

Yearly and seasonal changes of specific IgE to Japanese cedar pollen in a young population

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Background: There have been no detailed long-term observations of the relationship between specific IgE production and stimulation by various naturally occurring allergens.

Objective: This study was conducted to elucidate the yearly and seasonal changes of specific IgE antibody production to Japanese cedar pollen, an allergen of Japanese cedar pollinosis, in young adults.

Methods: The numbers of Japanese cedar pollen were counted over a period of 9 years. Changes in the percentages of antibody carriers to Japanese cedar pollen and mite were examined during these years. Changes in Japanese cedar pollen-specific IgE levels between a low exposure year and a high exposure year in individual subjects were also investigated.

Results: From 1987 to 1995, the percentages of Japanese cedar pollen-IgE carriers varied from about 30% to 50% with the intensity of pollen stimulation, and carriers tended to increase yearly. The rates of anti-mite IgE carriers changed little. In the spring which is the pollen season, Japanese cedar pollen-IgE levels in low exposure years were weaker than those in high exposure years in individual subjects. Levels in autumn, which is not the pollen season, showed equivalent levels in both high and low exposure years. Anti-mite IgE levels in individual subjects varied little during these years.

Conclusions: A long-term follow-up study supported that Japanese cedar pollen-IgE production is mainly associated with the degree of allergen exposure.

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INTRODUCTION

Seasonal changes of symptoms, total IgE, and specific IgE are closely related to allergenic stimulation. Berg and Johansson reported that changes in specific IgE antibodies were more pronounced than those of total IgE in pollen-allergic children and that the serologic course was characterized by a

rapid increase in reaginic activity during the pollen season.¹ Yunginger and Gleich also described elevation of serum IgE levels and nasal IgE concentrations during the ragweed pollination season in patients with ragweed hay fever.^{2,3} Deuschl reported that in patients with allergic rhinitis to timothy pollen, the total IgE and specific IgE antibodies in both serum and nasal secretions reached significantly higher levels in samples taken during and after than before the pollen seasons.⁴ Many studies in adults have also shown specific IgE levels and skin reactivity to be influenced by age, sex, race, and environmental or occupational exposure.^{5–8}

Previously, we examined age-related changes in Japanese cedar pollen-specific and mite-specific IgE production in a general population including a proportion of allergic individuals, and the highest levels of specific IgE were

found in young adults.^{9,10} There have, however, been no detailed long-term studies of year-to-year or seasonal changes of IgE levels and allergen exposure in a population.

In this study, we describe the relationship between changes of pollen counts of Japanese cedar pollen and the percentages of pollen-specific IgE in a young Japanese population from 1987 to 1995. We also investigated the changes in Japanese cedar pollen-IgE levels between a low exposure year and a high exposure year in individual subjects. As control studies, the changes in the incidence of mite-specific IgE positivity were observed during these years.

MATERIALS AND METHODS

Counts of Japanese Cedar Pollen

Japanese cedar pollen grains were counted in Funabashi city around Kanto district from the beginning of February to the end of April. Durham's standard apparatus was used for sampling. The slides, coated with white vaseline, were exchanged daily at 9 AM and prepared for counting following Sugaya's glycerine jelly method.¹¹ The pollen grains in an area of 10 cm² or 3.24 cm² on slides were counted and the results were expressed as number/cm²/season.¹²

Preparation of Antigen for Fluorescence-Enzyme Linked Immunosorbent Assay (fluorescence-ELISA)

Japanese cedar pollen extract was prepared from *Cryptomeria japonica* pollen by the method of Yasueda et al.¹³ Pollen grains were collected from sterile flowers and dried thoroughly. The precipitate fraction obtained from the pollen by 80% saturation with ammo-

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nium sulfate was dissolved, dialyzed against cold deionized water overnight and then lyophilized for storage. Mite extract prepared from *Dermatophagoides farinae* was a gift from Torii Yakuhin Co, Ltd (Japan). Prior to use, both extracts were dissolved in 0.05 M carbonate buffer, pH 9.6, at a final protein concentration of 25 µg/mL and used for antigens. Protein concentration was determined by BIO-RAD dye binding protein assay (Biorad Co., Ltd, USA) using bovine serum albumin as a standard.

