

Seasonal Rise in Interleukin-4 during Pollen Season is Related to Seasonal Rise in Specific IgE for Pollens but not for Mites

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Since IL-4 plays a key role in inducing and increasing the generation of not only primary polyclonal but also secondary specific IgE responses by B lymphocytes, a seasonal increase in IL-4 is likely to be involved in such seasonal rises in specific IgE in seasonal allergic rhinitis. The first aim of this study was to investigate the possible seasonal increase in serum IL-4 in patients with seasonal allergic rhinitis due to Japanese cedar pollens. If serum IL-4 increases in response to seasonal pollen exposure and is responsible for the seasonal increase in pollen-specific IgE in sera, this increase in IL-4 might theoretically affect specific IgE synthesis for other allergens. The second aim was to investigate the effect of natural pollen exposure on serum concentrations of house dust mite-specific IgE in patients who have seasonal allergic rhinitis and concurrent perennial allergic rhinitis due to house dust mites. This study included 55 adult patients with seasonal and perennial allergic rhinitis due to Japanese cedar pollens and *Dermatophagoides farinae* (*D. farinae*). Venous blood was collected twice from each patient, before and during the cedar pollen season 1996, to determine IL-4, cedar pollen-specific IgE and *D. farinae*-specific IgE in sera. Both IL-4 and pollen-specific IgE in sera were significantly increased during the pollen season, and the seasonal increase rate in pollen-specific IgE was significantly correlated with the seasonal increase rate in IL-4. By contrast, *D. farinae*-specific IgE was not changed during the pollen season in these patients. In conclusion, an elevation of IL-4 in sera during the pollen season may play an important part in the seasonal rise in pollen-specific IgE, and enhancement of specific IgE synthesis is likely to need not only an increase in IL-4 but also an increase in the number and/or capacity of specific IgE-secreting B cells. **Key words:** interleukin-4, seasonal allergic rhinitis, perennial allergic rhinitis, specific IgE synthesis, Japanese cedar pollen.

INTRODUCTION

Interleukin-4 (IL-4) plays the most critical role in the switching of B lymphocytes to produce IgE (1, 2) and may therefore be of critical importance in the development of allergic diseases. This role of IL-4 in isotype switching is reinforced by the observation that mice with germline disruption of IL-4 gene fail to generate Th-2 cells *in vivo* and to produce IgE antibodies (3). Seasonal rises in pollen-specific IgE in pollen-sensitive patients have been well established (4, 5). Since Th-2 cell-derived IL-4 plays a key role in inducing and increasing the generation of not only primary polyclonal but also secondary specific IgE responses by B lymphocytes (6), a seasonal increase in IL-4 is likely to be involved in such seasonal rises in specific IgE in seasonal allergic rhinitis. However, it has been reported that *in vitro* IgE production by peripheral blood mononuclear cells (PBMC) in the presence of IL-4 is not significantly different between atopic and nonatopic donors (7). *In vivo* studies sometimes support hypotheses that have been generated from *in vitro* systems, but sometimes indicate that *in vitro* observations have little *in vivo* applicability. Therefore, the first aim of this study was to investigate the possible seasonal increase in serum IL-4 in patients with seasonal allergic rhinitis due to Japanese cedar (*Cryptomeria japonica*) pollen.

If serum IL-4 increases in response to seasonal pollen exposure and is responsible for the seasonal increase in pollen-specific IgE in sera, the next question is whether patients who had seasonal allergic rhinitis due to the pollen and concurrent perennial allergic rhinitis due to house dust mites, have a resulting seasonal increase not only in pollen-specific IgE but also in house dust mite-specific IgE during the pollen season. However, little is known of the possible influence of pollen exposure on the other allergen-specific IgE. Therefore, the second aim of this study was to investigate the effect of natural pollen exposure on serum concentrations of mite-specific IgE in patients with seasonal allergic rhinitis due to Japanese cedar pollens and concurrent perennial allergic rhinitis due to house dust mites.

