

ORIGINAL PAPER

Seasonal changes in antigen-specific T-helper clone sizes in patients with Japanese cedar pollinosis: a 2-year study

S. Horiguchi*, Y. Tanaka†, T. Uchida*, H. Chazono*, T. Ookawa*, D. Sakurai* and Y. Okamoto*

*Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan and †Department of Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan

Clinical and
Experimental
Allergy

Summary

Background Allergic rhinitis (AR) is a typical type I allergic disease that occurs through the induction of allergen-specific effector T cells. Once established, new effector T cells derive mostly from memory T cells that are capable of surviving for extended periods, although the mechanisms by which these memory functions are maintained have not yet been clarified. In particular, the exact life-span of memory T cells is still not well understood.

Objective Pollinosis patients seemed to be suitable subjects to investigate because such patients are exposed to antigens strongly for only a limited period once a year. We compared the seasonal changes in memory T-helper type 2 (Th2) between pollinosis and perennial allergic subjects.

Methods The clone sizes of the Japanese cedar pollen-specific memory Th cells were measured by an ELISPOT assay using specific peptides from the patients with cedar pollinosis, and the seasonal changes were noted. This study was performed for 2 years. The cedar-specific IgE levels in the peripheral blood were also studied. Mite allergy patients were also enrolled in the study.

Results The Japanese cedar-specific IL-4-producing Th2 cells were detected in all patients examined, although the number of cells was low. These Th 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. The cedar-specific IgE levels exhibited changes similar to the cedar-specific Th cells. On the other hand, there was no drifting of Th memory clone size with the mite allergics, and the IgE levels also did not change.

Conclusions While pollen-specific Th cells decreased after pollen exposure, their memory functions continued. Memory clone size maintenance therefore requires repetitive antigen irritation.

Keywords allergic rhinitis, clone size, IgE, memory T cell, Th2

Submitted 25 January 2007; revised 6 September 2007; accepted 16 October 2007

Correspondence:

Shigetoshi Horiguchi, Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan.
E-mail: horiguti@faculty.chiba-u.jp

Introduction

In recent years, many countries have experienced an increase in the prevalence of allergic rhinitis (AR) [1, 2]. In Japan, Japanese cedar (*Cryptomeria 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 [3, 4].

Pollinosis is thought to be an adaptive immune response that manifests as a type I allergic reaction, and it occurs as a consequence of fundamental allergenic me-

chanisms involving the induction of pollen-specific T-helper type 2 (Th2) effector cells from naïve Th0 cells [5]. Most effector T cells are short-lived, but few effector T cells become long-lived memory T cells. Once a memory T cell is established, it retards the induction of new effector T cells from naïve Th0 cells, according to the principle of the 'original antigenic sin'. Hence, most effector T cells are derived from memory T cells [6–10]. This concept describes a phenomenon in which the antibody response elicited in an individual after a secondary viral infection reacts more strongly to the viral variant that originally infected the individual. A similar phenomenon

is likely to be observed in Th cell responses as they play critical roles in promoting antibody responses.

Patients with type I allergy are thought to have allergen-specific memory Th cell clones. Immune-therapeutic intervention directed at diminishing the size of these clone memory Th2 cells and shifting the cytokine type of memory Th clones is thought to play a considerably important role in finding a complete cure.

However, the mechanism by which the antigen-specific Th memory clone is maintained in an allergic patient has not yet been clarified. There are several reports describing the peripheral blood to be re-stimulated by a specific antigen, while the Th2 cytokine response was measured both in season or/and out of season [11–13] as well as before and after immunotherapy [14–16]. In those studies, peripheral blood mononuclear cells (PBMCs) were stimulated by an antigen, and measured Th2 cytokine or its mRNA. However, those cytokine responses that were re-stimulated by whole antigen reflected various kinds of cells such as T cells, B cells, macrophages and antigen-presenting cells and did not precisely reflect the function or the exact number of antigen-specific Th cells. Because the Th cell response is restricted in major histocompatibility complex class II, it is necessary to use the Th cell epitope of class II restrictive to measure the reaction only for Th cell clones respond to allergen. Our purpose is to estimate the duration of life-span of allergen-specific memory T cells. We therefore directly examined the number of specific Th2 cells to respond to class II restrictive T cell epitope, which matched with Japanese human leukocyte antigen (HLA) variation. These kinds of studies have not yet been carried out. Pollinosis seemed to be a suitable subject to investigate the life-span of Th memory clones because patients are exposed to the antigen for only a limited period. In the present study, we examined the specific memory clone size, which is a population size of memory T cells that recognizes the same specific HLA restrictive epitope and produces isologous cytokine. We tried to detect IL-4 productive cells using only seven T cell epitopes; as a result, the total summation of these seven kinds of clones is the clonal size.

