Int Arch Allergy Immunol* 2003;**131**:39-45.
11. Horiguchi S, Okamoto Y, Chazono H, Sakurai D, Kobayashi K. Expression of membrane-bound CD23 in nasal B cells from patients with perennial allergic rhinitis. *Ann Allergy Asthma Immunol* 2005;**94**:286-91.
12. Horiguchi S, Tanaka Y, Uchida T *et al.* Seasonal changes in antigen-specific Th clone sizes in patients with Japanese cedar pollinosis: A 2-year study. *Clin Exp Allergy* 2008;**38**:405-12.
13. Lockey RF, Nicoara-Kasti GL, Theodoropoulos DS, Bukantz SC. Systemic reactions and fatalities associated with allergen immunotherapy. *Ann Allergy Asthma Immunol* 2001;**87**(Suppl 1):47-55.
14. Cox LS, Linnemann DL, Nolte H, Weldon D, Finegold I, Nelson HS. Sublingual immunotherapy: a comprehensive review. *J Allergy Clin Immunol* 2006;**117**:1021-35.
15. Burastero SE. Sublingual immunotherapy for allergic rhinitis: an update. *Curr Opin Otolaryngol Head Neck Surg* 2006;**14**:197-201.
16. Passalacqua G, Lombardi C, Guerra L, Compalati E, Fumagalli F, Canonica GW. Sublingual immunotherapy: no more doubts. *Eur Ann Allergy Clin Immunol* 2005;**37**: 314-20.
17. Horiguchi S, Okamoto Y, Yonekura S *et al.* A randomized controlled trial of sublingual immunotherapy for Japanese cedar pollinosis. *Int Arch Allergy Immunol* 2008;**146**:76-84.
18. Yamamoto H, Okamoto Y, Horiguchi S, Kunii N, Yonekura S, Nakayama T. Detection of natural killer T cells in the sinus mucosa from asthmatics with chronic sinusitis. *Allergy* 2007;**62**:1451-5.
19. Taniguchi M, Harada M, Kojo S, Nakayama T, Wakao H. The regulatory role of Valpha14 NKT cells in innate and acquired immune response. *Annu Rev Immunol* 2003;**21**: 483-513.
20. Kawano T, Cui J, Koezuka Y *et al.* CDld-restricted and TCR-mediated activation of Valpha14 NKT cells by glycosylceramides. *Science* 1997;**278**:1626-9.
21. Akbari O, Stock P, Meyer E *et al.* Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nat Med* 2003;**9**: 582-8.
22. Akbari O, Faul JL, Hoyte EG *et al.* CD4+ invariant T-cell-receptor+ naturel killer T cells in bronchial asthma. *N Engl J Med* 2006;**354**:1117-29.

2007, at the patient's request. After approximately 1½ years of normal eosinophil levels, the patient's eosinophilia worsened during late 2008 (Fig 1). A single osseous plasmacytoma was found and irradiated but progressed to multiple lytic lesions and multiple myelomas confirmed by means of bone marrow biopsy. The patient died in December 2008 as a result of this disease.

HES can occur as a myeloproliferative, lymphoproliferative, or, most frequently, idiopathic variant. Some myeloproliferative patients respond to imatinib mesylate[3] and possess a mutant gene on chromosome 4.[4] Deletion of approximately 800 kB on chromosome 4 results in a *FIP1L1/PDGFRA* fusion gene and formation of a kinase potently inhibited by imatinib mesylate. In our patient the *FIP1L1/PDGFRA* fusion gene was not detected, and imatinib mesylate failed to control eosinophilia. In the lymphoproliferative HES variant, T-lymphocyte clones produce cytokines, especially IL-5, that stimulate eosinophil production in the bone marrow. Mepolizumab, an anti–IL-5 drug, is used to inhibit eosinophil proliferation stimulated by IL-5; however, for unknown reasons, 16% of patients do not respond to mepolizumab.[5] In this case mepolizumab had no effect, suggesting that eosinophilia was not IL-5 dependent or that other cytokines, such as IL-3 or GM-CSF, were supporting eosinophil growth. Alternatively, the patient might have been producing so much IL-5 that the levels might have outpaced mepolizumab injections. IFN-α treatment is often effective because of a shift in the cytokine milieu from T_H2, which is supportive of eosinophil growth, to a T_H1-type response. In this case IFN-α caused a decrease in eosinophil counts, although not to normal levels, and the patient experienced the side effects of IFN-α.