Sera

All serum samples were obtained from a general population of volunteers including a proportion of allergic individuals. Year-to-year changes in the percentages of Japanese cedar pollen-IgE carriers and mite-IgE carriers were observed using approximately 180 sera obtained from yearly freshmen students in Gunma University School of Health Sciences. The sera were collected from the subjects in the middle of May each year, directly after the end of the pollen season. For examination of Japanese cedar pollen-IgE levels between low (1989 and 1994) and high pollen exposure years (1990 and 1995) in individual subjects, sera were collected from the same subjects in the middle of May of both years. For comparative studies, sera were also collected from a group for health examinations in the middle of October, which is not the pollen season, in both 1989 and 1990.

Measurement of Specific IgE Antibody

Serum levels of specific IgE antibody were measured by fluorescence-ELISA according to the method described by Nakazawa et al¹⁴ with β-D-galactosidase-conjugated goat anti-human IgE as the secondary antibody and 4-methylumbelliferyl-β-D-galactoside as the enzyme substrate. All serum samples were tested at a dilution of 1:4. At the final reaction step, the hydrolyzed probe 4-methylumbelliferone released from the substrate was

measured with a MicroFLUOR reader (Dynatech Product, VA, USA). Results are expressed in relative fluorescence units (RFU). The RFU increased linearly in proportion to the amount of 4-methylumbelliferone present.

Criteria for Positivity

The RFU criteria for specific IgE positivity were set as follows: (1) Japanese cedar pollen-IgE and mite-IgE levels in all tested sera were measured by fluorescence-ELISA and each RFU was plotted on a histogram. (2) The specific IgE-negative population was determined from the curve of the histogram, and then the mean value and standard deviation (SD) of the negative population were calculated. (3) Positivity for specific IgE was defined as any value above the cut-off limit of the mean RFU + 3 SD. Weakly positive RFU were defined those ranging from the mean RFU + 2 SD to the mean + 3 SD. The positive, weakly positive, and negative responses for Japanese cedar pollen-IgE were respectively determined as ≥43, 34–42, and ≤33. For mite antibodies, the positive, weakly positive, and negative responses were respectively determined as ≥39, 32–38, and ≤31. Positive and negative control sera were kept at –20 °C and thawed before use.

Materials

Polystyrene black microplates were purchased from Dynatech Product (VA, USA). β-D-Galactosidase-conjugated goat anti-human IgE was obtained from MBL K.K., Nagoya, Japan. 4-Methylumbelliferyl β-D-galactoside was obtained from Sigma Co, Ltd (MO, USA). All other chemicals used were of special grade and purchased from Wako Pure Chemicals Ind, Ltd. (Tokyo).

RESULTS

Year-to-Year Changes of Japanese Cedar Pollen-IgE Carriers

Figure 1 shows changes of pollen scattering numbers from 1987 to 1995 and the percentages of Japanese cedar pollen-IgE and mite-IgE carriers. Pollen counts varied with every passing year from 121 to 3200 pollen grains/cm²/season; ie, the pollen counts were greatest in 1995 (3,200/cm²/season), while they were equally low in 1989 (121/cm²/season) and 1994 (158/cm²/season). The percentages of Japanese cedar pollen-IgE carriers in 1987 and 1989 were about 30% although those in several other years were about 45% to 50%. The percentages of carriers in 1992 and 1994 showed slight