The limited seasonal nature of antigen exposure is useful for elucidating the mechanisms used in the maintenance of Th memory clone sizes. We examined the Japanese cedar pollen-specific Th clone sizes and the associated seasonal changes in patients with cedar pollinosis, while also comparing the yearly change in the clone size due to pollinosis with that due to perennial mite allergies that were detected by 14 T cell epitopes.

Materials and methods

A total of 41 patients with Japanese cedar pollinosis were enrolled in this study. The ages of the 20 males and 21 females ranged from 20 to 51, with an average of 31.1

years. The diagnosis of Japanese cedar pollinosis was based on the occurrence of typical nasal symptoms during the cedar pollen season and the detection of Japanese cedar-specific IgE by CAP-RAST (score: 2 or more). All patients had symptoms for at least 3 years. None of the patients received immunotherapy or immunosuppressive drugs (including steroids) within 8 weeks before the start of the study. The study received prior approval from the Ethics Committee of the Chiba University (Chiba, Japan). A written, witnessed informed consent was obtained from all patients. The study design is shown in Table 1. From 2003 to 2004, 23 patients participated in this study, and from 2004 to 2005, another 18 patients were enrolled. Twenty-two patients with perennial AR due to mite were also enrolled in this study. The ages of the 10 males and 11 females with perennial AR were from 20 to 48, with an average of 28.8 years. The diagnosis of mite allergy was based on the occurrence of typical nasal symptoms and the detection of Der f IgE by CAP-RAST (score: 2 or more).

Ten healthy subjects were also enrolled as controls. They were all negative for symptom episodes and allergen IgE.

The blood samples were collected every 3 months after July, and the PBMCs were obtained by the Ficoll–Hypaque method from the patients. The samples were stored in liquid nitrogen until analysis.

Clinical symptoms

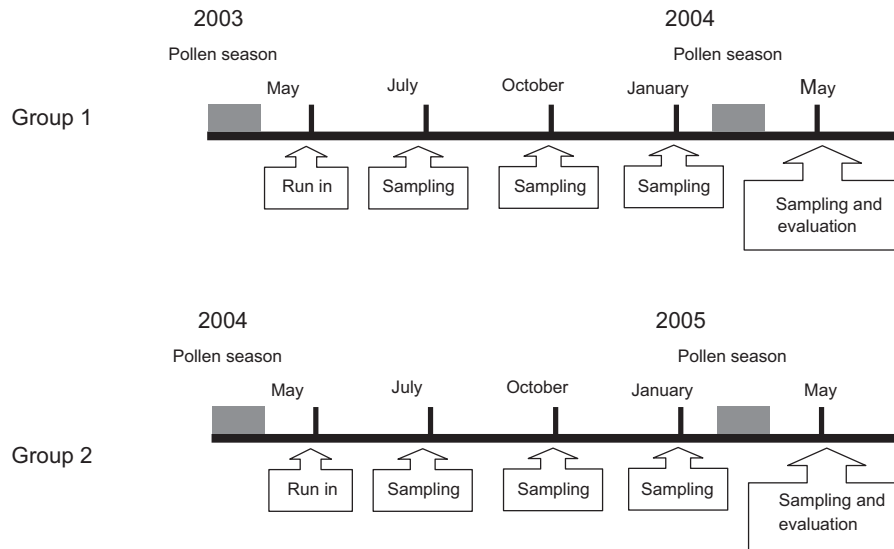
The nasal symptoms were evaluated on a scale from 0 to 4 in accordance with the practical guidelines for the treatment of AR [17], as follows: 0, no sensation; 1, mild; 2, moderate; 3, severe; and 4, extremely severe. Daily episodes of sneezing and nose blowing were rated 0 to 4, as follows: 0, none; 1, 1–5 episodes; 2, 6–10 episodes; 3, 11–20 episodes; and 4, >20 episodes. The medication was also recorded according to drug characteristics and duration of usage, according to the guidelines, as follows: antihistamine, mast cell stabilizers and vasoconstrictor were 1, and topical ocular or nasal steroids were 2.

Reagents

Antibodies. The monoclonal antibodies (MoAb) used for the ELISPOT assay were acquired from MABTECH (Stockholm, Sweden). For coating, MoAb 82-4 and MoAb 1-DIK were used to coat human IL-4 and IFN- γ , respectively. For detection, MoAb 12-1 and 7B6-1 were used to detect human IL-4 and IFN- γ , respectively. For the FACS analysis, anti-human Cy5-conjugated CD4, FITC-conjugated IFN- γ and PE-conjugated IL-4 were purchased from Dako (Tokyo, Japan).