Alemtuzumab is an anti-CD52 antibody that can bind to both eosinophils and T cells, potentially inhibiting either the myeloproliferative, lymphoproliferative, or idiopathic variant.[6] CD52 is a glycosylphosphatidylinositol-anchored molecule expressed on human eosinophils, lymphocytes, macrophages, and monocytes but not on neutrophils.[7] Alemtuzumab is approved for the treatment of B-cell chronic lymphocytic leukemia and is also used to treat small lymphocytic lymphoma and mantle cell lymphoma in conjunction with other treatments. Side effects include infusion reactions (often severe), lymphopenia, anemia, thrombocytopenia, and infections. Alemtuzumab was used successfully as a treatment for HES in 2 prior cases, 1 lymphoproliferative and 1 myeloproliferative, both of which did not respond to imatinib mesylate or IFN-α and that were not tested for the *FIP1L1/PDGFRA* fusion.[8,9] Alemtuzumab controlled our patient's eosinophilia for 1½ years, and the patient's quality of life appeared improved. Our patient most likely had the idiopathic HES variant. However, the occurrence of thromboembolism and an increased B12 level point to a possible myeloproliferative HES variant. The patient had a plasmacytoma and then multiple lytic lesions and multiple myelomas, suggesting involvement of 2 cell lineages by a single mutation or possibly independent mutations. Overall, the results in our patient and the previously reported cases suggest that alemtuzumab might be an effective treatment for the myeloproliferative, idiopathic, and lymphoproliferative HES variants.

Lori A. Wagner, PhD[a]
Stephen Speckart, MD[c]
Bruce Cutter, MD[c]
Gerald J. Gleich, MD[a,b]

From the Departments of [a]Dermatology and [b]Medicine, the University of Utah School of Medicine, Salt Lake City, Utah, and [c]Montana Oncology, Missoula, Mont. E-mail: lori.wagner@hsc.utah.edu.

Disclosure of potential conflict of interest: L. A. Wagner has received research support from the National Institutes of Health. G. J. Gleich has received research support from the National Institutes of Health and Dyax. The rest of the authors have declared that they have no conflict of interest.

REFERENCES

1. Klion AD, Bochner BS, Gleich GJ. Approaches to the treatment of hypereosinophilic syndromes: a workshop summary report. J Allergy Clin Immunol 2006;117:1292-302.
2. Roufosse F, Cogan E, Goldman M. The hypereosinophilic syndrome revisited. Annu Rev Med 2003;54:169-84.
3. Gleich GJ, Leiferman KM, Pardanani A, Tefferi A, Butterfield JH. Treatment of hypereosinophilic syndrome with imatinib mesilate. Lancet 2002;359:1577-8.
4. Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med 2003;348:1201-14.
5. Rothenberg ME, Klion AD, Roufosse FE, Kahn JE, Weller PF, Su H, et al. Treatment of patients with hypereosinophilic syndrome with mepolizumab. N Engl J Med 2008;358:1215-28.
6. Dumont FJ. Alemtuzumab (Millennium/Ilex). Curr Opin Investig Drugs 2001;2:139-60.
7. Elsner J, Hochstetter R, Spickermann K, Kapp A. Surface and mRNA expression of the CD52 antigen by human eosinophils but not by neutrophils. Blood 1996;88:4684-93.
8. Pitini V, Teti D, Arrigo C, Righi M. Alemtuzumab therapy for refractory idiopathic hypereosinophilic syndrome with abnormal T cells: a case report. Br J Haematol 2004;127:477.
9. Sefcick A, Sowter D, Dasgupta E, Russell NH, Byrne JL. Alemtuzumab therapy for refractory idiopathic hypereosinophilic syndrome. Br J Haematol 2004;124:558-9.