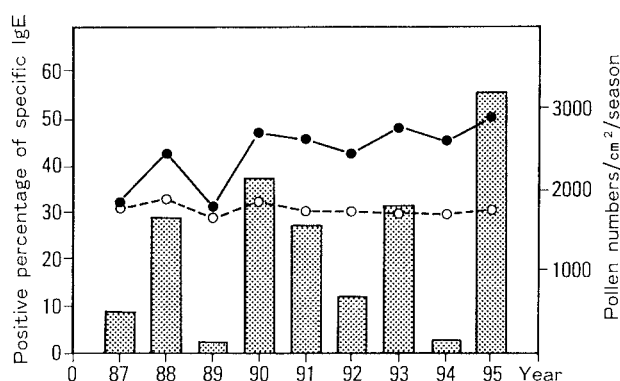


Figure 1. Changes in positive percentages of Japanese cedar pollen-IgE levels from 1987 to 1995 in a young Japanese population in relation to pollen scattering. Japanese cedar pollen was scattering from the beginning of February to the end of April. Durham's standard apparatus was used for sampling. The pollen grains in an area of 10 cm² or 3.24 cm² on slides were counted and the results were expressed as number/cm²/season. Allergen specific-IgE levels of the subjects were measured in 1:4 diluted sera by fluorescence-ELISA. ●—● Japanese cedar pollen-specific IgE, ○—○ mite specific-IgE, and ■ pollen number.

declines compared with those in the previous years. Changes in pollen count roughly corresponded to those in Japanese cedar pollen-IgE positivity rates, however, the positivity rates tended to show a yearly increase. Japanese cedar pollen-IgE positivity rates in 1989 and 1994 were also about 31% and 46%, respectively, although about the same low pollen counts were observed in both years. The percentages of mite-IgE carriers in the same populations changed little during the 9 years examined.

Changes of Specific IgE Levels in Individual Subjects in Low- and High-Exposure Years

The effects of allergen stimulation on the Japanese cedar pollen-IgE carriers were analyzed by comparing the specific IgE levels between an extremely low pollen exposure year (1989) and a high exposure year (1990) among subjects who had both Japanese cedar pollen-IgE and mite-IgE positive in 1989. As a control, mite-IgE levels were analyzed in the same population. Most subjects showed stronger specific IgE titers in 1990 than in 1989 (Fig 2A). Mite-IgE titers in both years varied little (Fig 2B). There was a significant correlation between anti-IgE titer in 1989 and that in 1990; the correlation coefficient was 0.846 ($y = 0.60x - 8.80$), and that between anti-mite IgE titer in 1989 and in 1990 was 0.929 ($y = 1.04x + 2.70$). These results indicate that the Japanese cedar pollen-IgE titer in 1989 was approximately 60% of that of 1990 in individual subjects although mite-IgE levels in both years were constant. Furthermore, the same analysis was also performed between an extremely low Japanese cedar pollen exposure year (1994) and a high exposure year (1995) in 76 individual subjects. As shown in Figure 2C, results similar to Figure 2A, were obtained. There was a significant correlation between Japanese cedar pollen-IgE titer in 1994 and that in 1995; the correlation coefficient was 0.875 ($y = 0.73x - 57.12$). Mite-IgE levels in both years were also constant as shown in Figure 2D. The correlation

coefficient of anti-mite IgE titers in 1994 and in 1995 was 0.974 ($y = 0.98x + 17.97$). On the other hand, similar examinations were conducted in 87 samples obtained from individual subjects at health checks in the middle of October, 1989 and 1990 which is not the pollen season. As shown in Figure 2E and 2F, the titers of Japanese cedar pollen-IgE and mite-IgE in the individual subjects were equivalent. The correlation coefficient between Japanese cedar pollen-IgE in the

autumns of 1989 and 1990 was 0.865 ($y = 1.00x + 28.77$), and that between mite-IgE during these periods was 0.981 ($y = 0.95x - 4.71$).