Peptides. A recombinant hybrid peptide was used for the ELISPOT assay. This peptide comprised of the seven CD4 T

Table 1. Time schedule for examination



The Group 1 patients were recruited after the pollen season of 2003 and the Group 2 patients were recruited after pollen season of 2004. The blood sampling was performed every 3 months starting in July. The last blood sampling was performed after the pollen season of May, and the samples were analysed simultaneously.

cell determinants of Cry j 1 and Cry j 2, and the major Japanese cedar pollen allergens. Almost all the patient populations responded to this hybrid peptide, which is comparable to the response to Cry j 1 and Cry j 2. Moreover, because the seven peptides do not contain IgE-binding residues of *C. japonica* allergens, the recombinant peptide will not directly influence IgE-bearing cells such as mast cells, basophils and B cells [18]. The recombinant peptides for mite allergy were also prepared. This peptide comprised of the 14 CD4 T cell determinants of Der f 1 [19] and Der f 2 [20], and the major mite allergens. Almost all the patient populations responded to these peptides, which is comparable to the response to Der f 1 and Der f 2. Those peptides were Class II restricted and recognized Th cells only.

ELISPOT

The ELISPOT assay was performed according to the manufacturer's instructions. Briefly, the anti-human IL-4 or IFN- γ MoAbs were diluted to a concentration of 15 $\mu\text{g/mL}$ in sterile, filtered (0.45 μm) PBS (pH 7.2), and 100 μL /well was added onto nitro-cellulose plates (Millititre, Millipore Corp., Bedford, MA, USA). The plates were incubated overnight at 4 °C and the unbound antibodies were washed with filtered PBS thereafter. After the last wash, the PBS was sucked through the membrane under vacuum

(Millipore Corp.). One hundred microlitres of the pre-stimulated cell suspension was added to each well in duplicate, and the plates were incubated for 10 h at 37 °C. The cells were subsequently washed before adding 100 μL of the biotinylated MoAbs (1 $\mu\text{g/mL}$), and were then incubated for 2 h at room temperature. The plates were then washed and incubated for 90 min at room temperature with 100 μL of streptavidin alkaline phosphatase (Mabtech, Stockholm, Sweden) at a dilution of 1 : 1000. The unbound conjugate was removed by another series of rinsing before 100 μL of BCIP/NBT substrate solution (Bio-Rad, Richmond, CA, USA) was added, and the plates were incubated at room temperature until dark spots emerged (1 h). The colour development was stopped by repeated rinsing with tap water. After drying, the spots were captured photo-electrically and counted by a computed analysis to avoid any visual bias, using Auto Counter (ImmunoScan, CTL, Gmünd, Germany).

Pollen counts

The combined annual cedar and cypress pollen counts were measured using Durham pollen samplers.

Statistical analysis

Wilcoxon's paired rank sum tests were used to compare the mean values. A Friedman two-way ANOVA was used to

analyse paired data. Pearson's tests were used to determine the correlation coefficients.

Results

The cedar and cypress pollen counts determined using the Durham samplers were 470 cm/season in 2004, which was 1/20 of the average for the last 10 years. In 2005, we obtained 7852 cm/season, which was threefold higher than that of the average for the last 10 years. The seasonal symptoms were comparatively mild in 2004, and comparatively serious in 2005. The mean symptom-medication scores during the pollen season in 2004 and 2005 were 1.4 ± 2.1 and 2.8 ± 2.3 (mean $\pm$ SD), respectively.

Before the study, we compared the peptide-specific Th2 clone size of the patients with that of healthy controls in October, which is off pollen season. After stimulation with 10 nmol/L of cedar-specific peptides, the mean values of the IL-4 spot from the healthy controls and patients with cedar pollinosis that were obtained are shown in Fig. 1. Positive spots were obtained only in the samples from the pollinosis patients, but not from controls (A), although spots were equally obtained in the samples from both patients and controls when stimulated by Con A as a pan T cell stimulant (B). We also confirmed peptides for mite. The IL-4 spots were obtained only in the samples from the patients, but not from the controls (Fig. 2a), although spots were equally obtained in the samples from both patients and controls when stimulated by Con A as a pan T cell stimulant (Fig. 2b). Peptide-specific IL-4 spots were detected in all samples examined. Approximately 10–100 peptide-specific IL-4 spots were observed in the wells