Available online April 2, 2009.
doi:10.1016/j.jaci.2009.01.069

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

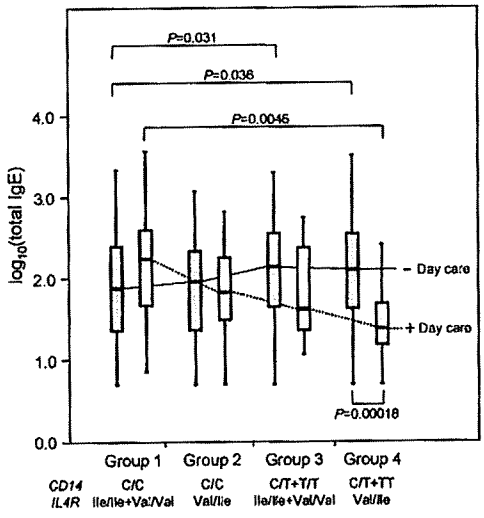

FIG 1. Total IgE levels in 4 groups of children classified based on a combination of *IL4R* and *CD14* genotypes. Box plot of $\log_{10}$(total IgE) values is shown for children who attended day care *(+Day care)* and for those who did not *(−Day care)*. Results are presented as medians and interquartile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

Yoichi Suzuki, MD, PhD[a]
Satoshi Hattori, MD[a]
Yoichi Mashimo, PhD[a]
Makiko Funamizu[a]
Yoichi Kohno, MD, PhD[b]
Yoshitaka Okamoto, MD, PhD[c]
Akira Hata, MD, PhD[a]
Naoki Shimojo, MD, PhD[b]

From the Departments of [a]Public Health, [b]Pediatrics, and [c]Otolaryngology, Graduate School of Medicine, Chiba University, Chiba, Japan. E-mail: ysuzuki@faculty.chiba-u.jp.

Supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and grants from the Ministry of Health, Labor, and Welfare, Japan.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

REFERENCES

1. Yang IA, Savarimuthu S, Kim ST, Holloway JW, Bell SC, Fong KM. Gene-environmental interaction in asthma. Curr Opin Allergy Clin Immunol 2007;7:75-82.
2. Celedon JC, Litonjua AA, Ryan L, Weiss ST, Gold DR. Day care attendance, respiratory tract illnesses, wheezing, asthma, and total serum IgE level in early childhood. Arch Pediatr Adolesc Med 2002;156:241-5.
3. Kramer U, Heinrich J, Wjst M, Wichmann HE. Age of entry to day nursery and allergy in later childhood. Lancet 1999;353:450-4.
4. Rothers J, Stern DA, Spangenberg A, Lohman IC, Halonen M, Wright AL. Influence of early day-care exposure on total IgE levels through age 3 years. J Allergy Clin Immunol 2007;120:1201-7.
5. Inoue Y, Shimojo N, Suzuki Y, Campos Alberto EJ, Yamaide A, Suzuki S, et al. CD14-550 C/T, which is related to the serum level of soluble CD14, is associated with the development of respiratory syncytial virus bronchiolitis in the Japanese population. J Infect Dis 2007;195:1618-24.
6. Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. Genes Immun 2006;7:95-100.
7. Hoffjan S, Nicolae D, Ostrovnaya I, Roberg K, Evans M, Mirel DB, et al. Gene-environment interaction effects on the development of immune responses in the 1st year of life. Am J Hum Genet 2005;76:696-704.
8. Fujii K, Matsubara Y, Akanuma J, Takahashi K, Kure S, Suzuki Y, et al. Mutation detection by TaqMan-allele specific amplification: application to molecular diagnosis of glycogen storage disease type Ia and medium-chain acyl-CoA dehydrogenase deficiency. Hum Mutat 2000;15:189-96.
9. Nisima S, Chisaka H, Fujiwara T. Surveys on the prevalence of pediatric bronchial asthma in Japan: a comparison between the 1982, 1992, and 2002 surveys conducted in the same region using the same methodology. Allergol Int 2009;58:37-53.

Available online May 18, 2009.
doi:10.1016/j.jaci.2009.03.035

Mucormycosis in chronic granulomatous disease: Association with iatrogenic immunosuppression

To the Editor:

Chronic granulomatous disease (CGD) results from mutations in either X-linked ($gp91^{phox}$) or autosomal ($p47^{phox}$, $p67^{phox}$, and $p22^{phox}$) genes encoding the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Impaired generation of reactive oxygen species predisposes to recurrent life-threatening bacterial and fungal infections. Septated hyaline molds (particularly *Aspergillus* species) are the primary fungal pathogens in CGD. Fungi of the order Mucorales (pauciseptated molds) are environmentally ubiquitous and cause mucormycosis in select immunocompromised patient populations, such as those with diabetic ketoacidosis and hematologic malignancy and recipients of transplants or deferoxamine. We investigated the prevalence of mucormycosis in patients with CGD.