MATERIALS AND METHODS

Patients

This study included 55 adult patients (29 females and 26 males, age range: 18–59 years) with seasonal allergic rhinitis due to Japanese cedar pollens and concurrent perennial allergic rhinitis due to *Dermatophagoides farinae* (*D. farinae*), who gave informed consent to participation in the study. All patients

were selected from our outpatients who completely satisfied the following conditions before treatment: (i) well-documented history of typical perennial allergic rhinitis and seasonal aggravation during the pollen season of Japanese cedar, (ii) positive skin tests to *D. farinae* and Japanese cedar pollens, (iii) positive nasal provocations to *D. farinae* and Japanese cedar pollens, and (iv) Japanese cedar pollen-specific IgE and *D. farinae*-specific IgE in sera before the pollen season of 1996 were above the lower limit of detection and quantitatively detectable. In addition, none of them had previously been treated with immunotherapy or systemic steroid treatment.

Study design

The period for pollination of Japanese cedar in 1996 was from the first week of February to the second week of April. All patients enrolled in this study were treated with non-sedating antihistamine tablets during the pollen season of 1996. Venous blood was collected twice from each patient, before the 1996 cedar pollen season (from December 1995 to January 1996) and during the 1996 cedar pollen season (first week of April 1996). Serum samples were separated, stored at -80°C , and never retrieved prior to use for the present determination. This study design was followed the principals outlined in the Declaration of Helsinki (8).

Determination of specific IgE and IL-4

Japanese cedar pollen-specific IgE, *D. farinae*-specific IgE and IL-4 in sera were determined at the same time. Pollen-specific IgE and *D. farinae*-specific IgE were measured by the CAP system (9). IL-4 was measured with our chemiluminescent enzyme-linked immunosorbent assay (ELISA) (10). In brief, microtiter plates were coated with mouse monoclonal anti-human IL-4 antibody (R & D Systems, Minneapolis, MN, USA) and after incubation with serum samples or purified recombinant IL-4 (R & D Systems) plates were developed with biotinylated mouse anti-human IL-4 antibody (PharMingen, San Diego, CA, USA) followed by avidine-alkaline phosphatase conjugate (Tago, Camarillo, CA, USA). Then CSPD (Boehringer Tropic, Bedford, MA, USA) with enhancer (Emerald, Tago) was added to each well. The chemiluminescence intensity was determined by a micro luminater MR 1000 (Dynatech Laboratories, Chantilly, VA, USA). The method employed is capable of detecting 0.75–100 pg/ml of IL-4, and does not measure the presence of other cytokines. The seasonal increase rates in specific IgE and IL-4 were expressed as the percentage deviation from the respective values before the pollen season.

Statistical analysis

For comparison of the paired values, Wilcoxon's signed-rank test was used, and significant differences were accepted when p was less than 0.05. Spearman's correlation coefficient (r_s) was calculated to analyse possible correlations between two different parameters, and significant correlation was accepted when p was less than 0.05 according to the Spearman's rank correlation analysis.

RESULTS

Serum concentrations of IL-4 before the pollen season were not quantitatively determined in 13 patients because the values were below the lower limit of detection. For these 13 patients, serum concentrations of IL-4 during the pollen season were also below the lower limit of detection in six patients, but were above the lower limit of detection in the remaining seven patients. In contrast, serum concentrations of IL-4 in the remaining 42 patients were above the lower limit of detection and could be quantitatively measured before as well as during the pollen season. The following statistical analysis concerning seasonal change in IL-4, seasonal change in pollen-specific IgE, seasonal change in *D. farinae*-specific IgE, and correlation between seasonal increase rate in IL-4 and seasonal increase rate in specific IgE was performed for the 42 patients whose paired values of IL-4 (before the season and during the season) could be quantitatively measured.

Seasonal change in IL-4

Serum concentrations of IL-4 were above the lower limit of detection in 42 patients. The serum levels (mean $\pm$ SD) of IL-4 were 2.81 ± 2.10 pg/ml before the pollen season and 4.46 ± 3.24 pg/ml during the

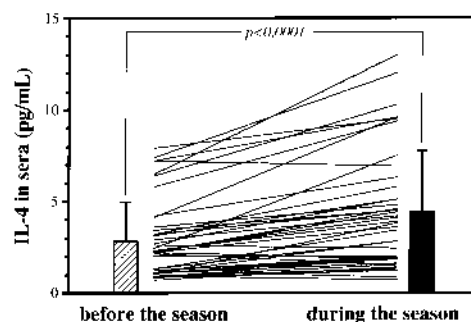


Fig. 1. Seasonal changes in IL-4 in sera. Individual values of IL-4 are expressed with solid lines. Levels of IL-4 during the pollen season are significantly higher than those before the pollen season.