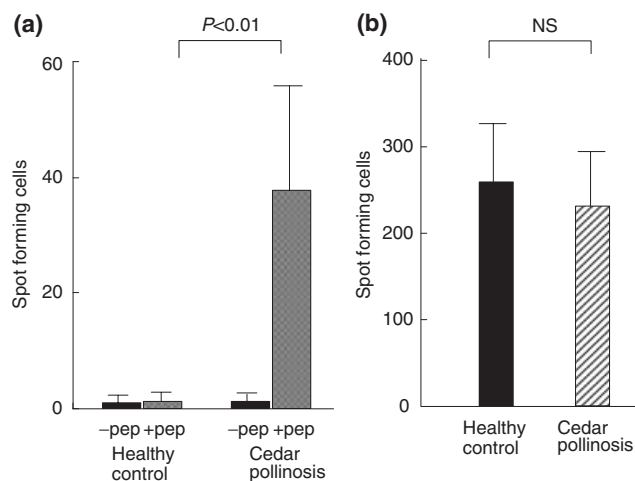


Fig. 1. The mean values of IL-4 spots in the samples from the healthy control subjects and patients with cedar pollinosis are shown. (a) 1×10^5 cells were stimulated by a peptide derived from Cry j 1 and Cry j 2. The spots were obtained only in the samples from the pollinosis patients, but not from the controls. (b) 1×10^4 cells were stimulated by Con A, a pan T cell stimulant. The spots were equally obtained in the samples from both the patients and the controls. NS, not significant.

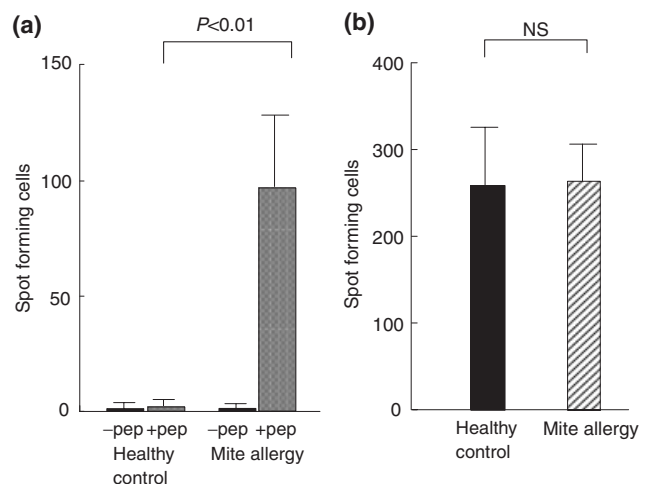


Fig. 2. The mean values of IL-4 spots in the samples from the healthy control subjects and patients with cedar pollinosis are shown. (a) 1×10^5 cells were stimulated by a peptide derived from Der f 1 and Der f 2. The spots were obtained only in the samples from the pollinosis patients, but not from the controls. (b) 1×10^4 cells were stimulated by Con A, a pan T cell stimulant. The spots were equally obtained in the samples from both the patients and the controls. NS, not significant.

incubated with 10^5 PBMC based on the ELISPOT assay. These spots were analysed in triplicate in each of the experiments and exhibited good reproducibility.

We carried out a depletion assay in advance with whole PBMC from three patients with Japanese cedar pollinosis using an antibody-conjugate magnetic bead kit (MACS system, Miltenyi Biotec GmbH, Tokyo, Japan). When CD4 was depleted, the spots of ELISPOT disappeared; however, after the depletion of CD8, the number of spots was equal to that with whole PBMC. The depletion of CD28 also caused the spots to disappear. These results show that the present ELISPOT assay using a hybrid peptide was CD4 restricted and the CD28 expression was indispensable for the detection of the spots (data not shown).

The seasonal changes in peptide-specific IL-4 spots are shown in Fig. 3. The IL-4 spots decreased after July, and were at their lowest in January before the onset of the cedar pollen season, which were almost 60% of those observed in July. The IL-4 spots increased during the cedar pollen season in 2004 despite the small amount of pollen (Fig. 3a). The same trend in seasonal changes was obtained during the 2005 season. The IL-4 spots decreased after July, and were at their lowest in January before the onset of the cedar pollen season, which were almost 60% of those observed in July (Fig. 3b). Interestingly, the clone size on May 2005 was 40% larger than that on July 2004. This phenomenon was not seen during the 2004 season.