PRIMERS FOR *IL4R* GENOTYPING (5′ TO 3′)

TaqMan probe (FAM-TACAGGTGACCAGCCTAACCCAGC CCCTGT-TAMRA); common primer (TGGAGGCATGTCCCG GACAC); Ile (A) allele primer (CGCCTCCGTTGTTCTCAG GGGT); and Val (G) allele primer (CGCCTCCGTTGTTCTC AGGGGC).

*Allergology International. 2009;58:***-***
DOI: 10.2332/allergolint.08-RAI-0072

REVIEW ARTICLE

Sublingual Immunotherapy for Japanese Cedar Pollinosis

Kimihiro Okubo[1] and Minoru Gotoh[1]

ABSTRACT

The prevalence of pollinosis caused by cedar pollen has increased by 10% these ten years of 26.5% in the investigation of 2008 in Japan. The pharmacotherapy is a main treatment tool for pollinosis, and the surgical treatment is not acknowledged to the treatment of pollinosis internationally. Moreover, allergen immunotherapy enters a special treatment method, and is an important therapeutic procedure. The allergen immunotherapy is unique for having possibility of curing allergen specific allergic diseases. However the side effect of allergen subcutaneous immunotherapy (SCIT), such as anaphylaxis is kept at a distance in a medical situation in Japan. Then, a sublingual immunotherapy (SLIT) that was safer than it, developed in Europe for pollinosis induced by grass or ragweed, but not in Japan. As a result, the effect of SLIT was proven in the cedar pollinosis in Japan as high level evidence. A whole body immunity induction is thought in the appearance of the effect, and, in addition, it is necessary to be going to be cleared the accurate mechanism of the effect in the future. Moreover, the development of a special SLIT and the import of an overseas product are needed in Japan.

KEY WORDS

Pollinosis, QOL, SCIT, Sublingual immunotherapy (SLIT)

INTRODUCTION

After Dr Noon begins to appear the conventional allergen specific subcutaneous immunotherapy (SCIT) in 1911, and is continuing treatment method.[1] The effect of SCIT on pollinosis caused by cedar pollen is low though the high therapeutic gain is admitted for the perennial allergic rhinitis in Japan. It is because the effect of SCIT has decreased relatively because this depends on the amount of pollen to which the symptoms of pollinosis and the amount of dispersion increases in recent years or the administering allergen of SCIT is a little. The problem of anaphylaxis in cause that SCIT has not become general treatment though effectiveness is confirmed.[2] An alternative immunotherapy to change the allergen administering route in Europe and United States to decrease the number of side effects of SCIT is done considerably than before. There are alternative route via the nose, sublingual, and the oral in the method development is not done respectively in Japan as for the double blind test comparison examination though effectiveness has been proven either. Therefore, it explains around sublingual immunotherapy (SLIT) that we are doing without the relation of the pharmaceutical company in Japan.

DEVELOPMENT IN JAPAN

In SLIT, high effectiveness is shown in Europe, and the few reports of the anaphylaxis have shown in randomized double blind placebo controlled (RCT) comparison examination evaluation.[3-5] It was one asthma case, and it was one diarrhea case in the SLIT 115 cases in three theses. It is recorded that it is not an anaphylaxis though the asthmatic attack is not described detailed. Moreover, that has not arrived importantly though the reaction of one case's near anaphylaxis externals less than ten times of allergen dose administration was observed by a recent report.[6]

To receive a lot of these reports, and to make SLIT adjust to pollinosis caused by cedar pollen from which the amount of the dispersion pollen was thought most, the research was started. We did the ex vivo culture experiment of the first human mouth mucous membrane incised by the time of surgery for analysis of allergen aspiration to the mucosal membrane. The double of the amount of the allergen dose

[1]Department of Otorhinolaryngology, Nippon Medical School, Tokyo, Japan.
Correspondence: Kimihiro Okubo, MD, PhD, Department of Otorhinolaryngology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan.
Email: ent-kimi@nms.ac.jp
Received 8 December 2008.