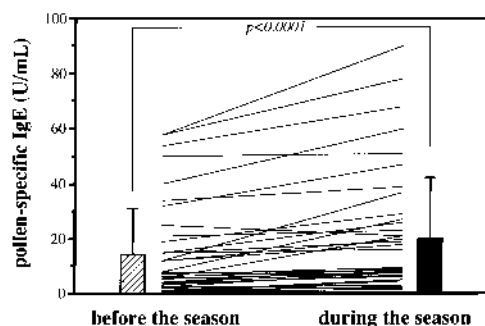


Fig. 2. Seasonal changes in cedar pollen-specific IgE in sera. Individual values of cedar pollen-specific IgE are expressed with solid lines. Levels of cedar pollen-specific IgE during the pollen season are significantly higher than those before the pollen season.

pollen season. The serum levels of IL-4 during the pollen season were significantly higher than those before the pollen season ($p < 0.0001$, Fig. 1).

Seasonal change in cedar pollen-specific IgE

The serum levels of cedar pollen-specific IgE during the pollen season (20.02 ± 21.86 U/ml) were significantly higher than those before the pollen season (13.94 ± 16.40 U/ml, $p < 0.0001$, Fig. 2).

Seasonal change in *D. farinae*-specific IgE

The serum levels of *D. farinae*-specific IgE during the pollen season (30.49 ± 33.50 U/ml) were not significantly different from those before the pollen season (31.25 ± 33.62 U/ml, $p = 0.9571$, Fig. 3).

Correlation between seasonal increase rate in IL-4 and seasonal increase rate in specific IgE

Seasonal increase rates in cedar pollen-specific IgE ($77.80 \pm 86.30\%$) were significantly correlated with

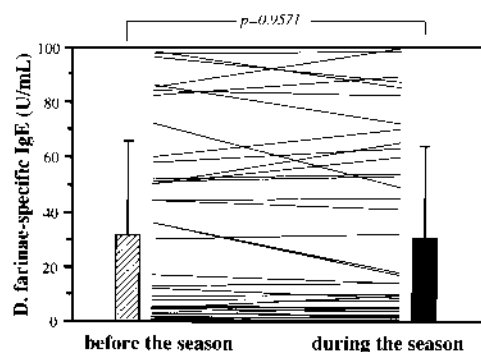


Fig. 3. Seasonal changes in *D. farinae*-specific IgE in sera. Individual values of *D. farinae*-specific IgE are expressed with solid lines. Levels of *D. farinae*-specific IgE during the pollen season are not significantly different from those before the pollen season.

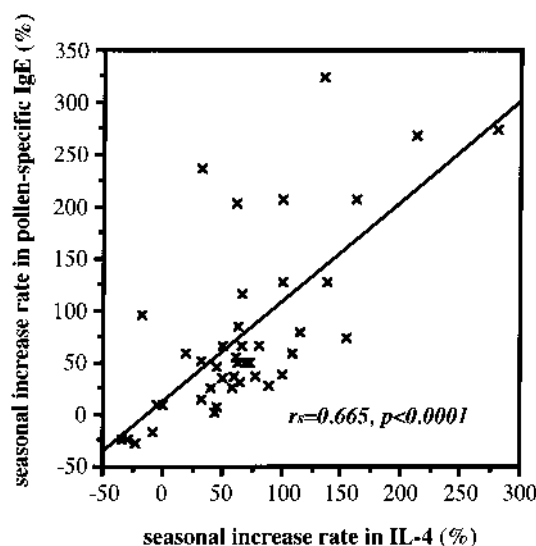


Fig. 4. Correlation between seasonal increase rates in IL-4 in sera and seasonal increase rates in cedar pollen-specific IgE in sera. Seasonal increase rates in IL-4 are significantly correlated with seasonal increase rates in cedar pollen-specific IgE.

seasonal increase rates in IL-4 ($67.73 \pm 62.27\%$, $r_s = 0.665$, $p < 0.0001$, Fig. 4). However, seasonal increase rates in *D. farinae*-specific IgE ($4.80 \pm 34.32\%$) were not significantly correlated with seasonal increase rates in IL-4 ($67.73 \pm 62.27\%$, $r_s = 0.248$, $p = 0.1128$, Fig. 5).