The seasonal changes in the cedar-specific IgE levels in the serum of the patients are shown in Fig. 4. While the cedar-specific IgE decreased after the pollen season in 2003, it did not increase during the cedar pollen season in

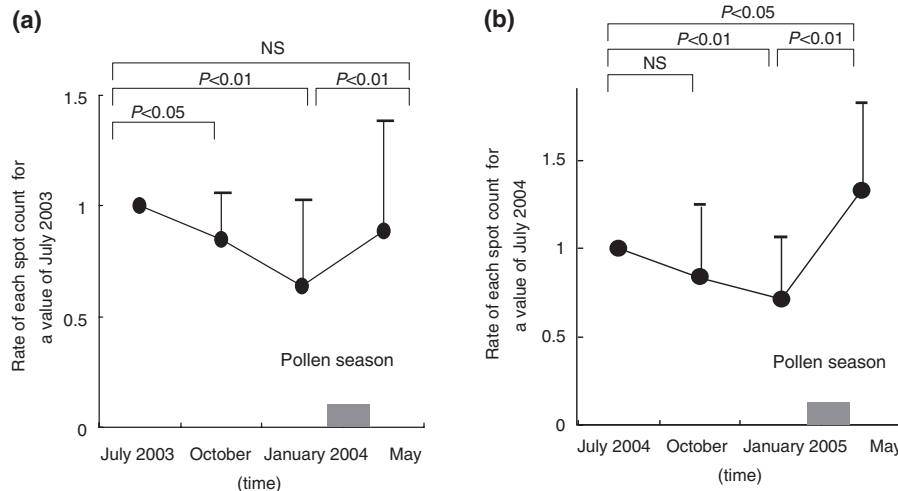


Fig. 3. The samples of each time-point were analysed simultaneously. The Cry j 1 and 2 peptide-specific IL-4-producing cells were counted using the ELISPOT technique. The rate of each spot count for the value in July is shown. (a) Twenty-three patients were enrolled during the 2004 season, which was characterized by very little pollen scattering. (b) Eighteen patients were enrolled during the 2005 season, which was characterized by massive pollen scattering. NS, not significant.

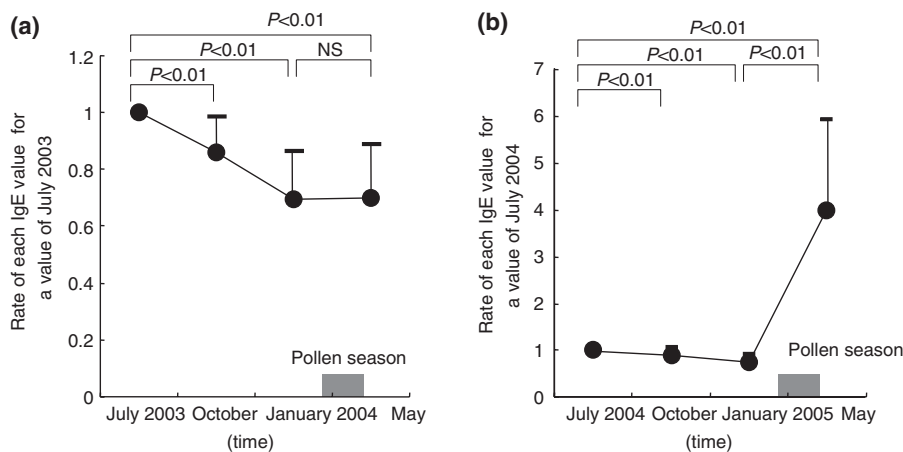


Fig. 4. The samples of each time-point were analysed simultaneously. Cedar-specific IgE were analysed using the RAST technique. The rate of each IgE for the value in July is shown. (a) Twenty-three patients were enrolled during the 2004 season, which was characterized by very little pollen scattering. (b) Eighteen patients were enrolled during the 2005 season, which was characterized by massive pollen scattering. NS, not significant.

2004 (Fig. 4a). The specific IL-4 spots decreased during the pollen season in some patients, and the cedar-specific IgE levels decreased during the pollen season in all patients. During the 2004–2005 season, the cedar-specific IgE levels decreased after the pollen season in 2004, but increased fivefold during the pollen season in 2005 (Fig. 4b). The number of cedar-specific IL-4 spots did not show a correlation with the cedar-specific IgE levels.

The yearly changes in the mite-specific IgE levels and Der f peptide-specific IL-4 spots were also examined in the samples from 22 patients with mite allergy. The yearly changes in mite-specific IgE and mite-specific memory Th2 clone size could not be clearly obtained (Fig. 5).

The patients with mite AR had persistent nasal symptoms all year around. Although the symptom scores varied among the patients as well as the seasons, the deviation was occasionally large; overall, no significant difference was observed. The pollinosis only demonstrated significant symptoms during the pollen dispersal season; however, the patients' nasal symptom scores during the pollen season were higher than those of the mite AR patients as shown in Fig. 6.