Approximately 3% to 5% of a weakly positive response was found in each population. Changes of the Japanese cedar pollen-IgE levels in the individual subjects weakly positive were observed between an extremely low exposure year (1989) and high exposure year (1990). Fourteen of the 15 subjects weakly positive in 1989

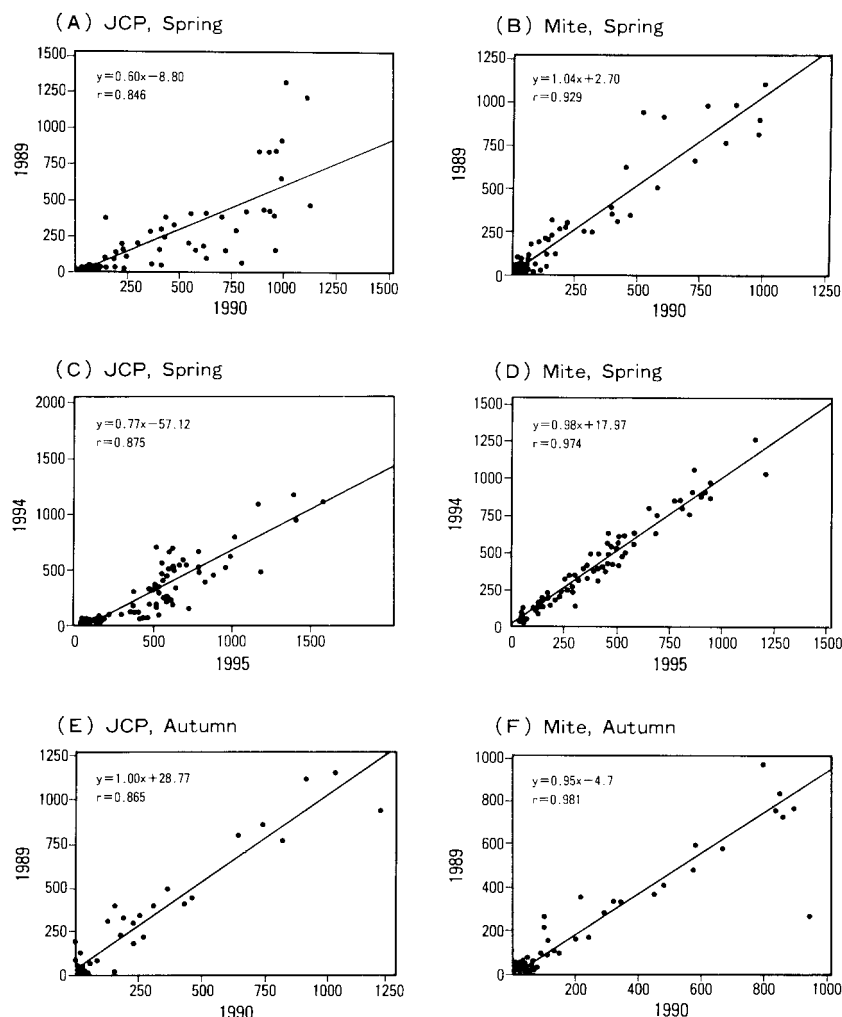


Figure 2. Correlation between anti-Japanese cedar pollen-IgE antibody levels (RFU) in a low pollen exposure year and a high exposure year in individual subjects. The abscissa and ordinate represent specific-IgE levels (RFU). JCP-IgE, Japanese cedar pollen-specific IgE; mite-IgE, mite specific-IgE, RFU; and relative fluorescence units obtained from fluorescence-ELISA. (A) JCP-IgE in spring of 1989 and 1990, (B) mite-IgE in spring of 1989 and 1990, (C) JCP-IgE in spring of 1994 and 1995, (D) mite-IgE in spring of 1994 and 1995, (E) mite-IgE in autumn of 1989 and 1990, and (F) mite-IgE in autumn of 1989 and 1990.

showed elevation to the RFU of over 100 in 1990. Then, approximately 12% of subjects with a negative result in 1989 became Japanese cedar pollen-IgE positive in 1990.