Table 1 Allergen administration schedule (increasing dosing)

	1st week (2 JAU)	2nd week (20 JAU)	3rd week (200 JAU)	4th week (2000 JAU)	5th week (2000 JAU)
1st day	1 drop	1 drop	1 drop	1 drop	20 drops
2nd day	2 drops	2 drops	2 drops	2 drops	
3rd day	3 drops	3 drops	3 drops	4 drops	
4th day	4 drops	4 drops	4 drops	8 drops	
5th day	6 drops	6 drops	6 drops	12 drops	20 drops
6th day	8 drops	8 drops	8 drops	18 drops	
7th day	10 drops	10 drops	10 drops	20 drops	

[After sixth week to pollen dispersed season, 20 drops of allergen extract was administered once a week sublingually. After pollen dispersed season, same dose was administered once in two weeks.]

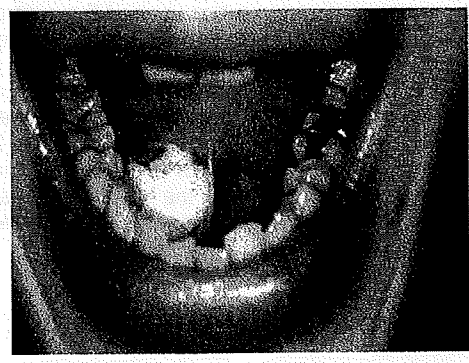

Fig. 1 How to be adapted the allergen extract and bit of bread.

in SCIT is almost the same dose aspirated by SLIT. So SLIT may act as the case of the SCIT is achieved is guessed by over the double dose of allergen at SCIT.[7]

HOW TO DO

The approval of the Nippon Medical School ethics committee was received to the pollinosis caused by cedar pollen patient and it went from some examinations including this basic experiment in SLIT. The allergen for SLIT, standardized Japanese cedar pollen allergen (2000 JAU [Japanese Allergology Unit]/ml, Torii Pharmaceutical, Tokyo, Japan), especially for SCIT products, was used for our SLIT trial. The allergen was able to be put on sublingual by using the bit of bread for the allergen to flow in actual sublingual and so as not to go out, then the allergen was kept to maintain at least for two minutes, and to present the antigen enough to the lymphatic tissue in the mouth.

The allergen administration was every day according the administration schedule from beginning to the forth week. On the first week, 2 JAU of allergen was administered from 1 drop to 10 drops, on the second week, 20 JAU of allergen was administered from 1 drop to 10 drops, on the third week, 200 JAU of allergen was administered from 1 drop to 10 drops, and then on forth week 2000 JAU of allergen was administered from 1 drop to 20 drops, as the final dose. On the fifth week twice a week after the sixth week, 2000 JAU/ml was administered to sublingual 20 drops as the final highest dose by once a week (Table 1, Fig. 1). There is tablet allergen for SLIT against grass pollinosis in Europe. There are some different allergen characters between Japanese cedar and grass. We cannot make the tablet allergen for SLIT of Japanese cedar pollinosis caused by its sticky character now.

THE EFFECT AND THE SIDE EFFECTS IN JAPANESE CEDAR POLLINOSIS

The Japanese cedar and cypress pollen dispersion was about 12000 grains, a large amount of dispersion in 2005 for these ten years. The RCT comparison by 60 cases was examined for making the first evidence in Japan. The SLIT group was intentionally low total symptom score (TSS) compared with the placebo (Fig. 2). This RCT of SLIT has shown to have lowered the symptom score more intentionally than the placebo in late pollen season.[8] SLIT had no significant difference with the drug therapy in the symptom score in the comparison research with the current drug therapy. However, the quality of life (QOL) score evaluated standardized Japanese Rhinitis Quality of Life Questionnaire (JRQLQ), is significantly decreased by SLIT group than placebo group, up to half level of score. QOL deterioration is significantly inhibited by SLIT (Fig. 3).

Moreover, it was confirmed though the side effect was completely fewer. Itchy of the tongue and the mouth when the antigen was administered, the feeling of numbness, nasal secretion increases, itchy of the skin, and hives were admitted at total of frequency of about 10% through the experiment, there were neither an anaphylaxis nor an asthmatic attack.