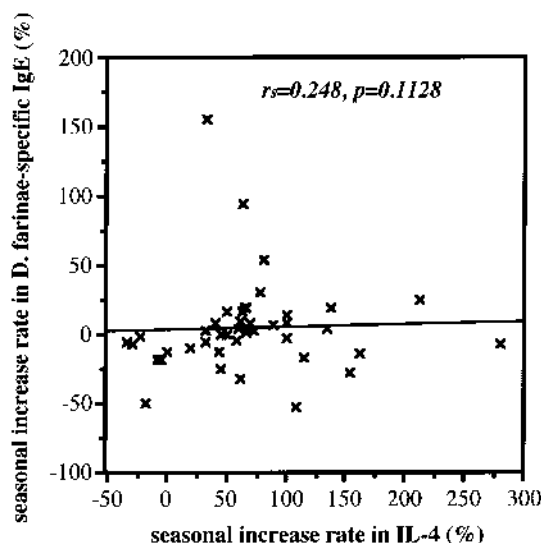


Fig. 5. Correlation between seasonal increase rates in IL-4 in sera and seasonal increase rates in *D. farinae*-specific IgE in sera. Seasonal increase rates in IL-4 are not significantly correlated with seasonal increase rates in *D. farinae*-specific IgE.

DISCUSSION

Allergic diseases are characterized by the increased productivity of IgE. Th-2 cell-derived IL-4 plays a key role in inducing and increasing the generation of both primary polyclonal and secondary specific IgE responses by B lymphocytes (6). At least four studies have documented a significant elevation of serum IL-4 in atopic individuals compared with non-atopic controls (9, 11–13). It is therefore possible to speculate that seasonal rises in serum IL-4 might be responsible for seasonal rises in specific IgE in seasonal allergic rhinitis. However, little is known about the seasonal change in serum IL-4 in patients with seasonal allergic rhinitis. The first aim of this study was therefore to investigate the seasonal change in serum IL-4 in patients with seasonal allergic rhinitis. In our study, serum levels of IL-4 were significantly increased during the pollen season. In addition, the seasonal increase rate in IL-4 was significantly correlated with the seasonal increase rate in pollen-specific IgE. Our data may therefore suggest that this increase in IL-4 is related to and is probably responsible for the seasonal rise in specific IgE in pollen allergy.

However, it has been reported that *in vitro* IgE production by PBMC in the presence of IL-4 is not significantly different between atopic and non-atopic donors (7). In addition, during the pollen season IL-4-stimulated PBMC of atopic donors produced more IgE than IL-4-stimulated PBMC of non-atopic donors, whereas out of the season no difference was observed between these two groups (14). Taking these lines of evidence together, an elevation of circulating IL-4 is not always enough to increase specific IgE in sera. An elevation of specific IgE in sera might necessitate an increase in the number and/or capacity of specific IgE-secreting B cells in the circulation, together with an elevation of serum IL-4. If not, patients who had seasonal allergic rhinitis due to pollens and concurrent perennial allergic rhinitis due to house dust mites, would have not only a resulting seasonal increase in pollen-specific IgE but also a seasonal increase in house dust mite-specific IgE during the pollen season.

The second aim of this study was thus to investigate whether a seasonal increase in IL-4 during the pollen season could also increase *D. farinae*-specific IgE in sera in patients with seasonal allergic rhinitis and concurrent perennial allergic rhinitis due to *D. farinae*. In this study, serum levels of *D. farinae*-specific IgE during the pollen season were not significantly different from those before the season. *Dermatophagoides farinae*-specific IgE was not increased when serum levels of IL-4 were significantly

increased in these subjects. In addition, the seasonal increase rate in *D. farinae*-specific IgE was not correlated with the seasonal increase rate in IL-4. Therefore, our data may suggest that IL-4 in sera increases during the pollen season in patients sensitive to both the pollen and *D. farinae* and that this elevation of IL-4 is likely to increase the pollen-specific IgE synthesis but does not affect *D. farinae*-specific IgE synthesis. An inference may be derived from our study that enhancement of specific IgE synthesis needs not only an increase in IL-4 but also an increase in the number and/or capacity of specific IgE-secreting B cells.

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