Discussion

We examined the Japanese cedar-specific IL-4-producing memory T cells in the peripheral blood of patients with

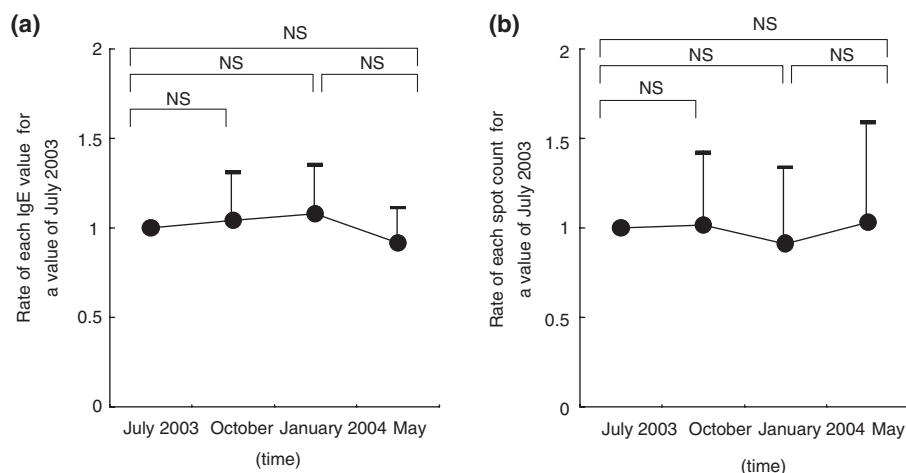


Fig. 5. The samples of each time-point were analysed simultaneously. Mite-specific IgE were analysed using the RAST technique (a). The Der f 1/2 peptide-specific IL-4-producing cells were counted using the ELISPOT technique (b). The rate of each value for the value in July is shown. NS, not significant.

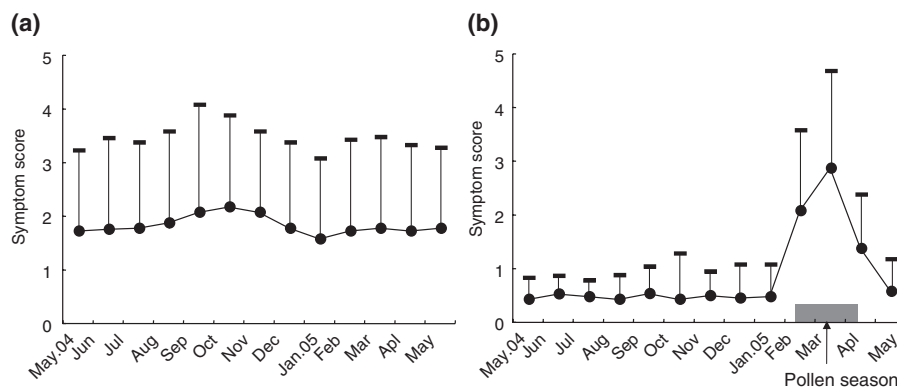


Fig. 6. The mean monthly symptom scores (mean + SD) from May 2004 to May 2005 are shown. (a) Mean nasal symptom scores of 22 patients with mite allergy. (b) Mean nasal symptom scores of 18 patients with Japanese cedar pollinosis.

Japanese cedar pollinosis by an ELISPOT assay using Japanese cedar-specific peptides. Although the number of cedar peptide-specific IL-4 T cells was low, the samples from all the patients examined had 10–100 spots/ 10^5 PBMC. In contrast, the healthy subjects had no positive IL-4 spot. These IL-4 T cells were thought to be cedar-specific Th2. The cedar peptide-specific IFN- γ T cells, which were thought to be cedar-specific Th1 cells, were detected in several patients. The cedar-specific peptides used in the ELISPOT assay were hybrid peptides that would only react with the T cells of those patients with Japanese cedar pollinosis, but not with the T cells of subjects not suffering from cedar pollinosis. Consequently, these findings could explain the low incidence and detection rates of cedar peptide-specific Th1 cells. The number of cedar peptide-specific IL-4 T cells (Th2 cells) did not correlate with the numbers of specific IFN- γ T cells (Th1 cells). More specifically, no relationship was observed between the cell types, and the cell numbers

appeared to be independent of each other (although cedar-specific serum IgE levels did not correlate with the number of cedar-specific IL-4 T cells, the patients who had specific IFN- γ T cells had lower specific IgE levels than those without specific IFN- γ T cells).