HOW TO ACT

The mechanism of the effect manifestation is known few up to the present time though the immunity induction of the limited part have some role on most of the effect of SLIT.[4] The mechanism of action for SCIT have been reported by the reduction of the effecter cells[9,10] and the increase of blocking antibody[11-14] in

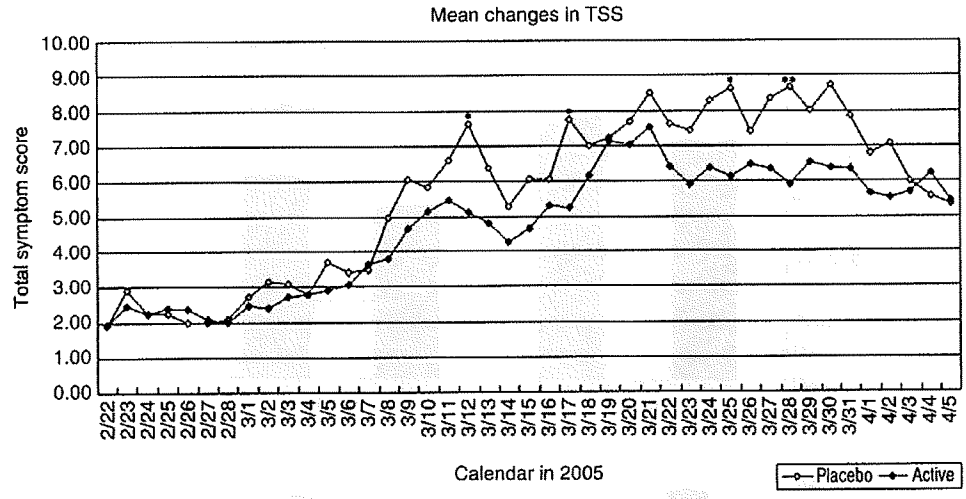

Fig. 2 Mean change of total nasal symptom score by SLIT and placebo group.

the conventional theories ten years ago. Recently, however, it has become widely accepted that SCIT may modify the T cell response to natural allergen because of T cell anergy and/or immune deviation[15-18] and regulatory T cell enhancement.[19]

For SLIT in particular, allergen administered to the oral mucosa accumulates in the sub-mandible lymph node, in which the immune response occurs[20] and peaks at approximately 2 h after administration.[21] An increase in stimulation index (SI) of PBMC at the early stage of the SLIT shows that the immunity induction of a sublingual allergen was at least caused in the general reaction.[22] It tried to reduce the side effect by reducing the effect throughout the body compared with past SCIT in SLIT. However, it has been understood that this result causes a general immunity induction. One more study of SLIT for Japanese cedar pollinosis was published by Chiba group also expressed the SLIT controlled the general Cry j-specific Th2 clone size.[23] The regulatory T cell enhancement in general by SLIT has reported in some papers recently.[24-26] So SLIT may act on generally, not just locally. It is necessary to clarify the exact effect mechanism of SLIT from the examination of the regional lymph node etc. by a similar examination that increased the number of cases or a detailed basic examination on animals in near future.

FOR THE FUTURE IN JAPAN

Approximately 15% of the Japanese population is affected by Japanese cedar pollinosis in 2002[27] and increase up to 26.5% in 2008.[28] The proportion of severe status patients is higher than with grass or ragweed pollinosis, which is the representative condition in other countries. The symptoms of Japanese cedar pollinosis persist for about 3 months, becoming a social issue. When the amount of pollen increases, patients show more severe symptoms, and the number of severe status patients is greatest in mid-March when the pollen count reaches its peak. Substantial antigen exposure enhances the antigen-antibody reaction in the airways (airway hypersensitivity), which is the mechanism involved in severe pollinosis, and immunotherapy with antigen-specific effects may control the exacerbation of the symptoms in the latter half of the cedar pollen season by inhibiting antigen-related enhancement of nasal mucosal hypersensitivity.

In SCIT for pollinosis treatment, the comments and responses of WHO are that the effect is verified from a lot of RCT comparison examinations.[29] However, it is a treatment method to which the medical treatment of Japan is kept at a distance because of the complexity, the possibility of the side effects, the cost and the enforcement under the present situation. The drug therapy is a main current in Japan where the allergy clinic has not been established from these problems for pollinosis. However, the immunotherapy that is fundamental treatment is an important method in the allergy management. The new SLIT shows the effect in pollinosis by cedar pollen was clarified in our examination in Japan. Any QOL fields and items became half QOL deterioration by the placebo in the evaluation using JRQLQ No1. This QOL questionnaire developed in Japan in the symptom score though the difference with the placebo was small in pharmacological treatment.[30] SLIT strongly controls the QOL deterioration in pollinosis rather than the symptom score to do effect is thought. Of the local immunotherapy modalities and SLIT is the most effective with a lower incidence of side effects, which complies with the WHO position paper on allergen