DISCUSSION

Allergen stimulation to peripheral blood lymphocytes from allergic patients in vitro increases the number of allergen-specific IgE-plaque-forming cells.¹⁵ The extent of allergen stimulation may therefore greatly influence specific IgE levels in vivo. Many studies have shown that specific IgE in serum and nasal secretions rises significantly in ragweed-sensitive or timothy-sensitive patients during the pollination season.¹⁻⁵ During the past 10 years, Japanese cedar pollen-sensitive patients have increased among Japanese, especially in young adults. Japanese cedar pollen is released from the beginning of February until April, and the degree of pollen release is markedly influenced by the temperature of the preceding summer. Sahashi et al described that high pollen scattering was observed when the mean temperatures in the previous July were higher than 26 °C.¹²

The positivity rate of Japanese cedar pollen-IgE in young adults in the Japanese has been reported to be more than 30%,^{9,16} and subjective symptoms have been noted in about 35% of antibody-positive subjects. The relationship between the extent of pollination and specific IgE production has not been studied over long-term periods, although such studies are very important for establishing methods to protect against and to reduce the symptoms of pollen allergy. We observed yearly changes in the extent of pollen release and in the prevalence of Japanese cedar pollen-IgE positivity among freshmen in our University from 1987 to 1995. Pollen release was extremely low in 1989, and 1994, and fairly low in 1987 and 1992 (Fig 1). The mean temperatures in each July of 1986, 1988, 1991, and 1993 were lower than those of the other years. The percentages of Japanese cedar pollen-IgE car-

riers were low in 1987 and 1989 (about 32% in each year), and also relatively low in 1992 (43%) and 1994 (46%) in comparison to those before or after these years. These findings suggest that the positivity rates of Japanese cedar pollen-IgE are mainly associated with the degree of Japanese cedar pollen stimulation. In addition, the percentages of Japanese cedar pollen-IgE carriers increased slightly during 9 years examined; that is, pollen released in 1989 and 1994 was equally low, 121/cm²/season and 158/cm²/season, respectively, while the IgE positivity rate in those years was 32.5% and 46%, respectively. The percentages of mite-IgE carriers in the same populations changed little during the 9 years examined.

Analysis of the Japanese cedar pollen-IgE levels obtained from individual subjects in 1989 and 1990 also suggested a close relationship between specific IgE levels and the intensity of pollen release. Japanese cedar pollen-IgE levels in individual subjects were higher in the spring of 1990 with strong allergen stimulation than in the spring of 1989 with weak stimulation, while there were no changes in anti-Japanese cedar pollen IgE levels of individual subjects in the autumn of 1990 and 1989 which was not pollen season, or in anti-mite IgE levels in spring or autumn. Also of interest was the observation that the RFU of almost all the subjects with weak positive result, against Japanese cedar pollen-IgE in 1989 increased to more than 100 in 1990, and then some of the subjects with a negative result in 1989 became Japanese cedar pollen-IgE-positive in 1990.

Our findings confirm that Japanese cedar pollen-specific IgE production is closely associated with pollen release similar to the data for other pollens such as ragweed and timothy. These findings also suggest that specific IgE production against naturally occurring allergen stimulation is mainly due to the allergen stimulation more than other factors.

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DURATION OF PROTECTION AFTER CESSATION OF MITE IMMUNOTHERAPY DEPENDS ON DURATION OF TREATMENT

In order to evaluate the duration of protection conferred by immunotherapy with house dust mite extract the authors studied 40 asthmatic patients treated with standardized *Dermatophagoides pteronyssinus* extract for 12–96 months. After cessation of treatment patients were followed at intervals of 6 months for up to 3 years. Relapses were defined as recurrence of symptoms of asthma for more than seven consecutive days or regular use of antiasthmatic drugs or FEV₁ less than 80% of that at the end of immunotherapy.

There were no relapses within 3 years in 45% of patients. Relapses occurred in 25% of patients within 3 months; 37% within 12 months. The duration of protection was related to the duration of immunotherapy; rate of relapse was significantly higher in those who had received therapy less than 35 months. Most patients who did not relapse had decreases in skin test reactivity at the end of therapy, whereas most who relapsed had no change in skin test reactivity.

These observations indicate the duration of protection after discontinuation of immunotherapy with *Dermatophagoides pteronyssinus* extract may depend upon the duration of therapy; treatment for at least 3 years may be more beneficial than shorter treatment. The authors do not comment on possible differences in exposure to mites in these patients.

—RMS

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