IgE production is likely to be controlled by many factors. IFN- γ T cells inhibit IgE production independently of the number of specific IL-4 T cells [21, 22]. Similarly, pollen-specific IgE is known to exhibit seasonal changes [23, 24], thus increasing during the pollen season and decreasing during the off-season. However, the amounts of cedar and cypress pollen in 2004 were extraordinarily low and the pollen counts, which were less than 1/20 of the annual average for the last 10 years, did not contribute to the enhancement of IgE production in the patients enrolled in this study. Conversely, the cedar peptide-specific IL-4 T cells increased by 30% despite the low levels of pollen exposure. The levels of Th2 clone size increased to levels equivalent to that in July 2003.

Although IL-4 is indispensable for IgE production, the cedar peptide-specific Th cells are thought to be very sensitive to exposure to cedar pollen.

In contrast, because the amount of pollen in 2005 was large, the cedar-specific IgE levels increased to five times after the pollen season. The specific Th2 clone size increased considerably after the pollen season, too, and the clone size increased by 70% after the pollen season. The levels of clone size increased to 1.4 times more than those observed in July 2004. A memory clone size might be affected by allergen exposition considerably. While cedar peptide-specific IL-4 Th2 cells decreased in number during the off-season, 8 months after the cessation of the pollen season, more than 60% of these memory Th2 cells were still found. Such annual drifting was absent in the perennial-allergic subjects. Therefore, pollinosis that undergoes exposition for a very limited period is different from mite perennial allergies.

Indeed, it is difficult to estimate the half-life of allergen-specific Th2. Generally, memory T cells are thought to have a long life-span, considering the duration of the vaccine effect of viral infections. Almost all reports of antigen-specific memory T cell have so far been about infectious diseases, while only a few reports of memory T cell are about type I allergy. The simple theory that an antigen creates a pool of long-lived antigen-specific memory T cells has been surprisingly difficult to prove and it is also still not completely accepted. The issue of longevity is much more controversial for CD4⁺ than for CD8⁺ T cells. Regarding CD4⁺ memory T cells, lymphocytic choriomeningitis virus (LCMV)-specific CD4⁺ memory T cells [25] and Sendai virus-specific CD4⁺ memory T cells [26] at first disappeared dramatically, whereas virus-specific CD8⁺ T cells were fairly stable in numbers over the same period. In humans, however, there have been several reports of vaccinia virus-specific CD4⁺ memory T cells with a long life-span. These cells were found to decline with a half-life of approximately 10 years, conferring long-term protection [27]. The question remains, however, as to whether these memory Th cells are maintained in the absence of an antigen or by contact with a persistent antigen, bound in the immune complexes on follicular dendritic cells, or by contact with other environmental cross-reactive antigens. Based on our results on memory Th2 cells from cedar pollinosis in comparison with those from perennial mite allergy, the clone size maintenance requires antigen irritation of repetition and clonal mitosis by it. Therefore, the half-life of allergen-specific Th2 might be estimated to be less than 1 year.

The mechanism wherein a daughter Th2 cell discharges Th2 cytokines such as IL-4 in the same way after mitosis from the original Th2 has not been well understood. The opening and closing of chromatin helps to determine the cellular characteristics. It is believed that acetylation and methylation of the chromatin are very stable. Therefore, it

is thought that the characteristics of a parent cell are transferred to the daughter cells after mitosis. However, a splitting enzyme for the acetylation and methylation has been discovered in a recent study, and it does not always seem to be stable [28]. It has recently been proposed that the opening and closing of chromatin is induced by an antigen, and that this is due to the Th cells' response [29, 30]. These changes in chromatin may therefore play an important role in retaining the memory functions of Th cells. Further studies need to be conducted on memory retention in order to clarify how these mechanisms could be applied to the development of an effective and fundamental solution for the treatment of allergic diseases.

References

- 1 Savage J, Roy D. Allergic rhinitis: an update. *J R Soc Health* 2005; 125:172–5.
- 2 Pawankar R, Fokkens W. Evidence-based treatment of allergic rhinitis. *Curr Allergy Asthma Rep* 2001; 1:218–26.
- 3 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.
- 4 Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. *Ann Allergy Asthma Immunol* 2003; 91:288–96.
- 5 Parronchi P, Macchia D, Piccinni MP *et al.* Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc Natl Acad Sci USA* 1991; 88:4538–42.
- 6 McMichael AJ. The original sin of killer T cells. *Nature* 1998; 394:421–2.
- 7 Davenport FM, Hennessy AV, Francis T. Epidemiological and immunological significance of age distribution of antibody variants of influenza virus. *J Exp Med* 1953; 98:641–56.
- 8 Fazekas de St G, Webster RG. Disquisitions of original antigenic Sin. I. Evidence in man. *J Exp Med* 1966; 124:331–45.
- 9 Muller S. Avoiding deceptive imprinting of the immune response to HIV-1 infection in vaccine development. *Int Rev Immunol* 2004; 23:423–36.
- 10 Auwaerter PG, Rota PA, Elkins WR *et al.* Measles virus infection in rhesus macaques: altered immune responses and comparison of the virulence of six different virus strains. *J Infect Dis* 1999; 180:950–8.
- 11 Tsuda M, Ohashi Y, Washio Y *et al.* Seasonal changes in phytohemagglutinin-induced cytokine synthesis by peripheral blood lymphocytes of patients with seasonal allergic rhinitis due to Japanese cedar pollens. *Acta Otolaryngol* 1998; 538 (Suppl.):156–68.
- 12 Ohashi Y, Nakai Y, Tanaka A *et al.* Seasonal rise in interleukin-4 during pollen season is related to seasonal rise in specific IgE for pollens but not for mites. *Acta Otolaryngol* 1998; 118:243–7.
- 13 Gabriellson S, Paulie S, Rak S *et al.* Specific induction of interleukin-4-producing cells in response to in vitro allergen stimulation in atopic individuals. *Clin Exp Allergy* 1997; 27:808–15.
- 14 Laaksonen K, Waris M, Makela MJ, Terho EO, Savolainen J. In vitro kinetics of allergen- and microbe-induced IL-4 and IFN-

- gamma mRNA expression in PBMC of pollen-allergic patients. *Allergy* 2003; **58**:62–6.
- 15 Moverare R, Elfman L, Bjornsson E, Stalenheim G. Cytokine production by peripheral blood mononuclear cells following birch-pollen immunotherapy. *Immunol Lett* 2000; **73**:51–6.
- 16 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.
- 17 Xiao JZ, Kondo S, Yanagisawa N *et al*. Probiotics in the treatment of Japanese cedar pollinosis: a double-blind placebo-controlled trial. *Clin Exp Allergy* 2006; **36**:1425–35.
- 18 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.
- 19 Matsuoka T, Kohroggi H, Ando M, Nishimura Y, Matsushita S. Dermatophagoides farinae-1-derived peptides and HLA molecules recognized by T cells from atopic individuals. *Int Arch Allergy Immunol* 1997; **112**:365–70.
- 20 Inoue R, Matsuoka T, Kondo N, Nishimura Y, Matsushita S. Identification of Dermatophagoides farinae-2-derived peptides and class II HLA molecules recognized by T cells from atopic individuals. *Int Arch Allergy Immunol* 1997; **114**:354–60.
- 21 Pene J, Rousset F, Briere F *et al*. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. *Proc Natl Acad Sci USA* 1988; **85**:6880–4.
- 22 So EY, Park HH, Lee CE. IFN-gamma and IFN-alpha posttranscriptionally down-regulate the IL-4-induced IL-4 receptor gene expression. *J Immunol* 2000; **165**:5472–9.
- 23 Sato K, Nakazawa T, Sahashi N, Kochibe N. Yearly and seasonal changes of specific IgE to Japanese cedar pollen in a young population. *Ann Allergy Asthma Immunol* 1997; **79**:57–61.
- 24 Kakinoki Y, Ohashi Y, Kato A *et al*. Seasonal increase in specific IgE in serum induced by natural Japanese cedar pollen exposure in asymptomatic and symptomatic sensitized individuals. *Acta Otolaryngol* 1998; **538** (Suppl.):152–5.
- 25 Homann D, Teyton L, Oldstone MB. Differential regulation of antiviral T-cell immunity results in stable CD8+ but declining CD4+ T-cell memory. *Nat Med* 2001; **7**:913–9.
- 26 Cauley LS, Cookenham T, Miller TB *et al*. Cutting edge: virus-specific CD4+ memory T cells in nonlymphoid tissues express a highly activated phenotype. *J Immunol* 2002; **169**:6655–8.
- 27 Hammarlund E, Lewis MW, Hansen SG *et al*. Duration of antiviral immunity after smallpox vaccination. *Nat Med* 2003; **9**:1131–7.
- 28 Shi Y, Lan F, Matson C *et al*. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 2004; **119**:941–53.
- 29 Yamashita M, Ukai-Tadenuma M, Kimura M *et al*. Identification of a conserved GATA3 response element upstream proximal from the interleukin-13 gene locus. *J Biol Chem* 2002; **277**:42399–408.
- 30 Yamashita M, Ukai-Tadenuma M, Miyamoto T *et al*. Essential role of GATA3 for the maintenance of type 2 helper T (Th2) cytokine production and chromatin remodeling at the Th2 cytokine gene loci. *J Biol Chem* 2004; **279**:26983–90.